**Universidade de São Paulo**
**Escola Superior de Agricultura "Luiz de Queiroz"**

# Inclusão de lisolecitina ou ß-glucanas na dieta líquida de bezerros leiteiros: efeitos no desempenho, saúde e metabolismo

**Maria Eduarda Reis**

Dissertação apresentada para obtenção do título de Mestra em Ciências. Área de concentração: Ciência Animal e Pastagens

**Piracicaba**
**2021**

**Maria Eduarda Reis**
**Médica Veterinária**

# Inclusao de lisolecitina ou ß- glucanas na dieta líquida de bezerros leiteiros: efeitos no desempenho, saúde e metabolismo

Orientadora:
Profa. Dra. **CARLA MARIS MACHADO BITTAR**

Dissertação apresentada para obtenção do título de Mestra em Ciências. Área de concentração: Ciência Animal e Pastagens

**Piracicaba**
**2021**

**Dados Internacionais de Catalogação na Publicação**
**DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP**

Reis, Maria Eduarda

Inclusão de lisolecitina ou ß- glucanas da dieta liquida de bezerros leiteiros: efeitos no desempenho, saúde e metabolismo / Maria Eduarda Reis. - - Piracicaba, 2021.

88 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura "Luiz de Queiroz".

1. Aditivo 2. Ganho de peso 3. Eficiência alimentar 4. Escore Fecal I. Título

**DEDICATÓRIA**

A família, Maria Cecilia, Antonio Carlos, Jaqueline, Marcelo e Vitoria que não mediram esforços e sempre acreditaram em mim. Sem vocês nada disso seria possível!

Dedico

## AGRADECIMENTOS

Agradeço,
A Professora Carla Maris Machado Bittar, a qual sou e sempre serei muito grata, não só pela oportunidade de ingressar no mestrado, mas tambem por todos ensinamentos, orientação e confiança desde a minha época de estagiária no grupo.
Ao Departamento de Zootecnia da Escola Superior de Agricultura "Luiz de Queiroz", ao programa de "Ciência Animal e Pastagens" e a todos os professores que contribuíram com seus ensinamentos.
A todos meus colegas do Grupo de Pesquisa em Metabolismo Animal da ESALQ, Ari, Paulinha , Marcos, Marina, Milaine e Gercino por toda a ajuda. Em especial a Ari e Paulinha por conduzirem parte do meu experimento junto e sempre me ajudarem e apoiarem em momentos mais difíceis.
A Fernanda e Marcos, pela amizade e por todo suporte desde que realizei meu estágio no bezerreiro.
Aos estagiários do Clube de Criação de Bezerros (CCB), Amandinha, Frete, Tak, Poti, Mei, Rafa, Xakra e Tiabeti não só pela ajuda, mas também por entenderem o quão importante é trabalhar em equipe. A nossa união tornou o ambiente de trabalho mais leve e descontraido.
A todos os estagiários que passaram pelo bezerreiro durante o período do experimento: Sophia, Cristiane, Grazi, Lum, Barbara, Marjorie, Caio, Mateus, Marina, Mariana, Shara, Fran.
À querida Idalina, que me recebeu de forma muito agradável no bezerreiro e sempre estava disposta a me ajudar e aconselhar.
Ao membros do Clube de Práticas Zootécnicas (CPZ) em especial ao Larguei por toda a ajuda e compreensão de que trabalhando juntos teríamos melhores resultados.
Aos funcionários do Setor de Não Ruminantes, Ednesio, Gilberto e todos os demais, que sempre nos auxiliaram nas dificuldades da rotina de trabalho.
Ao professor Joao Henrique Cardoso Costa pela oportunidade, ensinamento e confiança no meu período de estágio na Universidade do Kentucky.
A Coordenação de Aperfeiçoamento de Pessoal de Nivel de Superior (CAPES) pela concessão da bolsas de estudos (Processo: 88882.328645).
Ao Leandro Greco da Kemin America do Sul pelo auxílio no delineamento experimental e à Kemin pelo apoio financeiro.

MUITO OBRIGADA!

**"***Você não pode mudar o vento,*
*mas pode ajustar as velas do barco para chegar onde quer."*
***Confúcio***

# Sumário

## RESUMO

**Inclusão de lisolecitina ou ß-glucanas na dieta líquida de bezerros leiteiros: efeitos no desempenho, saúde e metabolismo**

O objetivo desse estudo foi avaliar o efeito da inclusão de lisolecitina ou ß-glucanas na dieta líquida de bezerros leiteiros em relação ao desempenho, saúde e metabolismo. Foram conduzidos dois experimentos com 32 bezerros da raça Holandesa cada em delineamento experimental de blocos casualizados, totalizando 16 animais por tratamento. Os animais foram distribuídos em blocos, de acordo com o peso ao nascer, data de nascimento e sexo, sendo distribuídos nos tratamentos do Experimento 1: 1) Controle; 2) Sucedâneo lácteo suplementado com lisolecitina (4 g/dia); e no Experimento 2: 1) Controle; 2) Sucedâneo lácteo adicionado de ß-glucanas (2g/dia). O consumo de alimentos, e o escore fecal e de saúde foram avaliados diariamente durante 56 dias de idade em ambos os experimentos. O desenvolvimento corporal foi acompanhado através da pesagem corporal semanalmente e medidas corporais quinzenalmente em ambos experimentos. Amostras de sangue foram coletadas semanalmente para a determinação de indicadores metabólicos. A inclusão de lisoleticina não apresentou efeito no consumo de matéria seca ($P > 0.05$). No entanto, melhorou o ganho médio diário ($P < 0.04$), o peso corporal final ($P < 0.05$), a eficiência alimentar ($P < 0.02$) e o escore fecal ($P = 0.04$). Os parâmetros sanguíneos também não foram afetados, exceto pelas concentrações de proteína total ($P < 0.01$), que foram menores nos bezerros suplementados com lecitina. Os principais efeito da inclusão de ß-glucanas para os bezerros durante o período de aleitamento foi a melhor eficiência alimentar ($P = 0.04$), peso corporal final ($P = 0.05$) Além disso, bezerros não suplementados tiveram uma maior incidência de diarreia quando comparado aos animais suplementados com ß-glucanas OR 22.46 (95% CI: 15.14-33.32; $P < 0.0001$). A inclusão de aditivos na dieta líquida de bezerros é uma alternativa que pode auxiliar na melhoria da eficiência alimentar, escore fecal e consequentemente no desempenho.


**Palavras-chave**: Aditivos; ganho de peso; eficiência alimentar; escore fecal.

## ABSTRACT

**Inclusion of lysolecithin or ß-glucans in the milk replacer for Holstein dairy calves: effects on performance, health and metabolism**

The study aimed to investigate how addition of β-glucans can affect calf's growth performance, health, and blood metabolites. Two experiments were conducted with thirty-two Holstein dairy calves each (16 calves per treatment/ experiment). Calves were individually housed in tropical shelters and blocked according to sex, date and weight at birth and randomly assigned to 1 of the treatments of Experiment 1: 1) Control; 2) Milk replacer supplemented with lysolecithin (4 g / day); and Experiment 2: 1) Control; 2) Milk replacer supplemented with ß-glucans (2g / day). Feed intake, and health score and fecal score were recorded daily during 56-d study in both experiments. Body weight were measured weekly and body measures biweekly in both experiments. Blood samples were collected weekly to determine metabolic indicators. Lysolecithin had no effect on dry matter intake (P >0.05). However, the lysolecithin improved average daily gain (P <0.04), final body weight (P <0.05), feed efficiency (P <0.02), and fecal score (P =0.04). Blood parameters were also not affected, except for total protein concentrations (P <0.01), which were lower in supplemented calves. In the experiment 2 the main effect of providing beta-glucans to Holstein dairy calves during preweaning period is the improvement in feed efficiency (P =0.04), final body weight (P =0.05). Moreover, control calves were more likely to have a diarrhea bout than β-glucans calves OR 22.46 (95% CI: 15.14-33.32; *P* <0.0001). Overall, lysolecithin and β-glucans supplementation may improve feed efficiency, final body weight and fecal score during the preweaning phase.


**Key words**: Additives; fecal health; feed efficiency; weight gain.

## 1. INTRODUÇÃO

Em propriedades leiteiras a criação de bezerras e novilhas representa o futuro da reposição do rebanho ou a possibilidade de ampliação futura, sendo essencial para estabilidade do sistema de produção. Para que isso aconteça de forma eficiente, se faz necessária a adoção de adequado manejo alimentar, sanitário e condições de bem-estar que contribuam significativamente não só com a produtividade, mas com a saúde e desenvolvimento, garantindo a sustentabilidade e lucratividade futura de uma fazenda leiteira (Tahmasbi et al., 2014).

As enfermidades dos bezerros têm um impacto significativo na economia da atividade devido às perdas diretas, além dos efeitos de longo prazo sobre o desempenho (Lorenz et al., 2011). Nesse sentido, a saúde dos bezerros pode ser destacada como um dos problemas mais significativos enfrentados na bovinocultura leiteira. A alta taxa de mortalidade de bezerras no sistema de criação é um fator preocupante visto que essas posteriormente serão matrizes leiteiras, o que influencia diretamente a futura produção de leite (López-Valencia et al., 2017). Enfermidades do trato gastrointestinal, como as diarreias, são as principais responsáveis pela alta taxa de mortalidade. Isso porque o período de maior susceptibilidade do sistema digestório ocorre nas duas primeiras semanas de vida (Hulbert e Moisá, 2016).

Logo após o nascimento, bezerros não têm imunidade inata, nem são ruminantes funcionais. Por isso enfrentam o desafio de adquirir imunidade e, se alimentar como não ruminantes nas primeiras semanas de vida até que o desenvolvimento e a função ruminal estejam estabelecidos (Kertz et al., 2017). Além disso, durante esse período bezerros estão expostos a diversos patógenos causadores de enfermidades. Dessa forma, é imprescindível boas práticas de nutrição e gerenciamento para garantir o crescimento e desenvolvimento na criação das bezerras durante todo o período de aleitamento.

A inclusão de aditivos na dieta de bezerros pode ser uma estratégia benéfica para o desempenho e saúde desses animais. Esses aditivos têm podem ter função de melhorar a eficiência de utilização de nutrientes, podendo também prevenir algumas enfermidades (FAO, 2019). O uso de fontes alternativas como as β-glucanas na dieta de bezerros resultou em melhora na composição da microflora intestinal (Zhou et al., 2009; Signorini

et al., 2012), melhor eficiência na digestão de nutrientes (Ma et al., 2014), e melhor saúde, uma vez que diminuiu a frequência de enfermidades e reduziu morbidade e mortalidade em bezerros (Magalhaes et al., 2008).

Os emulsificantes também são utilizados na dieta para melhorar o desempenho zootécnico, através de melhor absorção e nutrientes. Estes auxiliam a absorção de gordura no epitélio intestinal, aumentando o aproveitamento pelo animal (Silva Junior, 2009). Estudos recentes apresentaram maior ganho médio diário em leitões (Papadoulos, 2014) e melhor eficiência alimentar em vacas (Rico et al., 2017), ovelhas (Gallo et al., 2019) e frangos (Boontiam et al., 2019). Os estudos da inclusão de emulsificantes como aditivos na dieta de bezerros são escassos. Aparentemente, a lisolecitina apresenta um papel importante para a emulsificação nesses animais no período de aleitamento, mas o efeito relativo ainda precisa ser verificado (Garton, 1969).

Assim, o objetivo deste trabalho foi avaliar a inclusão de dois diferentes aditivos, β-glucanas ou lisolecitina, na dieta líquida de bezerras leiteiras e avaliar seus efeitos no desempenho, saúde e metabolismo dos animais.

## 2. REVISÃO DE LITERATURA

### 2.1. Desenvolvimento do sistema digestório de ruminantes

O sistema digestório dos ruminantes se desenvolve no estágio embrionário e é composto por rúmen, retículo, omaso e abomaso (Davis e Drackley, 1998). Porém, nas primeiras semanas de vida a digestão dos bezerros funciona similarmente à digestão de animais monogástricos (Baldwin et al., 2004). Para que se tornem ruminantes funcionais é necessário que passem por mudanças morfofisiológicas, sendo o tempo e o tipo de alimento fornecido aos bezerros fatores fundamentais neste processo (Silva; Bittar; Ferreira, 2011).

Com o avanço da idade ocorre o desenvolvimento ruminal e as proporções relativas de cada compartimento gástrico são alteradas, até que tenha proporções de um animal adulto (Tabela 1). Entre 12-16 semanas de idade, os compartimentos do trato digestório superior de bezerros, retículo-rúmen, omaso e abomaso apresentam proporções de 67%, 18% e 15% respectivamente, em termos de peso do tecido, valores semelhantes ao de um animal adulto (Davis e Drackley, 1998). O desenvolvimento depende de fatores como a colonização por microrganismos, o aumento da capacidade absortiva do tecido ruminal, a disponibilidade de água e a presença de alimentos sólidos (Quigley, 1996).

Tabela 1 - Proporção e peso dos compartimentos do trato digestório superior de bezerros ao longo do seu desenvolvimento

| | Semanas | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 8 | 12 | 17 | Adulto |
| Retículo-rúmen, % | 35 | 40 | 55 | 65 | 66 | 68 | 62 |
| Retículo-rúmen, g | 95 | 180 | 335 | 770 | 1.150 | 2.040 | 4.540 |
| Omaso, % | 14 | 15 | 11 | 14 | 15 | 18 | 24 |
| Omaso, g | 40 | 65 | 70 | 160 | 265 | 550 | 1.800 |
| Abomaso, % | 51 | 45 | 34 | 21 | 19 | 14 | 14 |
| Abomaso, g | 140 | 200 | 210 | 250 | 330 | 425 | 1.030 |

Adaptado de Church, 1988.

A fase de aleitamento é marcada por desafios imunológicos e alta dependência de dietas líquidas, o que lhe confere um custo dietético elevado (Oltramari et al., 2016). Nesse período, o trato gastrointestinal dos bezerros é constantemente desafiado pois está sujeito as alterações morfofisiológicas e ao estabelecimento do microbioma ruminal e intestinal e ainda se encontra em desenvolvimento.

Além da imaturidade anatômica, os bezerros apresentam limitações fisiológicas nas primeiras semanas de vida por não serem capazes de produzir ou produzirem quantidades insuficientes de enzimas responsáveis em digerir carboidratos (amilase, maltase e isomaltase), exceto a lactase, responsável pela digestão da lactose proveniente do leite. Devido a estes aspectos do sistema digestório dos bezerros e a menor capacidade de digestão no intestino, a utilização de ingredientes inadequados ou de produtos de origem vegetal na dieta líquida pode provocar distúrbios metabólicos, como a diarreia (Soares, 2013).

### 2.2. Substitutos de leite

Os substitutos de leite se apresentam como uma boa alternativa para alimentação de bezerros jovens, desde que apresentem boa qualidade. Uma vez que o fornecimento desse produto oferece maior segurança alimentar e maior consistência na composição de sólidos totais, é uma ótima alternativa ao fornecimento de leite de descarte (Drackley, 2008). A consistência dos ingredientes utilizados na dieta de bezerros é extremamente

importante pois reduz as chances de problemas digestivos, principalmente em situações em que esses animais são submetidos a situações de estresse derivados de clima ou doenças.

A composição de nutrientes do substituto do leite deve ser compatível com a fase em que os bezerros se encontram. De todos os nutrientes presentes, a gordura é a principal variável que resulta em diferenças no conteúdo energético, sendo que sua elevação aumenta os ganhos diários, mas podem diminuir o consumo inicial (Hill; Aldrich; Schlotterbeck, 2006). A inclusão de altos níveis de gordura vegetal ou animal nas dietas tem como objetivo melhorar o potencial de crescimento dos animais. Segundo Jenkins e Kramer (1986), a incorporação de gorduras na fabricação de sucedâneos apresenta resultados satisfatórios, além de boa digestibilidade pelos bezerros.

As gorduras de origem animal, como o sebo, são alternativas para composição de sucedâneos. Porém, a sua composição de ácidos graxos saturados o torna uma gordura pouco aproveitada pelo animal, visto que essa característica diminui digestibilidade. As gorduras de origem vegetal também são amplamente utilizadas (Azevedo, 2017).

É fato que as gorduras de origem láctea são as mais adequadas para a incorporação de sucedâneos, entretanto, devido ao alto valor comercial essas são substituídas produtos de origem vegetal como óleo de palma ou óleo de coco (Bittar; Ferreira; Da Silva, 2016). O óleo de coco apresenta característica de digestibilidade de ácidos graxos muito semelhante da encontrada na gordura do leite (Davis e Drackley,1998).

A inclusão de gorduras nos substitutos do leite ocorre através do processo conhecido como spray-dry, que auxilia na sua dispersão no sucedâneo e facilita o manuseio. As gorduras são insolúveis em água, o que impede a solubilização no trato gastrointestinal. Assim, precisam ser emulsionadas antes que as enzimas lipolíticas possam digeri-las. Em virtude da insolubilidade da gordura em água torna-se necessário a adição de agentes emulsificadores para digestão, sendo também importante para a adequada diluição de sucedâneos (Siyal et al., 2017). Dessa forma, os emulsificantes estabilizam uma emulsão e impedem a coalescência dos glóbulos da fase dispersa (Siyal et al., 2017). Um emulsificante na dieta auxilia no aumento da superfície enzimática total necessária para a digestão de gorduras (Roy et al., 2010).

## 2.3. Lisolecitina

Os lisofosfolipídeos são considerados emulsificantes de gordura, moléculas que elevam a capacidade de preservação dos nutrientes no processo de absorção de óleos e gorduras. Esses são melhores aproveitados em relação aos lipídios neutros pois apresentam características anfipáticas, ou seja, possuem porções hidrofílicas e hidrofóbicas. Os lisofosfolipídeos requerem uma menor concentração para formação de micelas (concentração micelar crítica) comparados aos surfactantes químicos sintéticos, o que possibilita maior absorção do ácido linoleico, em cerca de 17%, (Zubay; Adisson-Wesley, 1984). As adições dessas substâncias são utilizadas para estabilização no processo de suspensões de gordura, como é o caso dos monoglicerídeos lisofosfolipideos como a lecitina (Tomkins; Jaster, 1991). Além disso, a inclusão de lecitina na dieta animal apresenta-se como fonte energética altamente digestível.

A lecitina é composta por uma mescla de fosfolipídios (50%), triglicerídeos (35%) e glicolipídios (10%), carboidratos, pigmentos, carotenóides e outros microcompostos. As propriedades tensoativas da lecitina são provenientes da estrutura molecular dos fosfolipídios, componentes ativos da lecitina. Essa pode ser obtida da soja, e de diversas fontes de óleos vegetais como o óleo de palma, óleo de canola e o óleo de girassol, bem como leite. Possui capacidade de emulsificante, o que auxilia a utilização da gordura da dieta (Overland et al., 1993).

Emulsionantes sintéticos derivados da hidrólise enzimática da lecitina como lisolecitina ou lisofosfatidilcolina também executam a mesma função (Zhang et al., 2011). A lisolecitina é produzida naturalmente no intestino delgado de ruminantes através da hidrólise da lecitina através da enzima fosfolipase A2, o que auxilia na absorção de ácidos graxos (Figura 1) (Dawson, 1959).

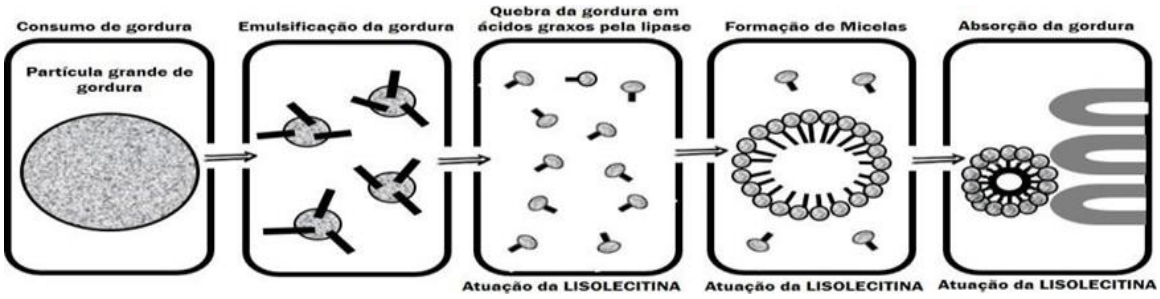


Figura 1 – Atuação da lisolecitina no processo de emulsificação.

A inclusão de lisofosfolipídios como lecitina e lisolectina resultaram em benefícios no desepenho de animais de produção. De acordo com Jin et al., (1998), a adição da lecitina na dieta de leitões contendo sebo melhorou a digestibilidade da matéria

seca, extrato etéreo, energia bruta e proteína bruta. O consumo de energia foi 19% maior em cordeiros alimentados com lecitina de soja, influenciando diretamente sobre o rendimento e qualidade de carcaça (Lough et al.,1991). Em frangos de corte a adição de emulsificantes exógenos na dieta resultou positivamente na digestibilidade, ganho de peso e conversão alimentar (Roy et al., 2010), assim como na energia metabolizável e na emulsificação do óleo de palma (Yordan et al., 2013).

Os fosfolipídios também apresentam ação semelhante aos antibióticos na flora intestinal, desestabilizando o equilíbrio iônico das bactérias (Silva Júnior et al., 2009). Esse modo de ação tem importância para bezerros visto que são susceptíveis às proliferações de microrganismos patogênicos em seu sistema gastrointestinal.

A utilização de emulsificantes na dieta de bovinos pode auxiliar o desempenho animal pois estes aumentam a absorção de nutrientes, principalmente através dos processos como digestão e absorção de gordura (Smits et al., 2000). Esses processos apresentam menor eficiência em recém-nascidos quando comparados aos animais com sistema digestório totalmente desenvolvido. Os órgãos como o pâncreas e fígado, fundamentais para o processo de absorção, respondem a um sistema enzimático que ainda está em desenvolvimento e, consequentemente, a concentração micelar crítica não consegue desenvolver uma de suas principais funções corretamente, a emulsificação(Ruckebusch; Dardillat; Guilloteau, 1983).

A lisolecitina apresenta propriedades de superfície ativa, essenciais para o processo de emulsificação de lipídios, ademais, podem influenciar a absorção de ácidos graxos no intestino delgado (Jenkins et al., 1989). Sendo assim, a utilização desse fosfolipídio no sucedâneo de bezerros pode contribuir com uma melhor absorção de lipídeos nas primeiras semanas de vida de bezerros.

**2.4. Colonização do trato gastrointestinal de bezerros**

Com o avanço da idade do animal, o número e o tipo de microrganismos que habitam o trato gastrointestinal (TGI) se alteram com o tipo de substrato (alimento) e mudanças na fermentação do mesmo. A população microbiana ruminal e intestinal nas primeiras semanas de vida é limitada em concentrações e cepas de microrganismos (Daneshvar et al., 2015). Essa situação se intensifica quando esses animais são submetidos a situações de estresse, como desaleitamento, ambientes inadequados e doenças (Davis e Drackley, 1998). Esses fatores influenciam diretamente na saúde gastrointestinal dos bezerros, ja que se esses animais não apresentam um sistema imunologico eficiente ou uma microbiota característica e estável, a funcionalidade passa

a ser ineficiente afetando assim a digestão e absorção de nutrientes da dieta oferecida (Celi et al., 2016).

O TGI dos animais domésticos contém comunidades microbianas densas e complexas, podendo ser compostas por archaea, bactérias, fungos, protozoários e vírus. Essas comunidades são responsáveis pela digestão e fermentação de polímeros vegetais no rúmen, função de extrema importância para os ruminantes. Além disso, a microbiota também é responsável pela síntese de vitaminas, estimulação do sistema imunológico (Bezirtzoglou e Stayropoulou, 2011), transformação metabólica de compostos tóxicos em resíduos não tóxicos, manutenção do peristaltismo e integridade da mucosa intestinal (Vorbach et al.,2003). Também, desempenha papel de barreira contra a colonização por patógenos e o armazenamento e gasto de energia obtida da dieta (Drissi et al., 2014; Angelakis, 2017). Ao contrário do rúmen, o intestino é revestido por uma única camada de células epiteliais que facilita a digestão e a absorção de nutrientes, além de atuarem como uma barreira contra microrganismos invasores, toxinas e antígenos alimentares (Celi et al., 2016).

As alterações na microbiota podem ser feitas através de alterações na composição da dieta (substratos), mas também através de inclusão de aditivos alimentares, como antibióticos, probióticos e prebióticos. Esses agentes normalmente são adicionados na dieta líquida ou sólida e quando chegam ao intestino formam uma barreira que reduz a proliferação de bactérias oportunistas e patogênicas, impedindo a colonização e aumentando a ingestão de energia (Angelakis et al., 2013).

**2.5. Alternativas a antibióticos**

Os antibióticos são comumente utilizados no sistema de produção para auxiliar no tratamento de doenças, promover o crescimento e a saúde dos animais (Hammer et al., 2016). Entretanto, devido à resistência das bactérias aos antibióticos e a preocupação com os resíduos em produtos de origem animal, se buscam alternativas a estes compostos. A inclusão de probióticos aparece como uma alternativa com intuito de melhorar a saúde e produtividade do rebanho (Chaucheyras-Durand e Durand, 2010; Timmerman et al.,2005). Os probióticos também podem ser utilizados no manejo profilático, podendo apresentar resultados positivos na saúde e desempenho de bezerros (Uyeno et al., 2015).

Probióticos são definidos como microrganismos vivos, na maioria das vezes bactérias gram-positivas como *Lactobacillus, Bacillus, Streptococcus, Pediococcus*, leveduras pertencentes às espécies *Saccharomyces cerevisiae*.Esses microrganismos, quando ingeridos, devem apesentar algumas características essenciais como resistência

as enzimas e ácidos presentes no trato digestório, se manterem viáveis no alimento até o momento do consumo (Coppola e Turnes, 2004). Além disso, podem apresentar efeitos nutricionais benéficos, melhorar a eficiência alimentar e agir positivamente sobre a saúde (Angelakis et al., 2012; Gaggia et al., 2010; Timmerman et al., 2005). Os efeitos benéficos dos probióticos estão atribuídos à manutenção da integridade do epitélio intestinal (Yirga,2015), além de outros aspectos resultantes do seu modo de ação (Tabela 2).

Os mecanismos de ação dos probióticos sobre os patógenos não são completamente conhecidos. Entretanto, sabe-se que um ou vários processos, associados ou não, alteraram a atividade e composição bacteriana do TGI de forma favorável ao hospedeiro. Os principais modos de ação dos probióticos no TGI de bezerros incluem: estimulação da resposta imunológica, metabolismo de compostos benéficos ao hospedeiro, produção de compostos antibacterianos, competição com patógenos para colonização da mucosa intestinal ou por nutrientes e produção ou estimulação de enzimas (Seo et al.,2010).

Tabela 2 - Principais mecanismos de ação de bactérias probióticas no trato gastrointestinal de bezerros

| |
|---|
| 1. Competição com patógenos para a colonização da mucosa e/ou de nutrientes |
| 2. Produção e/ou estimulação de enzimas |
| 3. Estimulação da resposta imunológica do hospedeiro |
| 4. Produção de compostos antibacterianos (antibióticos, ácidos, bacteriocinas) |
| 5. Desintoxicação e metabolismo de compostos benéficos ao hospedeiro |

Adaptado de Seo et al. (2010).

A utilização de probióticos em ruminantes durante o periodo de crescimento pode viabilizar a colonização adequada do ambiente intestinal por microrganismos, fazendo com que o sistema gastrointestinal funcione de maneira mais eficiente (Kung Jr., 2001). De acordo com Timmerman et al. (2005), a adição de probióticos no leite de bezerros

reduziu a incidência de diarreia e a taxa de mortalidade, além de melhorar a eficiência alimentar até a oitava semana de vida dos animais.

As leveduras são componentes do grupo dos probióticos que apresentam propriedades que favorecem fisiologicamente as condições do rúmen, fator importante para o crescimento bacteriano, especialmente das bactérias celulolíticas (Queiroz et al.,2004). O fornecimento de leveduras vivas (Saccharomyces cerevisiae) para bezerros desde os primeiros dias de vida favorece a colonização microbiana intestinal reduzindo patógenos como *Escherichia. coli* (Zhou et al., 2009). Alguns estudos mostraram que a suplementação com *Saccharomyces cerevisiae* resultou em melhora na saúde, redução na morbidade e mortalidade (Magalhães et al., 2008); melhor eficiência alimentar (Leimester et al., 2004) e redução no índice de diarreias (Fomenky et al., 2017) em bezerros durante o período de aleitamento.

**2.6. Saccharomyces cerevisiae**

A produção do extrato da levedura Saccharomyces cerevisiae para fornecimento como probiótico passa por processos como a autólise das células, centrifugação para isolar os componentes da parede celular que posteriormente são lavados e desidratados pelo método spray-dry gerando uma fração insolúvel, a qual é composta por mananoligossacarídeos e β-glucanas (Figura 2; Spring et al., 2000).

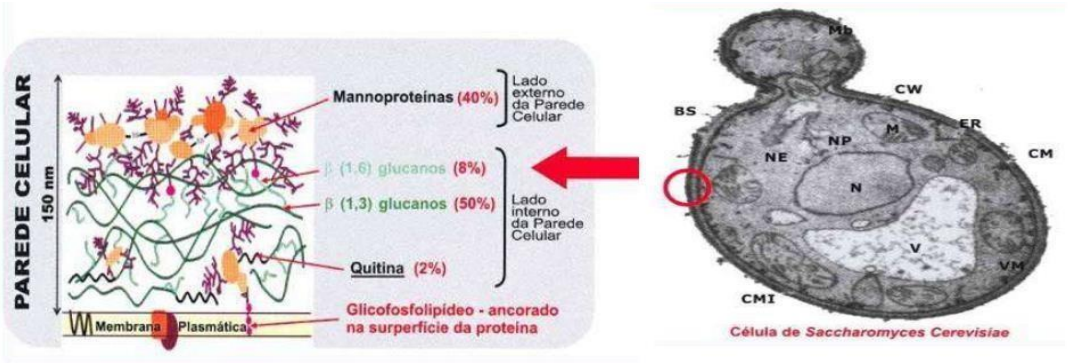


Figura 2 – Parede celular da levedura Saccharomyces Cerevisiae.
Fonte: www.Ifa-america.com

A levedura Saccharomyces cerevisiae é utilizada como aditivo microbiano na alimentação de ruminantes, mesmo que em quantidades reduzida (Carvalho et al., 2009). Em vacas leiteiras, as leveduras vivas mostraram resultados positivos em relação a maior produção de leite, desempenho ruminal, aumento do consumo de matéria seca, ácidos graxos voláteis (AGV) e diminuição da concentração de lactato ruminal (Chaucheyras-Durand e Durand, 2010).

Existem alguns trabalhos relacionados ao período pré-ruminante o qual se encaixa para bezerros, analisando o estado de saúde desses animais, considerando principalmente a diarreia. Alguns trabalhos comparam a incidência e o grau de severidade de diarreia e transporte de microrganismos patogênicos, além dos parâmetros econômicos, mostrando benefícios do aditivo (Gaggia et al., 2010). A redução na incidência de diarreia também foi observada com o fornecimento de leite fermentado com bactérias L. acidophilus ou Saccharomyces cerevisiae (Agarwal et al., 2002). A taxa de mortalidade, incidência de diarreia e coliformes fecais em bezerros diminuiu através da adição de probióticos contendo Lactobacillus spp. de origem humana e bovina (Timmerman et al., 2005). Em bezerros, a suplementação com algumas cepas bacterianas e de leveduras auxiliaram no ganho de peso e desenvolvimento ruminal (Adams et al., 2008) no período de desaleitamento e redução da colonização de patógenos (Chaucheyras-Durand e Durand, 2010).

Uma das porções insolúvel das leveduras Saccharomyces cerevisiae é conhecida como β-glucanas, as quais possuem características que estimulam o sistema imune, especificamente os macrófagos, auxiliando na defesa contra patógenos (Franklin et al.,2005). As β-glucanas são componentes estruturais importantes da parede celular na levedura e em algumas bactérias e algas como a *Euglena gracilis*.

## 2.7 Euglena gracilis

Uma fonte alternativa de β-glucanas para rações animais pode ser fornecida a partir de *Euglena gracilis*, uma microalga de água doce que pode servir como biomediador para resíduos agrícolas e sintetizar nutrientes de alto valor (Kottuparambil, Thankamony, & Agusti, 2019; Krajčovič, et al., 2015*)*. Os estudos dessas algas se devem a sua biologia molecular e por sua produção de uma variedade de bioprodutos incluindo proteína contendo aminoácidos essenciais, vitaminas, lipídios e o paramilon β-1,3-glucano (Pollak et al., 2012). Essa alga pode acumular grandes quantidades de paramilon β-1,3-glucano, que pode constituir mais de 80% do peso (Sun et al., 2018). Quando cultivado em um ambiente heterotrófico, Euglena pode armazenar grandes quantidades de paramilon, e devido às propriedades insolúveis em água desta molécula e seu alto grau de cristalinidade incomum, é possível recuperar paramilon β-1,3-glucano a um custo menor quando comparado a outras fontes de glucanas (Krajčovič et al., 2015). Os β-1,3-glucanos são de interesse especial por causa de suas

bioatividades imunoestimulatórias e antimicrobianas relatadas (Russo et al., 2017; Gissibl et al., 2018). Porém, as β-glucanas também podem contribuir para maior imunidade do animal (Bohn; BeMiller, 1995).

## 2.8. β-glucanas

A atividade biológica da β-glucanas é estudada pela habilidade que essa apresenta em ativar o mecanismo de defesa do hospedeiro (Kim et al., 2006). Após a ingestão, as β-glucanas vão para o intestino delgado onde são absorvidos pelas placas de Peyer, que são aglomerados de nódulos linfáticos responsáveis pela produção de imunoglobulinas e anticorpos (Krehbiel e Zhang, 2016). Em nível molecular as β-glucanas ativam as células do sistema imune e intensificam sua capacidade de combater as doenças.

As células do sistema imune (macrófagos, monócitos, neutrófilos e células natural killer) reconhecem as β-glucanas através de receptores da superfície celular. Esse reconhecimento pode ser feito pelo seu grau de ramificação (Chorvatovicová; Machová e Sandula ,1996) ou solubilidade do polissacarídeo (Tokunaka et al.,2002). Os macrófagos presentes no TGI englobam essas β-glucanas, as transportam por todo o corpo para sítios linfoides secundários (medula óssea, baço, linfonodos, etc.) e simultaneamente liberam os fragmentos de β-glucanas, o que desencadeia a liberação de moléculas de sinalização, como citocinas (Berner et al. ,2005; Moon et al., 2005). Essas moléculas sinalizadoras são responsáveis pela ativação de outras células do sistema imunológico e promovem seu recrutamento para o local infectado. Os macrófagos ativados e as células do sistema imune combatem e destroem efetivamente células não reconhecidas e organismos causadores de doenças (Tizard, 2014).

O reconhecimento pelo sistema imunológico está associado a padrões moleculares (PAMPs), que comumente são fundamentais para sobrevivência de patógenos microbianos. Uma das PAMPs mais conhecidas são as β-glucanas que estimulam respostas das células de defesa protegendo o hospedeiro contra invasão de organismos causadores de doenças, caracterizando a imunidade inata de organismos superiores (Brown; Gordon, 2005). O efeito imunomodulatório das β-glucanas foi efetivo em infecções causadas por vírus (Jung et al., 2004); parasitas (Holbrook; Cook; Parker, 1981); fungos (Meira et al., 1996) e bactérias (Liang et al., 1998).

Em bovinos, essa ativação do sistema imune contribui positivamente, principalmente quando esses animais são expostos a situações de estresse, doenças ou até no período em que os animais jovens estão desenvolvendo seu sistema imunológico (Celi et al., 2016). O aumento na imunidade protetora de bezerros é de extrema importância para os produtores, visto que esses animais são muito suscetíveis a doenças nas primeiras semanas de vida (Krehbiel e Zhang, 2016). Sendo assim, minimizar o impacto dos desafios de doenças no início da vida do animal pode trazer consequências diretas sobre o ganho de peso, conversão alimentar e probabilidade de disseminação de doenças. A inclusão de β-glucanas associadas com antibióticos no tratamento de bezerros diagnosticados com diarreia infecciosa, contribuíram com diminuição na taxa de mortalidade, levando a uma redução de vinte pontos percentuais na mortalidade de bezerros diagnosticados com crypitosporidium spp (López-Valencia et al., 2017).

Esse resultado positivo pode estar correlacionado com a longa permanência das β-glucanas no organismo de mamíferos, que por não apresentarem as β-glucanases (responsáveis pela metabolização desses polímeros) se acumulam por mais tempo no sistema, contribuindo para maior eficiência na ativação da resposta imune do hospedeiro. A atividade biológica também está correlacionada com o peso molecular das β-glucanas que quando apresentam alto peso molecular ativam os leucócitos diretamente, desencadeando a atividade citotóxica e a capacidade fagocítica, bem como a produção de mediadores pró-inflamatórios (Brown; Gordon, 2003). Ademais, são encontrados outros efeitos benéficos com utilização das β-glucanas como anti-inflamatório, antimutagênico, hipocolesterolêmico e hipoglicêmico (Behall et al., 2006; Kim et al., 2006). Esses efeitos são totalmente dependentes de algumas características das β-glucanas como a estrutura complexa, propriedades físico-químicas, e da habilidade que as células intestinas apresentam para absorvê-las (Muckosová; Babicek ;Pospisil, 2001).

A administração de β-glucanas por via oral reforça as funções dos linfócitos intraepiteliais presentes no intestino auxiliando na absorção (Tsukada et al.,2003). Dependendo da via, dose e tempo de administração as β-glucanas podem estimular ou suprimir a resposta imunológica do hospedeiro (Tzianabos, 2000). A ingestão contínua de β-glucanas pode reduzir o risco de doenças crônicas em animais. Kogan e Kocher (2007) adicionaram esse biomodulador na dieta de suínos e observaram a influência no estímulo do sistema imunológico, principalmente nas mucosas, áreas que mais entram em contato com os patógenos.

Gosh and Mehla (2012) utilizaram a β-glucanas extraida de levedura como suplemento em pré- ruminantes e obtiveram resultados satisfatórios no consumo de matéria seca, eficiência alimentar e ganho de peso. Os estudos sobre a inclusão de β-glucanas na dieta líquida de bezerros apresentam benefícios na digestibilidade de nutrientes e aumento no pH ruminal (Kim et al., 2011); no consumo medio diário e redução no índice de diarreia de bezerros durante 56 dias de idade (Nargeskhani et al., 2010). Porém, esses benefícios podem ser observados apenas quando os animais estão em situações de estresse (Frizzo et al., 2010; Krauze et al., 2010). Além disso, a inclusão desse suplemento no sucedâneo e sua influência no sistema imunológico e saúde de bezerras em aleitamento e desaleitamento ainda não são bem compreendidos (Foote et al., 2007). Grande parte dos estudos utilizam β-glucanas extraída de leveduras, sendo escassos os trabalhos com β-glucanas derivadas de algas. Visto que as β-glucanas extraidas de alga tem maior potencial torna-se necessario explorar os efeitos dessa na dieta de bezerras para minimizar os fatores que prejudicam o desempenho desses animais.

## 3. REFERÊNCIAS

ADAMS, M.C.; LUO, J.; RAYWARD, D.; KING, S.; GIBSON, R.; MOGHADDAM, G.H. Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. **Animal Feed Science and Technology** 145, p.41–52, 2008.

AGARWAL, N.; KAMRA, D.N.; CHAUDHARY, L.C.; AGARWAL, L.; SAHOO, A.; PATHAK, N.N..Microbial status and rumen enzyme profile of crossbred calves on different microbial feed additives. **Letters in Applied Microbiology** 34, p.329336, 2002.

ANGELAKIS E.; BASTELICA D.; A., BEN AMARA; EL FILALY A; DUTOUR A.; MEGE J.L.;ALESS M.C.; RAOULT I, D. An evaluation of the effects of Lactobacillus ingluviei on body weight, the intestinal microbiome and metabolism in mice, **Microb. Pathog** v.52, p. 61-68, 2012.

ANGELAKIS E.; MERHEJ V.; RAOULT D. Related actions of probiotics and antibiotics on gut microbiota and weight modification**, Lancet Infect**. Dis. 13, p.889-899, 2013.

ANGELAKIS, E. Weight gain by gut microbiota manipulation in productive animals, **Microbial Pathogenesis** v.106, p. 162-163, 2017.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analyses of the **Association of Official Analytical Chemists.** Arlington: AOAC, p. 500, 1990.

AZEVEDO, R. **Sucedaneos lacteos para bezerros leiteiros.** Gir leiteiro, 2017. Disponivel em: < http://gir- leiteiro.com/artigo-sucedaneos-lacteos-para-bezerros-leiteiros>

BALDWIN R. L. V. I.; CLEOD K.R. M.; KLOTZ, J. L.; HEITMANN, R. N. Rumen development,intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. **Journal of Dairy Science**, v. 87(Suppl. E), E55–E65, 2004.

BALDWIN, R. L.; MCLEOD V; K. R.; KLOTZ, J. L.; HEITMANN, R. N.2004. Rumen development,intestinal growth and hepatic metabo- lism in the pre- and post-weaning ruminant. **Journal of Dairy Science**. 87, 2004.

BALDWIN, R.L., K.R. MCLEOD, J.L. KLOTZ, AND R.N. HEITMANN. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. **J. Dairy Sci**. 87:55–65, 2004.

BALLOU, M.A. Immune responses of Holstein and Jersey calves during the preweaning and immediate postweaned periods when fed varying planes of milk replacer. **Journal of Dairy Science**, v.95, I. 12, 7319 – 7330, 2012.

BAYATKOUHSAR, J., A. TAHMASEBI, A. NASERIAN, R. MOKARRAM, and R. VALIZADEH.2013. Effects of supplementation of lactic acid bacteria on growth performance, blood metabolites and fecal coliform and lactobacilli of young dairy calves. **Anim. Feed Sci. Technol.** n.186, p.1–11, 2013.

BEHALL, K. M.; SCHOLFIELD, D. J.; HALLFRISCH, J. G.; LILJEBERG-ELMSTÅHL, H. G. M.Consumption of both resistant starch and β-glucan improves postprandial -plasma glucose and insulin in women. **Diabetes Care**, Alexandria, v. 29, n. 5, p. 976-981, 2006.

BERNER, M. D.; SURA, M. E.; ALVES, B. N.; HUNTER- Jr. K, W. IFN-gamma primes macrophages for enhanced TNF-alpha expression in response to stimulatory and non-stimulatory amounts of microparticulate β-glucan**. Immunology Letters, Amsterdam**, v. 98, n. 1, p. 115-122, 2005.

BEZIRTZOGLOU, E. E.; STAVROPOULOU E. Immunology and probiotic impact of the newborn and young children intestinal microflora, **Anaerobe** 17, p.369, 2011.

BITTAR, C.M.M.; FERREIRA, L.S.; DA SILVA, J.T. Sucedaneos lácteos para bezerras leiteiras.**Cadernos Técnicos de Veterinária e Zootecnia**, n 8, p. 64 , 2016.

BOHN, J. A.; BeMILLER, J. M. (1-3)-β-D-Glucans as biological response modi ers: a review of structure- functional activity relationships. **Carbohydrate Polymers, Barking**, v. 28, n. 1, p. 3-14, 1995.

BOONTIAM, W., Y. K. HYUN, B. JUNG, AND Y. Y. KIM.Effects of lysophospholipid supplementation to reduced energy, crude protein, and amino acid diets on growth performance, nutrient digestibility, and blood profiles in broiler chickens. **J. Poult. Sci.** 98,6693–6701,2019.

BROWN, G. D.; GORDON, S. Fungal β-glucans and mammalian immunity. **Immunity**, **Cambridge**, v.19, n. 3, p. 311-315, 2003.

CARVALHO, E. R.; SANTOS, S. C.; CAETANO, T. F.; GOUVÊA, V. N.; LIMA, M. L.M.; FERNANDES, J. J. R. Bicarbonato de sódio e leveduras como aditivos de dietas para vacas leiteiras mestiças. **Ciência Animal Brasileira**. Goiânia, v.10, n.2, p. 511- 526, 2009.

CHAUCHEYRAS-DURAND F.; DURAND H., Probiotics in animal nutrition and health, **Benef.Microbes** v.1, p.3-9, 2010.

CHAUCHEYRAS-DURANT, F. FONTY, G. Influence of a probiotic yeast (Saccharomyces cerevisiae CNCM I-1077) on microbial colonization and fermentations in the rumen of newborn lambs**. Microbial Ecology in Health and Disease, Mississippi**, v.11, n.1, p.30-36, 2002.

CHO YI, YOON KJ. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J Vet Sci. 2014 Mar;15(1):1-17. https://doi.org/10.4142/jvs.2014.15.1.1

CHORVATOVICOVÁ, D.; MACHOVÁ, E.; SANDULA, J. Effect of ultrasonicated carboxymethylglucan on cyclophosphamide-induced mutagenicity. **Mutation Research**, Amsterdam, v. 371, n. 1-2, p. 115-120, 1996.

CHURCH, D.C. **The ruminant animal**: digestive physiology and nutrition. p. 564, 1988.

COPPOLA, M. M.; TURNES, C. G. Probióticos e a resposta imune. **Ciência Rural**, Santa Maria, v. 34, p. 1297-1303, 2004.

DANESHVAR, D.M.; KHORVASH, E.; GHASEMI, A.H.; MAHDAVI, B.; MOSHIRI, M.; MIRZAEI,A. PEZESHKI, H. ;GHAFFARI, M. The effect of restricted milk feeding through conventional or step-down methods with or without forage provision in starter feed on performance of Holstein bull calves. **Journal of dairy Science**, Champaing, v.93, p.3979–3989, 2015.

DAVIS, C.L.; DRACKLEY, J.K. **The development, nutrition and management of the young calf**, p.329, 1998.

DRACKLEY, J.K. Calf Nutrition from Birth to Breeding. **Veterinary Clinical Food Animal Practice**, v. 24, p.55-86, 2008.

DRISSI, V. MERHEJ, E. ANGELAKIS, A. EL KAOUTARI, F. CARRIERE, B. HENRISSAT, D.RAOULT, Comparative genomics analysis of Lactobacillus species associated with weight gain or weight protection**, Nutr. Diabetes** 4 e.109, 2014.

EICHER S. D.; WESLEY, I. V.; SHARMA, V. K.; JOHNSON, T. R. Yeast cell-wall products containing beta-glucan plus ascorbic acid affect neonatal Bos taurus calf leukocytes and growth after a transport stressor. **Journal of Animal Science**, v.88, p.1195–1203, 2010.

FAO. Environmental performance of feed additives in livestock supply chains – Guidelines for assessment (Draft for public review). **Livestock Environmental Assessment and Performance** (LEAP) Partnership. FAO, Rome, Italy. 2019.

FOMENKY, B. E., CHIQUETTE, J., BISSONNETTE, N., TALBOT, G., CHOUINARD, P. Y., & IBEAGHA-AWEMU, E. M. Impact of Saccharomyces cerevisiae boulardii CNCMI-1079 and Lactobacillus acidophilus BT1386 on total lactobacilli population in the gastrointestinal tract and colon histomorphology of Holstein dairy calves. **Animal Feed Science and Technology,** 234, 151–161, 2017.

FRANKLIN, S. T.; NEWMAN, M. C.; NEWMAN, K. E.; MEEK, M. I. Immune parameters of dry cow fed mannan oligosaccharide and subsequent transfer of immunity to calves. **Journal of Dairy Science.Champaign**.v. 88, n.2, p. 766-775, 2005.

FRIZZO, L.S., SOTO, L.P., ZBRUN, M.V., BERTOZZI, E., SEQUEIRA, G., ARMESTO, R.R., ROSMINI, M.R.Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. **Anim. Feed Sci. Technol.** 157, 159-167,2010.

GAGGIA, F.; MATTARELLI P.; BIAVATI, B. Probiotics and prebiotics in animal feeding for safe food production**, Int. J. Food Microbiol**. 1, p.15, 2010.

GALLO, S.B., T. BROCHADO, L. BROCHINE, D. PASSARELI, S.F. COSTA, I.C. DA S. BUENO, DA S., J.C. DE C. BALIEIRO, R. FRANZOLIN, AND L.O. TEDESCHI. Effect of biosurfactant added in two different oil source diets on lamb performance and ruminal and blood parameters. **Livest Sci.** 226,66-72, 2019.

GARTON, G. Digestion and absorption of lipids in the ruminant. Proc. **Nutr. Soc.**28: 131-139,1969.

GHOSH, S., AND R.K. MEHLA. Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. **Trop anim health prod.** 44:617-622, 2012.

GISSIBL A, SUN A, CARE A, NEVALAINEN H AND SUNNA A . Bioproducts From Euglena gracilis: Synthesis and Applications. **Front. Bioeng. Biotechnol**. 7:108,2019.

HAMMER, T. J.; FIERER, N.; HARDWICK B.; A. SIMOJOKI A.; SLADE E.;, TAPONEN, J.;VILJANEN H., ; ROSLIN T.. 2016. Treating cattle with an- tibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles**. Proc. R. Soc. B**. 283, 2016.

HILL, T.M.; ALDRICH, J.M.; SCHLOTTERBECK.Effects of feed in grate and concentrations of protein and fat of milk replacers fed to neonatal calves. **The Professional Animal Scientist** 2006.

HOLBROOK, T. W.; COOK, J. A.; PARKER, B. W. Immunization against Leishmania dovavani: glucana as an adjuvant with killed promastigotes. **American Journal of Tropical Medicine and Hygiene**, Mclean, v. 30, n. 4, p. 762-768, 1981.

HULBERT, L. E.; S. J. MOISÁ. 2016. Stress, immunity, and the man- agement of calves. **Journal of Dairy Science**, 2016.

JENKINS, K.J.; KRAMER, J.K.G. Influence of low linoleic and linolenic acids in milk replacer on calf performance and lipids in blood plasma, heart, and liver. **Journal of Dairy Science**, 1986.

JENKINS, T. C.; FOTOUHI, N. Effects of lecithin and corn oil on site of digestion, ruminal fermentationand microbial protein synthesis in sheep. **Journal of Animal Science**. Champaign, v. 68, p. 460- 466, 1990.

JUNG, K.; HA, Y.; HA, S.-K.; HAN, D. U.; KIM, D.-W, MOON, K. W.; CHAE, C. Antiviral effect of Saccharomyces cerevisiae β-glucan to swine in uenza by increased production of interferon-γ and nitric oxide. **Journal of Veterinary Medicine**, Chicago, v. 51, n. 2, p. 72-76, 2004.

KIM, M.H., J.K. SEO, C.H. YUN, S.J. KANG, J.Y. KO, AND J.K. HA. Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. **Animal.** 5:953-960,2011.

KIM, S. Y.; SONG, H. J.; LEE, Y. Y.; CHO, K.-H.; ROH, Y. K. Biomedical issues of dietary ber β- glucan. **Journal Korean of Medical Science**, Seoul, v. 21, n. 10, p. 781-789, 2006.

KOGAN, G.; KOCHER, A. Role of yeast cell wall polysaccharides in pig nutrition and health protection.**Livestock Science**, Amsterdam, v. 109, n. 1-3, p. 161- 165, 2007.

KOTTUPARAMBIL, S., THANKAMONY, R. L., & AGUSTI, S. Euglena as a potential natural source of value-added metabolites. A Review. **Algal Research**, 37, 154–159,2019.

KRAJČOVIČ, J., VESTEG, M., & SCHWARTZBACH, S. D. (2015). Euglenoid flagellates: A multifaceted biotechnology platform. **Journal of Biotechnology**, 202, 135–145,2015.

KRAUSE, D.O., BHANDARI, S.K., HOUSE, J.D., NYACHOTI, C.M. Response of Nursery Pigs to a Synbiotic Preparation of Starch and an Anti-Escherichia coli K88 Probiotic. **Appl.Environ. Microbiol**. 76, 8192-8200, 2010.

KREHBIEL, C.; ZHANG, G. Modulation of Gut Health in Beef and Dairy Cattle. **Oklahoma State University**, 2016.

KUEHN, C.S.; OTTERBY, L. The effect of dietary energy concentration on calf performance. **Journal of Dairy Science**, 1994.

KUNG JR., L. Direct fed microbials and enzymes for ruminants. In: ANNUAL FLORIDA RUMINANT NUTRITION SYMPOSIUM, 12. 2001. Gainesville. **Proceedings... Gainesville**: University of Florida, p. 11-12, 2001.

LESMEISTER, K. E.; TOZER, P. R.; HEINRICHS, A. J. 2004. Development and analysis of a rumen tissue sampling procedure. **Journal of Dairy Science**, v.87, p.1336–1344, 2004.

LIANG, J.; MELICAN, D.; CAFRO, L.; PALACE, G.; FISETTE, L.; ARMSTRONG, R.; PATCHEN, M. L. Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. **International Journal of Immunopharmacology**, New York, v. 20, n. 11, p. 595-614, 1998.

LÓPEZ-VALENCIA,G.;ZAPATA-RAMÍREZ,O.;NÚÑEZ-GONZÁLEZ,L.;NÚÑEZ-BENÍTEZ,V.;LANDEROS-LÓPEZ,H.;LÓPEZSOTO, M.;BARRERAS,A.;GONZÁLEZ,V.;ESTRADAÂNGULO,A.;ZINN,R.;PLASCENCIA , A. Effective use of probiotic-glyconutrient combination as an adjuvant to antibiotic therapy for diarrhea in rearing dairy calves. **Turk J Vet Anim Sci** ,2017.

LORENZ, I; FAGAN, J. MORE, S.J. Calf health from birth to weaning: II.Management of diarhoea in pre-weaned calves. **Irish Vet Journal**, 64,9-14, 2011.

MA, T., TU, Y., ZHANG, N., DENG, K., ZHOU, ZI., YUN, QIANG AND DIAO, Q. Effects of dietary yeast β-glucan on nutrient digestibility and serum profiles in pre-ruminant Holstein calves. **Journal of integrative agriculture** v.14, p. 749-757, 2014.

MEIRA, D. A.; PEREIRA, P. C. M.; MARCONDES- MACHADO, J.; MENDES, R. P.; BARRAVIERA, B.; PELLEGRINO, J. R. J.; REZKALLAH-IWASSO, M. T.; PERAÇOLI, M. T. S.; CASTILHO,L. M.; THOMZAINI, I.; SILVA, C. L.; FOSS, N. T.; CURRI, P. R. The use of glucan as immunostimulant in the treatment of paracoccidioidomycosis. **American Journal of Tropical Medicine and Hygiene,** Mclean, v. 55, n. 5, p. 496-503, 1996.

MOON, S.-H.; HEO, J.-C.; FINE, R.-L.; KIM, H.-M.; KIM, S.-U.; YOON, B.-D.; LEE, S.-H. BRD-glucan exhibits potent immunochemotherapeutic activity in vitro and in vivo. **International Journal of Oncology**, Athens, v. 26, n. 2, p. 395-404, 2005.

MUCKSOVÁ, J.; BABÍCEK, K.; POSPISIL, M. Particulate 1,3-β-D-Glucan, Carboxymethylglucan and Sulfoethylglucan - In uence of their oral or intraperitoneal administration on immunological respondence of mice. **Folia Microbiologica, Praha**, v. 46, n. 6, p. 559-563, 2001.

MURRAY RK. Glycoproteins. In: Murray RK, Granner DK, Rodwell VW. **Harpers Illustrated Biochemistry**. 27, 2006.

NARGESKHANI A.,N. DABIRI ,S. ESMAEILKHANIAN, A. M. MOHAMMAD,M. BOJARPOUR. Effects of Mannanoligosaccharide–β Glucan or Antibiotics on Health and Performance of Dairy Calves. **Animal Nutr and Feed Tech**. 10(1):29-36, 2010.

OLTRAMARI, C.E.; NÁPOLES, G.G.O.; DE PAULA, M.R.; SILVA, J.T.; GALLO, M.P.C.; SOARES,M.C.; BITTAR, C.M.M. Performance and metabolism of dairy calves fed starter containing citrus pulp as a replacement for corn. **Animal Production Science**, 2016.

PAPADOPOULOS, G. A., K.MÜLLER, D. SCHERTLING, AND M. DI BENEDETTO. Supplementation of lysolecithin in combination with a multi-non-starch polysaccharides enzyme improves the feed efficiency during the post-weaning period in piglets. Acta Agric. Scand. **A Anim. Sci**. v.64,p.130–136, 2014.

POLLAK, D.W., BOSTICK, M.W., YOON, H., WANG, J., HOLLERBACH, D.H., HE, H.Isolation of a Δ5 desaturase gene from Euglena gracilis and functional dissection of its HPGG and HDASH motifs. **Lipids** 47, 913–926,2012.

QUEIROZ, R. C.; BERGAMASCHINE, A. F.; BASTOS, J. F. P.; SANTOS, P. C.; LEMOS, G. C. Usode produtos à base de enzimas e leveduras na dieta de bovinos:digestibilidade dos nutrientes e desempenho em confinamento. **Revista Brasileira de Zootecnia**, Viçosa, v.33, n.6, p. 1548- 1556, 2004.

QUIGLEY J. D. Effects of lasalocid in milk replacer and calf starter on growth, intake, and fecal oocyst shedding in calves challenged with Eimeria. **Journal of Dairy Science**, Champaing. v.79, p. 154, 1996.

RICO, D.E., Y. YING, AND K.J. HARVATINE. Short communication: Effects of lysolecithin on milk fat synthesis and milk fatty acid profile of cows fed diets differing in fiber and unsaturated fatty acid concentration. **Journal of Dairy Science**. 100, 9042-9047, 2017.

ROY, A.S.; HALDAR, S.; MONDAL, T.; GHOSH, K. Effects of supplemental exogenous emulsifieron performance, nutrient metabolism, and serum lipid profile in broiler chickens. **Veterinary Medicine International**: Art. ID 262604, 2010.

RUCKEBUSCH Y., DARDILLAT, C.; GUILLOTEAU, P. Development Of Digestive Functions In The Newborn Ruminant. **Annales de Recherches Vétérinaires,** INRA Editions, 1983, 14 (4), pp.360-374, 1983.

RUSSO, R., BARSANTI, L., EVANGELISTA, V., FRASSANITO, A.M., LONGO, V., PUCCI, L., et al. Euglena gracilis paramylon activates human lymphocytes by upregulating pro-inflammatory factors. **Food Sci. Nutr**. 5, 205–214, 2017.

SEO, J.K.; KIM, S.W.; KIM, M.H.; UPADHAYA, S.D.; KAM, D.K.; HA, J.K. Direct-fed Microbials for Ruminants Animals. **Asian-Australasian Journal of Animal Science**. v.23, n.12, p. 1657-1667, 2010.

SIGNORINI, M.L., SOTO, L.P., ZBRUN, M.V., SEQUEIRA, G.J., ROSMINI, M.R., FRIZZO, L.S. Impact of probiotic administration on the health and fecal microbiota of young calves: a metaanalysis of randomized controlled trials of lactic acid bacteria. **Res. Vet. Sci**. 93, 250-258, 2012.

SILVA JUNIOR, A. Interações químico-fisiolóficas entre acidificantes, probióticos, enzimas e lisofosfolipídeos na digestão de leitões. **Revista Brasileira de Zootecnia**. Viçosa, v. 38, suplemento especial, p. 238-245, 2009.

SILVA, J.T.; BITTAR, C.M.M.; FERREIRA, L.S. Desempenho e desenvolvimento ruminal em resposta ao fornecimento de substâncias húmicas para bezerros leiteiros em sistema de desaleitamento precoce.Acta Scientiarum**. Animal Sciences**. Maringá, v. 33, n.4, p. 395-401, 2011.

SIYAL F. A.;BABAZADEH, D.; WANG C.; ARAIN, M.A.; SAEED, M. ;AYASAN, T.; ZHANG L.;WANG, T. Emulsifiers in the poultry industry. **World's Poultry Science Journal**, v. 73, 2017.

SMITS, C.H.M., MOUGHAN, P.J. and BEYNEN, A.C. The inhibitory effect of a highly viscous carboxymethylcellulose on dietary fat digestibility in the growing chicken is dependent on the type of fat. **Journal of Animal Physiology** 83: 231-238, 2000.

SOARES, M. C. **Diarreia e acidose metabólica em bezerros leiteiros: efeito da composição do concentrado inicial e avaliação de probiótico**. 2013. 168 f. Dissertação (Mestrado) - Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, 2013.

SPRING, P.; WENK, C.; DAWSON, K.A.; NEWMAN, K.E. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bactéria in the ceca of Salmonella-challenged broiler chicks. **Poultry Science,** Champaign, v.79, p.205-211, 2000.

SUN, A., HASAN, M.T., HOBBA, G., NEVALAINEN, H., AND TE'O, J. Comparative assessment of the Euglena gracilis var. saccharophila variant strain as a producer of the β-1,3-glucan paramylon under varying light conditions. **J. Phycol**. 54, 529–538,2018.

TAHMASBI, A. M.; HEIDARI JAHAN ABADI, S.; NASERIAN, A. A. The effect of 2 liquid feeds and 2 sources of protein in starter on performance and blood metabolites in Holstein neonatal calves**. Journal of Dairy Science**, 2014.

The growing importance of de ning gut “health” in animal nutrition and health. CELI, P.; COWIESON, A. J.; FRU-NJI, F.; KLUENTER, A. M.; VERLHAC, V. The growing importance of defining gut "health" in animal nutrition and health. Journal of Animal Science.Vol. 94, E-Suppl. 5 **Journal of Dairy Science**. Vol. 99, E-Suppl. 1. 2016.

TIMMERMAN, H. M., L. MULDER, H. EVERTS, D. VAN ESPEN, E. VAN DER WAL, G. KLAASSEN, S. ROUWERS, R. HARTEMINK, F. ROMBOUTS, and A. BEYNEN. Health and growth of veal calves fed milk replac- ers with or without probiotics**. Journal Dairy Science**. v.88, p.2154–2165, 2005.

TIZARD, I. Imunologia veterinária. 8. ed**.,Elsevier**, 2009.

TOKUNAKA, K.; OHNO, N.; ADACHI, Y.; MIURA, N. N.; YADOMAE, T. Application of Candida solubilized cell wall β-glucan in antitumor immunotherapy against P815 mastocytoma in mice.**Internacional Immunopharmacology**, v.2, n. 1, p.59-67, 2002.

TOMKINS, T; JASTER E.H. Preruminant calf nutrition. Vet Clin North Am Food Anim Pract 1991. TSUKADA, C.; YOKOYAMA, H.; MIYAJI, C.; ISHIMOTO, Y.; KAWAMURA, H.; ABO, T.Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of β- glucan**. Cellular Immunology**, v. 221, n. 1, p. 1-5, 2003.

TZIANABOS, A. O. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. **Clinical Microbiology Reviews**, v. 13, n. 4, p. 523-533, 2000.

UYENO Y, SHIGEMORI S, SHIMOSATO T. Effect of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes and Environments v.30, p.126-132, 2015.VON BUENAU, R.; JAEKEL, L.; SCHUBOTZ, E.; SCHWARZ, S.; STROFF, T.; KRUEGER, M..Escherichia coli strain Nissle 1917: significant reduction of neonatal calf diarrhoea. **Journal of Dairy Science** 88, 318-323, 2005.

VORBACH C.; HARRISON, R.; CAPECCHI, M.R., Xanthine oxidoreductase is central to the evolution and function of the innate immune system, **Trends Immunol**. v.24, p.512-515, 2003.

WANG, Z.; L.A. The use of MIXED models in the analysis of animal experiments with repeated measures data **Canadian Journal of Animal Science**, 2004.

WEISS, W.P. Predicting energy values of feeds. In SYMPOSIUM: PREVAILING CONCEPTS IN ENERGY UTILIZATION BY RUMINANTS**. Journal of Dairy Science**, v. 76, p. 1802-1811, 1993.

YIRGA H. The use of probiotics in animal nutrition. **Prob Health** 3, p.2, 2015.

ZHANG, B.; HAITAO, L.; ZHAO, D.; GUOAND, Y.; BARRI, A. Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids and apparent metabolisable energy content. **Feed Science and Technology** v. 163, p.177- 184, 2011.

ZHOU, Y., DIAO, Q., TU, Y. AND YUN Q. Effects of yeast β-glucan on gastrointestinal development in early-weaning calves. **Chinese Journal of Animal Nutrition** v.21,p. 846-852, 2009.

# 3. SUPPLEMENTATION OF LYSOLECITHIN IN MILK REPLACER FOR HOLSTEIN DAIRY CALVES: EFFECTS ON GROWTH PERFORMANCE, HEALTH, AND METABOLITES[1]


## ABSTRACT

Lysolecithin is an anti-inflammatory emulsifier associated with improved apparent digestibility of total dietary fat and improved feed efficiency in dairy cattle. However, it is unknown if lysolecithin improves performance in calves. Moreover, since many conventional milk replacers use vegetable sourced fat (e.g. palm oil), nutrient absorption and fecal score may be affected in neonatal calves. Thus, the objective of this study was to evaluate the effects of lysolecithin **(LYSO)** supplemented in milk replacer on performance, metabolites, and gut health of pre-weaned dairy calves. Holstein calves (n=32) with passive transfer were assigned in pairs (16 blocks) balanced by birth weight, date of birth and gender at 1 d of age to randomly receive either LYSO (mixed in two milk replacer feedings at a rate of 4g/d Lysoforte®, Kemin Industries, Inc., USA), or a milk replacer control (nothing added). Both treatments were fed 6 L/d milk replacer (22.5 % crude protein, 16.2% crude fat (vegetable oil fat source) on a DM basis at 14% solids) by bucket in two daily feedings for 56 days. Calves were individually housed in wooden hutches and offered a commercial calf starter (24.6% CP and 13.9% NDF) and water by bucket ad libitum. Feed refusals and calf health was assessed daily. Weights, and blood metabolites (glucose, total serum protein, albumin, creatinine, triglycerides, and cholesterol) were sampled weekly, and calves completed the study prior to weaning at 56 d of age. The effect of lysolecithin on calf ADG, FE, and blood metabolites, were evaluated using a linear mixed model with time as a repeated measure, calf as the subject, and block as a random effect in SAS. The effect of lysolecithin to improve the odds of


[1] Artigo aprovado para publicação no Journal of Dairy Science, com previsão de publicação em 2021.

abnormal fecal score was evaluated using a logistic model. Supplementation of LYSO increased average daily gain (control 0.28 ± 0.03 kg; LYSO 0.37 ± 0.03 kg; LSM ± SEM) and increased feed efficiency (gain-to-feed; control 0.25 ± 0.03; LYSO 0.32 ± 0.03). Similarly, LYSO calves had a higher final body weight at d 56 (control 52.11 ± 2.33 kg; LYSO 56.73 ± 2.33 kg). Interestingly, total DMI was not associated with LYSO despite improved average daily gain (total DMI control 1088.7 ± 27.62 g; total DMI LYSO 1124.8 ± 27.62 g). Blood glucose, albumin, creatinine, triglycerides, and cholesterol were not associated with lysolecithin. Indeed, only total serum protein had a significant interaction with LYSO and age at weeks 5, and 6. Moreover, control calves had a 13.57 (95% CI: 9.25-19.90) times greater odds of having an abnormal fecal score on any given day during the diarrhea risk period from d1 to 28. The inclusion of LYSO as an additive in milk replacer in a dose of 4g/d may improve performance, and calf fecal score, preweaning. Further research should investigate the mechanisms behind LYSO's effects on fat digestibility in calves fed 6 L/d of milk replacer with vegetable sourced fat.

**Keywords**: dairy calf, diarrhea, emulsifier, feed efficiency

## 4.1. INTRODUCTION

One significant challenge for neonatal calves is development of the lower gastrointestinal tract (**GIT**; as reviewed by Khan et al., 2016). Neonatal calves lack certain digestive enzymes; thus, nutrient digestion and lipid absorption are self-limiting (as reviewed by Jones and Henrich, 2017). Lipid digestion in calves begins in the mouth where salivary lipase hydrolyses milk fat; however, only 30% of total fat is hydrolyzed before reaching the small intestine (as reviewed by Davis and Drackley, 1998). Once fat reaches the small intestine, pancreatic lipases, lysolecithin, and bile salts form micelles maximizing milk fat digestibility to approximately 97% (as reviewed by Thornsberry et al., 2016). Despite low lipase activities in young calves, milk fat digestibility is high since

short-chain fatty acids (**SCFA**), are easily hydrolyzed by pregastric esterase, a plentiful enzyme in the neonatal calf (Edwards-Webb, 1983). However, conventional milk replacer formulations often use economical vegetable fat sources, which lack SCFA and are less digestible for calves (Toullec and Guilloteau, 1989). Thus, research which improves feed efficiency for calves fed conventional milk replacers are needed.

The primary purpose of including fat in a milk replacer is to serve as a concentrated source of energy. While whole milk fat is highly digestible for calves, it is expensive to feed (Hawkins et al., 2019). Thus, vegetable sourced fat such as palm oil and coconut oil are often used as fat sources in milk replacers (Huuskonen et al., 2005). One limitation to using vegetable fat sources in milk replacers is that the long chain fatty acids are poorly digested, decreasing the nutritive value for the calf (as reviewed by Kertz et al., 2017). Therefore, several studies have attempted to improve fat digestion in calves by adding fat emulsifiers to milk replacers (as reviewed by Kertz et al., 2017). Indeed, research from 70 years ago evaluated the importance of emulsifier inclusion in milk replacer, mainly to facilitate the dilution process when formulating milk replacers (as reviewed by Kertz et al., 2017). Furthermore, a consequence of including emulsifiers in milk replacers was improved health, and performance through increased digestion and absorption of fat by calves (Kastelic et al., 1950; Huff et al., 1951). Today, many products do not contain emulsifiers due to improvements in biotechnology for fat formulation. Consequently, the issue with digestion of vegetable sourced fat in milk replacers for calves remains a challenge.

Since dairy calves are fed high volumes of the liquid diet per meal, an emulsifier's importance is more critical for fat metabolism and absorption. A key feature of fat digestion in the GIT is micelle formation, which occurs after complex lipid digestion and emulsification (McFadden, 2019). Bile salts act as emulsifiers, stabilizing an emulsion,

and preventing dispersed phase globules from a fusion in the gastrointestinal tract (Siyal et al., 2017). Indeed, synthetic emulsifiers (e.g. lysolecithin, lysophosphatidylcholine) are derived from the hydrolysis phrase of lecithin and exert the same function (Zhang et al., 2011). Similarly, lysolecithin is a potent emulsifier produced naturally in the small intestine of ruminants, enhancing fatty acid digestion via hydrolysis in pancreatic juice (Dawson, 1959). According to Zhang (2011), the ability of lysolecithin to emulsify lipids is much greater than that of common phospholipids, explaining effects on lipid metabolism. It appears that in the case of pre-ruminant calves, lysolecithin is an important aid to emulsification, but the relative effect has still to be ascertained (Garton, 1969). Therefore, more research is needed to investigate the effects of lysolecithin supplementation on not only performance, but blood metabolites in calves.

Other roles of lysolecithin supplementation may be associated with improved GIT function by providing a source of exogenous phospholipids. For example, research has shown that lysolecithin supplementation to broilers resulted in improved GIT function, including longer villus length and up-regulation of several genes associated with collagen, the extracellular matrix, and integrin pathways (Brautigan et al. 2017), and others observed similar effects in broilers more recently (Boontiam et al., 2019). This research suggests that in broilers, lysolecithin improves GIT function, and this increased GIT performance. Indeed, it is likely that improved GIT function is why research has shown that lysolecithin supplementation improved gain-to-feedand performance in dairy cattle (Rico et al., 2017), and lambs (Gallo et al., 2019). However, the literature is scarce in the evaluation of emulsifiers to improve dairy calf performance and associated effects on blood metabolites. Similarly, feeding vegetable sources in MR was associated with deleterious degradation of the GIT microvilli in calves (Seegraber and Morrill, 1986). Therefore, it is possible that lysolecithin supplementation may not only improve

performance, but potentially reduce a calf's chance of abnormal feces.

This study aimed to evaluate the effect of lysolecithin supplementation on milk replacer, and the associated effects on growth performance, selected blood metabolites (glucose, total serum protein, albumin, creatinine, cholesterol, and triglycerides) and likelihood of improved fecal status of pre-weaned dairy calves up to 56 days of life. We hypothesized that the supplementation of lysolecithin in a milk replacer with vegetable sourced fat might improve calf performance and fecal score through increased gain-to-feedand less days spent with an abnormal fecal score.

## 4.2 MATERIALS AND METHODS

The Animal Research Ethics Committee of the Luiz de Queiroz College of Agriculture/University of São Paulo approved all procedures involving animals in this study (protocol nº 2019-11).

### *4.2.1 Experimental Design and Treatments*

This study was conducted at the Experimental Calf Facility of the Animal Science Department at "Luiz de Queiroz" College of Agriculture, University of Sao Paulo, Piracicaba. The average temperature during the study period was 22.3°C (max. of 29.4 °C and min. of 16.2°C); the relative humidity was 72.5%, and the average rainfall was 80.5 mm/mo.

Thirty-two newborn Holstein male and female calves (BW = 35.76 ± 1.5 kg; Mean ± SD) were separated from their dams at birth, transferred to the experimental facility, weighed and fed a high-quality colostrum (> 22 % Brix) by bottle within the first 6 h of life, a volume corresponding to 10% of birth weight (Godden et al., 2009). A blood sample was collected from the jugular vein, 48h after colostrum feeding, to ensure passive transfer through evaluation of total serum protein (**TSP**). All calves had passive transfer

using a threshold of 5.2 g/dL (Deelen et al., 2014) and TSP averaged 6.17 ± 0.20 g/dL. The calves were blocked in pairs by date of birth, birth BW, and gender, resulting in 16 blocks, and were randomly assigned to receive either: Lysolecithin (**LYSO**; mixed in the two milk replacer feedings at a rate of 4g/d Lysoforte®, Kemin Industries, Inc., USA), or a milk replacer control (nothing added).

### *4.2.2 Management and Feeding*

All calves were housed in individual wood shelters (1.35m in height, 1m in width, and 1.45 m in depth) with buckets for feed and water, and wood shelters were distributed in a trimmed grassy field. The animals were bucket-fed with 6 L of a commercial MR (Sprayfo Azul, 14% solids, 22.46% CP, 16.20% fat, Sloten do Brazil Ltd., Santos, SP, Brazil), split into two meals (0700 h and 1700 h). The MR fed did not contain emulsifiers in its formulation, and the fat was composed of palm oil (67%) and coconut oil (33%). A precision scale was used to weigh the amount of LYSO per feeding. Every day, 4 g of LYSO (2 g/meal) was added and sufficiently mixed to the diluted MR (3 L/meal) just before feeding. Water and a commercial calf starter (24.6% CP and 13.9% NDF; Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda., Rio Claro, SP, Brazil) were available for ad libitum intake throughout the 56-d study. The starter was offered every morning, just after milk feeding, and was available until the following morning, when orts were weighted for daily intake calculations. Feed efficiency was calculated as gain to feed ratio. Calves were enrolled on this study for the preweaning period, d 1 to d 56, prior to weaning. Calves were gradually weaned after the trial ended.

### *4.2.3Feed Analysis*

Samples of MR and starter were collected weekly for analysis. DM was measured by drying at 100°C in a forced-air oven for 24 h, and ash by furnace incineration at 550 ° C for 4 hours (AOAC International, 2002; method 942.05). Ether extract (**EE**) was determined using petroleum ether (AOAC International, 2002; method 920.39), with

acidification with glacial acetic acid for the MR samples. Crude protein was analyzed according to the Dumas method (Wiles et al., 1998), with an N analyzer (FP-528; LECO, St. Joseph, MI, USA). Determination of free-ash NDF was done according to Van Soest et al. (1991) and ADF, according to Goering and Van Soest (1970), using sodium sulfite and thermostable amylase. The NFC of the starter and MR were estimated according to the following equation: NFC (%) = 100% - (% NDF + % CP + % fat + % ash), according to Mertens (1997). Dry matter of MR was used for total DMI and to calculate gain to feed ratio (Table 1).

**Table 1. Chemical composition of calf starter and milk replacer fed to calves throughout the 56d trial.**

| Item | Calf starter[1] | Milk replacer [2] |
|---|---|---|
| DM % | 89.3 | 96.1 |
| Ash, % DM | 9.6 | 8.8 |
| CP, % DM | 24.7 | 22.4 |
| EE, % DM | 5.2 | 16.20 |
| NDF, % DM | 13.89 | 0.06 |
| ADF, % DM | 5.5 | - |
| NFC, % DM | 46.6 | 52.2 |

[1] Commercial calf starter (Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda., Rio Claro, SP, Brazil).

[2] Milk replacer (Sprayfo Azul, Sloten do Brazil Ltd., Santos, SP, Brazil) was fed to both treatments diluted to 14.5% of solids.

### *4.2.4 Measurements and Blood Sampling*

Animals were weighed once weekly before the morning liquid diet feeding, on a

mechanical scale (ICS-300; Coimma Ltd., Dracena, SP, Brazil) until the final weight after weaning at 56 d of age. Body measures were collected every other week and included hearth girth (Bovitec, Sao Paulo, SP, Brazil), wither height, and hip-width (Carci, Sao Paulo, SP, Brazil).

Health exams and interventions were performed daily on all calves by the same veterinarian to assess for Bovine Respiratory Disease, diarrhea, and navel ill according to the UW Calf Health Scoring App. In brief, the following symptoms (e.g. 0 normal to 3 abnormal) were collected daily on each calf: presence of abnormal nasal discharge, coughing, ear tilt, eye discharge, a rectal temperature recorded by a digital thermometer (TS-101 Colors Techline digital, Techline Sao Paulo, SP, Brazil), and navel status, (McGuirk and Peek, 2014). Fecal consistency was scored on a scale of 0 to 3, where fecal score of 0 = normal consistency, 1 = semiformed or pasty, 2 = loose feces, and 3 = watery feces. A fecal score $\geq 2$ was considered a diarrhea bout when it occurred for more than 2 consecutive days. Oral rehydration solution (2 L/d of warm water with 50 g of dextrose, 20 g of sodium bicarbonate, and 10 g of sodium chloride) was offered between milk feedings, for every calf that presented diarrhea until the fecal consistency was $\leq 1$. Antibiotic therapy was administered only when the animal showed fever or/and depression symptoms, such recumbence and decreased or refused milk intake.

Blood samples were collected weekly, 2 h after the morning milk feeding via jugular vein puncture with evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The samples were collected in three different tubes containing either sodium fluoride as an anti-glycolytic and potassium EDTA as an anticoagulant to obtain plasma; K3 EDTA to obtain plasma; or a clot activator to obtain serum. An aliquot of blood from the K3 EDTA tube was used for capillary hematocrit using a microhematocrit centrifuge (Model SPIN 1000, MICROSPIN). The remaining samples were centrifuged at 2,000 x g

for 20 minutes at 4 ° C to obtain plasma or serum. The determination of selected blood metabolites was performed on an Automatic Biochemistry System - Model SBA - 200 (CELM, Barueri, SP, Brazil) using commercial kits (LABTEST Diagnóstica S.A., Lagoa Santa, MG, Brazil). The selected metabolites were chosen to understand the effects of LYSO in the intermediate metabolism of fat [cholesterol (Ref. 76) and triglycerides (Ref. 87)]; protein [albumin (Ref. 19), creatinine (Ref. 35), TSP (Ref. 99)] and carbohydrates [glucose (Ref. 85)].

### *4.2.5 Statistical Analysis*

Sample size calculations were performed using R software and the power package (R Studio). This study was powered for sample size based on the most limiting factor, if LYSO reduced the odds of a diarrhea bout in calves. A power analysis was conducted for health outcome measures (diarrhea). This power analysis showed that a total of 28 calves (n = 14 per treatment) was required at 80% power to detect an expected difference of 50% in diarrhea incidence between the treatment and control group. We powered this study at half-widths of 0.05, and an expected herd incidence of diarrhea at 80%. This incidence and targeted difference were selected based on the potential that this MR with vegetable fat sources increased fecal fluidity in calves (De Paula et al., 2017). The exploration of use of emulsifiers to reduce diarrhea in calves is quite limited in the literature. Thus, a total of 14 calves per treatment were required to detect a difference, but 16 calves were enrolled per treatment in case of a loss-to-follow up.

All other statistical procedures were processed in SAS (version 9.4, SAS Institute Inc., Cary, NC). Normality distribution was assessed by the Shapiro-Wilk test, and homogeneity of the variances using the Levene test. Performance, health, and blood metabolites were analyzed as time-repeated measures. The analysis was performed as repeated measures over time (weeks of age), using the MIXED procedure of SAS

statistical package), according to the following model:

$Yijk= \mu + Ti + Bj + eij + Wk + (TW)ik + Eijk$

Where, Yijk = response variable; μ = general average; Ti = fixed effect of treatment (control or LYSO); Bj = random block effect; eij = residual error A; Wk = fixed age effect (days of life); (TW)ik = fixed effect of the diet × age interaction; Eijk =residual error B. The covariance matrices "compound symmetry, heterogeneous compound symmetry, autoregressive, autoregressive heterogeneous, unstructured, banded, ante-dependence, variance components, toeplitz, and heterogeneous toeplitz" were tested and defined according to the lowest value obtained for "Akaike's Information Criterion Corrected" (AICC) and calf was the subject. For all the response variables, the means were obtained through the LSMEANS command. The effect of treatments was performed by test F in the analysis of variance. Significance was declared when $P \leq 0.05$ and a tendency when $0.05 \leq P \leq 0.07$.

Since the diarrhea risk period in calves is the first 28 days of life, the effect of LYSO on a diarrhea bout during the first 28 days was first explored using a general linear model (Proc Glimmix), with the solution option to estimate the random effect of block nested by calf as the subject. However, since the random effect was not significant in the model, a logistic model was chosen. The effect of LYSO on the odds of having a diarrhea bout during the first 28 days on the study was calculated with logistic modeling (Proc Logistic), with age and the treatment interaction explored as fixed effects. Similarly, the effect of LYSO on the odds of having an abnormal fecal score ≥ 2 on any given day from day 1 to 28 was also evaluated.

## 4.3. RESULTS

All performance parameters were affected by age ($P$ <0.02), although there was no significant effect of the interaction treatment x age ($P$ <0.05; Table 2). In brief, LYSO

increased average daily gain (control 0.28 ± 0.03 kg; LYSO 0.37 ± 0.03 kg; $P$ <0.04; LSM ± SEM). Moreover, LYSO increased feed efficiency (control 0.25 ± 0.03; LYSO 0.32 ± 0.03; $P$ <0.02). Similarly, LYSO calves weighed more at final weight at 56 d (control 52.11 ± 2.33 kg; LYSO 56.73 ± 2.33 kg; $P$ <0.05; Table 2; Figure 1). Despite better ADG in LYSO calves, LYSO was not associated with EE intake, and starter DMI ($P$ <0.05; Table 2). Similarly, total DMI for the duration of the study was also not associated with lysolecithin (control 1088.70 ± 27.62; LYSO 1124.80 ± 27.62; $P$ >0.10).

**Table 2. Feed intake and performance of calves supplemented or not with LYSO on milk replacer.**

| Item | Treatment | | SEM | P value [2] | | |
|---|---|---|---|---|---|---|
| | Control | LYSO [1] | | T | A | T×A |
| Total DMI, g/d | 1088.7 | 1124.8 | 27.62 | 0.35 | <0.01 | 0.20 |
| Total EE intake[3], g/d | 149.0 | 149.6 | 1.65 | 0.79 | < 0.01 | 0.58 |
| Starter DMI, g/d | 249.9 | 284.7 | 28.07 | 0.37 | < 0.01 | 0.20 |
| ADG, kg | 0.28 | 0.37 | 0.03 | 0.04 | < 0.01 | 0.45 |
| Feed Efficiency | 0.25 | 0.32 | 0.03 | 0.02 | 0.02 | 0.58 |
| Initial BW, 0 d | 35.5 | 36.0 | 1.49 | 0.67 | - | - |
| Final BW, 56 d | 52.1 | 56.7 | 2.33 | 0.05 | - | - |
| Body measures, cm | | | | | | |
| Withers height | 80.8 | 81.0 | 0.83 | 0.82 | < 0.01 | 0.49 |
| Heart girth | 80.6 | 81.2 | 0.99 | 0.47 | < 0.01 | 0.89 |
| Hip-width | 20.6 | 21.2 | 0.38 | 0.11 | < 0.01 | 0.48 |

[1] LYSO = 4g of lysolecithin fed with 6L of the milk replacer.

[2] T= treatment effect; A= age effect; T x A= interaction treatment x age effect.

[3] MR fat intake = 136.08 g/d for both treatments, since MR volume fed was fixed and there were no refusals.

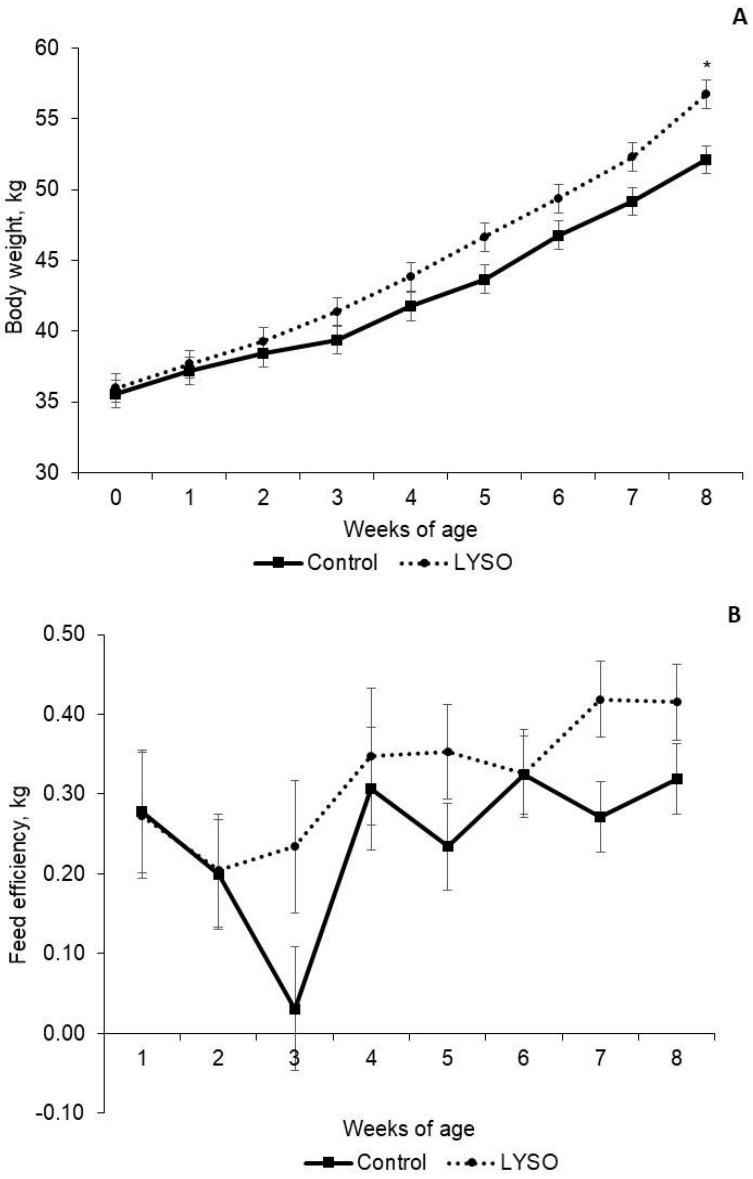


Figure 1. Body weight (A) and gain-to-feed(B) of calves supplemented with or without LYSO on milk replacer (4g of lysolecithin fed with 6L of the MR), according to weeks of age. *Denotes difference with $P<0.05$.

For disease, one LYSO calf died due to pneumonia at 12 d of age. Therefore 15/16 LYSO and 16/16 control calves had health outcomes assessed. In total, 47% (7/16) LYSO had a bout of diarrhea while 100% (16/16) control calves had at least one bout of diarrhea. There were 10 calves requiring antibiotic intervention for diarrhea (control 6 and LYSO 4). There were no other health outcomes (navel ill, Bovine Respiratory Disease) observed. Age, and the treatment interaction were not associated with odds of a diarrhea bout and were not included in the model ($P$ >0.10). However, control calves had 15.34 times greater odds of having a diarrhea bout compared to LYSO calves (95% CI: 10.26-22.94; P <0.0001). Similarly, control calves had a 13.57 (95% CI: 9.25-19.90; $P$ < 0.001) times greater odds of having an abnormal fecal score ≥ 2 on any given day during the diarrhea risk period from d1 to 28.

Supplementation of LYSO had no effect on the following blood parameters: glucose, albumin, creatinine, cholesterol, and triglycerides (Table 3; $P$ > 0.10). However, there was a significant interaction of LYSO and age for TSP, with LYSO calves having higher TSP concentrations at weeks 5 and 6 (Figure 2; $P$ <0.04).

**Table 3. Blood parameters of calves supplemented or not with LYSO on milk replacer**

| Item | Treatment | | SEM | P value [2] | | |
|---|---|---|---|---|---|---|
| | Control | LYSO [1] | | T | A | T×A |
| Glucose, mg/dL | 85.78 | 86.40 | 2.70 | 0.86 | 0.81 | 0.23 |
| Total Serum Protein, g/dL | 5.43 | 5.57 | 0.12 | 0.25 | < 0.01 | 0.04 |
| Albumin, g/dL | 2.94 | 3.00 | 0.03 | 0.15 | 0.36 | 0.06 |
| Creatinine, mg/dL | 1.31 | 1.34 | 0.05 | 0.55 | < 0.01 | 0.77 |
| Cholesterol, mg/dL | 86.71 | 88.83 | 4.34 | 0.70 | 0.27 | 0.21 |
| Triglycerides, mg/dL | 29.84 | 30.92 | 2.44 | 0.73 | 0.28 | 0.99 |

[1] LYSO = 4g of lysolecithin fed with 6L of the milk replacer.
[2] T= treatment effect; A= age effect; T x A= interaction treatment x age.

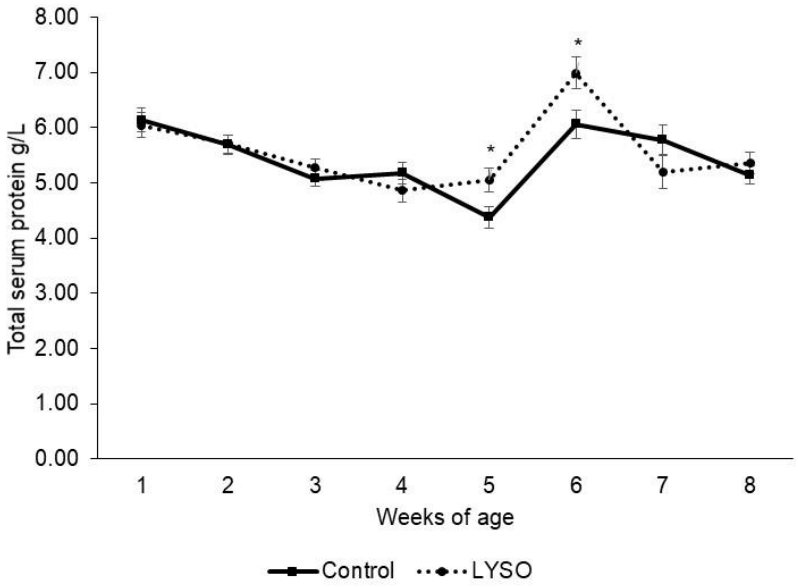


Figure 2. Total serum protein concentration of calves supplemented with or without LYSO on milk replacer (4g of lysolecithin fed with 6L of the MR), according to weeks of age. *Denotes difference with P<0.04.

## 4.4. DISCUSSION

In the current study, total DMI, EE, and starter intake were unaffected by LYSO supplementation, despite improved ADG and better final weights than control calves. While there is outdated literature on feeding emulsifiers to calves (Kastelic et al., 1950; Huff et al., 1951), our results agree with the calf literature that ADG and health outcomes are improved without affecting feed intake. More recent literature in other species also agrees with our results, LYSO improved growth without affecting DMI in piglets (Sun and Kim, 2019), and broilers (Roy et al., 2010). Research has also shown that lysolecithin added to the diets of dairy cattle did not affect DMI despite changing fatty acid profiles in the milk produced (Rico et al., 2017). Indeed, more recently, research has shown that abomasal infusion of an emulsifier improved fatty acid digestion, and increased milk fat production in dairy cattle (de Souza et al., 2020). Thus, this research agrees with our study and suggests that lecithin potentially improves fat digestion without compromising or increasing feed intake.

It is likely we observed similar EE intakes because a fixed amount of MR was offered to calves throughout the study and milk refusals did not occur. It can be hypothesized that LYSO calves grew better because the LYSO was added to a MR with vegetable sourced (palm and coconut) fat. For example, lysolecithin may have played a role in emulsification and hydrolysis of fat for better digestion. However, palm and coconut oil sources have reportedly high digestibility in calves (reported range: 92-96%, Toullec et al., 1980). Alternatively, since starter intake increased with age, the fatty acid profile reaching the small intestine may have also changed. However, since ADG was affected over the course of the study, it is most likely that adding an emulsifier to MR comprised of medium chain fatty acid fats likely improves FE and digestion by neonatal

calves. Years ago, research suggested that adding emulsifying agents to MR which used vegetable sourced fats improved calf performance and fecal health (Kastelic et al. 1950 and Huff et al. 1951). However, limitations to these studies were the inability to use statistical modeling for repeated measures, and the lack of controlling for variation in the environment (not balancing by birth weight, gender, and age at enrollment). Thus, since then, few studies have investigated the inclusion of emulsifiers in the calf diet. This is despite evidence that multiple commercial MR vary in fat content and sources, and some MRs utilize emulsifiers while others do not (as reviewed by Thornsberry et al., 2016). For example, in broilers, research has shown that the addition of lecithin to the diet improved the total tract digestibility of palmitic, oleic, and linoleic acids during the grower period (Zhang et al., 2011). These FA make up sources commonly used in commercial MR composition (Davis and Drackley, 1998). However, unfortunately, we did not measure fat digestibility changes as a function of LYSO supplementation, thus we can only hypothesize this mechanism and concede this is the primary limitation of this study.

Previous studies with exogenous emulsifiers used in non-ruminant diets revealed that these products could increase ADG in piglets (Papadopoulos, 2014) and broilers (Boontiam et al., 2019). Others have observed that feeding LYSO improved FE in broilers (Zhang et al., 2011; Boontiam et al., 2019) and pigs (Akit et al., 2018). Similarly, research has also shown that feeding LYSO led to a heavier final BW in weanling pigs (Xing et al.,2004) and broilers (Boontiam et al., 2017). In the present study, LYSO increased ADG, with no effect on DMI, resulting in higher feed efficiency. Besides improved digestion and absorption of vegetable FA, these findings may be related to the fact that several lysophospholipids, present in lysolecithin, induced physiological responses in the intestine to enhance nutrient absorption as was observed in broilers (Brautigan et al.,

2017). Indeed, broilers fed LYSO had different GIT gene expression, including upregulating genes associated with collagen production, the extracellular matrix, integrin pathways, and improved intestine traits, suggesting a nutrigenomic effect of this compound (Brautigan et al., 2017). Therefore, we suggest that effects of LYSO on calf intestinal gene regulation and morphology should be investigated in future research.

It could be hypothesized that LYSO improved calf FE because of meal size. However, our MR on a DM basis contained 16.2% fat, which is much lower than whole milk at 30% fat. Thus, our calves consumed 65 g of fat in 3 L. This is much lower fat per meal than a calf allowed dam access, who would consume around 12 L/d in several suckling meals, consuming around 100-125 g per meal (Khan et al., 2011). Indeed, it is much more likely that simply MR fatty acid composition differs from whole milk, and emulsification may be more important when vegetable sources of fat are fed (Raven and Robinson, 1964). Recent research has shown that feeding high-fat content MR, at the expense of lactose, can improve performance in intensively fed calves (Berends et al., 2020). However, one limitation to Berends et al., (2020) was the lack of description on which emulsifiers were used in the milk replacer. According to Boerman et al. (2015), for adult ruminants, FA digestibility is inversely correlated to intake and flow of FA, with FA profile being also a critical factor. Thus, because calves still have an immature enzyme system and since MR often contains vegetable oils, the recommended inclusion levels of emulsifiers in MR warrants further investigation.

One reason we believe FE was improved in calves is that the emulsification process improves fat stability, triggering hydrolysis of triglycerides affected by amphiphilic molecules present in the interphase such as bile salts, phospholipids, and lysophospholipids (Golding and Wooster, 2010). Furthermore, the ability to form smaller micelles in the guts of animals and produce larger surface areas can increment bile salt

micelle sizes, and consequently increase incorporation of FA (Boontiam et al., 2017). These emulsifier mechanisms could help alleviate the difficulties of FA digestibility and absorption imposed by the digestive system of pre-ruminants (Toullec and Guilloteau, 1989). According to Huerou-Luron et al. (1992) the lipase activity in the calf digestive system is low at birth and increases with age, improving performance. In our view, this improved growth performance could be related to enhanced digestibility of fat and other nutrients, which is consistent with previously cited research in broilers (Zhang et al., 2011; Papadoulos et al., 2018). Although LYSO improved growth performance, body measures were unaffected by supplementation, even though these measures are directly correlated with calf weight and development (Heinrichs et al., 2007). It is possible that we did not observe differences in heart girth, height, or hip circumference because LYSO is associated with improving fat digestion. Indeed, skeletal growth in calves is typically associated with accelerated protein diets (Davis Rincker et al., 2011). Thus, it is possible LYSO does not affect skeletal growth in calves.

While body measures were not affected, calf fecal health was improved. Indeed, we observed that LYSO ameliorated the odds of a diarrhea bout. We may speculate that since calves had diarrhea with a fever, diarrhea may have been pathogenic. However, others have observed that feeding MR with vegetable sourced fat may increase fecal fluidity in calves, independent of a pathogen (De Paula et al., 2017). Thus, while our calves likely experienced pathogenic diarrhea, LYSO calves likely had a faster emulsification process, and thus, absorption rate of fat, which was sufficiently increased to decrease diarrhea (Field, 2003). However, considering that the fed MR had a lower fat content than conventional cow's milk, lower diarrhea odds may be more related to the anti-inflammatory effects of phospholipids that make-up LYSO (Treed et al., 2007) and potentially improvements in villus health that were observed in broilers (Brautigan et al.

(2017). Thus, we suggest future research is needed to determine if LYSO may potentially lower GIT inflammation in diarrheic calves, and potentially enhance fat digestion which decreases the fat load for diarrheic associated lower GIT dysfunction.

The use of feed additives is a strategy to improve growth performance. Besides providing essential nutrients and optimizing feed utilization (Pandey et al., 2019), many additives could influence the intestinal microbiota balance for better digestion efficiency and animal health. Previous studies showed that lysophospholipids supplementation could influence changes in jejunal morphology, absorptive capacity, defensive mechanism, and substantial effects on performance and immunity in broilers (Boontiam et al., 2016) and laying hens (Witchanun et al., 2019). Numerous studies indicated that phospholipids have an essential role in the immunological process (as reviewed by Hartmann et al., 2009). Indeed, it was observed that phospholipids have destabilized the ionic balance of bacteria in the lower SI in piglets (Silva Júnior et al., 2009). This action mechanism matters to calves since they are susceptible to the proliferation of pathogenic microorganisms in gut microbiota. However, this needs further investigation since calf diarrhea is a multi-factorial disease and can be attributed to multiple enteric pathogens such as bacteria, viruses, and protozoa.

Blood parameters in our study (e.g. glucose, albumin, creatinine, cholesterol, triglycerides) were within the normal range for dairy calves during the preweaning phase (Pogliani and Birgel Junior, 2007). Total serum protein concentrations were affected by treatment at 5 and 6 weeks, indicating that protein metabolism and liver function were affected by supplementation. These results were expected since phospholipids have been observed to improve liver functions in laying hens (Attia et al., 2008). Like our results, Zangeneh et al. (2018) found that supplementation of lysolecithin to broilers decreased TSP with time. However, others have reported no significant effect of lysolecithin

supplementation on the TSP concentration in broilers (Roy et al., 2010; Raju et al., 2017). Lysophospholipids can change protein channel formation in the membranes of the lower GIT, increasing ion exchange (Maingret et al., 2000). This mechanism enhances the size and the number of membranous pores improving the flux rate of macromolecules across the cell membrane (Lundbak et al., 2010), which influences nutrient transport. Thus, it is possible that LYSO offered to our calves changed TSP due to an increased nutrient efficiency, which was also explained by increased feed efficiency.

Lysolecithin supplementation also did not affect creatinine levels, and concentrations were in a safe range (Hammon et al., 2002), suggesting normal renal and liver functions in these calves. Similar results were found by Lee et al. (2008), who compared calves fed milk replacers containing different amounts of energy and protein. Similarly, no treatment effects were observed for cholesterol and triglycerides on our study and agrees with findings in broilers (Raju et al. 2017; Zangeneh et al. 2018). However, serum triglycerides and cholesterol levels in neonatal calves are influenced by fat absorption and usually increase in preweaned calves fed higher fat diets (Egli and Blum, 1998). Thus, an increase in those concentrations was expected and it is unknown why changes were not observed in our calves. Future research should investigate the mechanisms behind LYSO added to MR and its effect on fat digestibility in calves.

In summary, LYSO improved calf ADG, and final weights without negating total dry matter intake. Based on the literature in broilers, piglets, lambs, and lactating dairy cattle, it is likely that LYSO emulsifies fat and provides a source of lysophospholipids to enhance fat digestion in the calf. Future research should investigate these effects of LYSO on calf fat digestibility. Similarly, we observed LYSO to ameliorate odds of abnormal fecal scores during the first 28 days of life. We hypothesize this mechanism is multifactorial by reducing inflammation in the lower GIT and improving fat digestion for

better lower GIT function. Moreover, LYSO can be supplemented to calves without negating liver, kidney, or blood glucose function. Supplementation of LYSO may improve calf performance, fecal health, and TSP status without negating total dry matter intake or kidney and liver function for calves offered 6 L/d milk replacer using vegetable soured fats.

## 4.5. CONCLUSION

Supplementation of LYSO at 4 g/d in milk replacer with vegetable fat sources improved gain-to-feed of dairy calves. Lysolecithin improves average daily gain, without negating feed intake, resulting in a higher body weight at weaning. It is likely that calves receiving LYSO had improved fat digestion and absorption than control calves. Lysolecithin may also ameliorate a calf's likelihood of diarrhea, which benefits health and performance. Future research is necessary to explore LYSO supplementation's effects on fat digestibility in dairy calves.

## ACKNOWLEDGEMENTS

The authors appreciate the partial financial support from Kemin South America. The authors acknowledge the assistance of the graduate and undergraduate students in the Experimental Calf Station "Evilasio de Camargo" (College of Agriculture "Luiz de Queiroz," University of Sao Paulo). The authors also appreciate the financial support provided by the Coordination for the Improvement of Higher Education Personnel (CAPES) as a scholarship for the first author.

## 4.6. REFERENCES

Akit, H., C. Collins, F. Fahri, A. Hung, D. D'Souza, B. Leury, and F. Dunshea. 2018. Dietary lecithin improves gain-to-feedwithout impacting meat quality in immunocastrated male pigs and gilts fed a summer ration containing added fat. Anim.

Nutr. 4:203–209. http://doi: 10.1016/j.aninu.2018.01.008.

AOAC International. 2002. Official Methods of Analysis. 18th ed. AOAC International Gaithersburg, MD.

Attia, Y.A., A.S. Hussein, A.E. Tag El-Din, E.M. Qota, A.I. Abed El-Ghany and A.M. El-Sudany.2008. Improving productive and reproductive performance of dual-purpose crossbred hens in the tropics by lecithin supplementation. Trop. Anim. Health Prod. 41:461-475. http://doi: 10.1007/s11250-008-9209-3.

Berends, H., H. van Laar, L.N. Leal, W.J.J. Gerrits and J. Martín-Tereso. 2020. Effects of exchanging lactose for fat in milk replacer on ad libitum feed intake and growth performance in dairy calves. J. Dairy Sci. 103:4275–4287. https://doi.org/10.3168/jds.2019-17382.

Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta regression. J. Dairy Sci. 98:8889–8903. https://doi.org/10.3168/jds.2015-9592.

Boontiam, W., Y. K. Hyun, B. Jung, and Y. Y. Kim, 2019. Effects of lysophospholipid supplementation to reduced energy, crude protein, and amino acid diets on growth performance, nutrient digestibility, and blood profiles in broiler chickens. J. Poult. Sci. 98: 6693–6701. https://doi:10.3382/ps/pex005.

Boontiam, W., B. Jung, and Y. Y. Kim. 2017.Effects of lysophospholipid supplementation to lower nutrient diets on growth performance, intestinal morphology, and blood metabolites in broiler chickens. J. Poult. Sci. 96:593–601. https://doi: 10.3382/ps/pew269.

Brautigan, D. L., R. Li, E. Kubicka, S.D. Turner, J.S. Garcia, M.L. Weintrau, and E.A. Wong. 2017. Lysolecithin as feed additive enhances collagen expression and villus length in the jejunum of broiler chickens. J. Poult. Sci. 96:2889-2898.

https://doi:10.3382/ps/pex078.

Davis, C.L. and J.K. Drackley. 1998. The development, nutrition, and management of the young calf. University Press, Ames.

Davis Rincker, L. E., M. J. Vandehaar, C. A. Wolf, J. S. Liesman, L. T. Chapin, and M. S. Weber Nielsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. J. Dairy Sci. 94(7):3554-3567. DOI: 10.3168/jds.2010-3923

Dawson A.M. and J. I. Kurt. 1959. Studies on lipid metabolism in the small intestine with observations on the role of bile salts. J Clin Invest.39:730-740. https://doi:10.1172/JCI104090.

de Paula, M.R., C.E. Oltramari, J.T. Silva, M.P.C. Gallo, G.B. Mourão, and C.M.M. Bittar. 2017. Intensive liquid feeding of dairy calves with a medium crude protein milk replacer: Effects on performance, rumen, and blood parameters. J. Dairy Sci.100:4448-4456.https://doi.org/10.3168/jds.2016-10859.

de Souza, J., M. Westerrn, and A. L. Lock. 2020. Abomasal infusion of an exogenous emulsifier improves fatty acid digestibility and milk fat yield of lactating dairy cows. J. Dairy Sci.103(7):6167-6177. https://doi.org/10.3168/jds.2020-18239

Deelen, S., T. Ollivett, D. Haines, and K. Leslie. 2014. Evaluation of a brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97: 3838-44. https://doi.org/10.3168/jds.2014-7939.

Edwards-Webb, J.B.1983. Digestive lipolysis in the pre-ruminant calf. The abomasal hydrolysis of butter oil, coconut oil, palm oil and tallow. J. Sci. Food Agric. 34:930-936. https://doi: 10.1002/jsfa.2740340906.

Egli, C. P. and J. W. Blum, 1998. Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling Simmental calves held in a cow-calf

operation. J. Vet. Med A. 45:99–118. https://doi.org/10.1111/j.1439-0442.1998.tb00806.x

Field M. 2003. Intestinal ion transport and the pathophysiology of diarrhea. J. Clin Invest. 111:931–943. https://doi.org/10.1172/JCI18326.

Gallo, S.B., T.Brochado, L.Brochine, D.Passareli, S.F. Costa, I.C. da S. Bueno, da S., J.C. de C. Balieiro,R. Franzolin, and L.O. Tedeschi. 2019. Effect of biosurfactant added in two different oil source diets on lamb performance and ruminal and blood parameters. Livest Sci. 226:66-72. https://doi.org/10.1016/j.livsci.2019.06.006.

Garton, G. 1969. Digestion and absorption of lipids in the ruminant. Proc. Nutr. Soc.28: 131-139. https://doi.org/10.1079/PNS19690024.

Godden, S. M., D. M Haines, and D. Hagman. 2009. Improving passive transfer of immunoglobulins in calves. I: dose effect of feeding a commercial colostrum replacer. J Dairy Sci. 92: 1750–1757. https://doi.org/10.3168/jds.2008-1846.

Golding, M., and T.J. Wooster. 2010. The influence of emulsion structure and stability on lipid digestion. Curr. Opin. Colloid Interface Sci. 15: 90-101. https://doi.org/10.1016/j.cocis.2009.11.006.

Goodell, G. M., J. Campbell, L. Hoejvang-Nielsen, W. Stansen, and P. D. Constable. 2012. An alkalinizing oral rehydration solution containing lecithin-coated citrus fiber is superior to a nonalkalinizing solution in treating 360 calves with naturally acquired diarrhea. J. Dairy Sci. 95(11):6677-6686. https://doi.org/10.3168/jds.2012-5605

Hammon, H.M. and J.W. Blum. 2002. Feeding different amounts of colostrum or only milk replacer modifies receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domest. Anim. Endocrinol. 22:155-168. https://doi.org/10.1016/S0739-7240(02)00122-4.

Hartmann, P., A. Szabo, G. Eros, D. Gurabi, G. Horvath, I. Nemeth, M. Ghyczy, and M.

Boros. 2009. Anti-inflammatory effects of phosphatidylcholine in neutrophil leukocyte-dependent acute arthritis in rats. Eur. J. Pharmacol. 622:58–64. https://doi.org/10.1016/j.ejphar.2009.09.012.

Hawkins, A., K. Burdine, D. Amaral-Phillips, and J. H. Costa. 2019. An economic analysis of the costs associated with pre-weaning management strategies for dairy heifers. Animals 9(7):471. https://doi.org/10.3390/ani9070471

Heinrichs, A. J., H. N. Erb, G. W. Rogers, J. B. Cooper, and C. M. Jones. 2007.Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. Prev. Vet. Med. 78: 333–338. https://doi.org/10.1016/j.prevetmed.2006.11.002.

Huerou-Luron, I. L., P. Guilloteau, C. Wicker-Planquart, J.A. Chayvialle, J. Burton, A. Mouats,

R. Toullec, A. Puigserver. 1992. Gastric and pancreatic enzyme activities and their relationship

with some gut regulatory peptides during postnatal development and weaning in calves. J. Nutr, 122: 1434–1445. https://doi.org/doi:10.1093/jn/122.7.1434.

Huff, J.S., R.K. Waugh, and G.H. Wise.1951. Effect of glycerol-mono-stearate on fat absorption, growth, and health of calves. J. Dairy Sci. 34:1056-1063. https://doi.org/10.3168/jds.S0022-0302(51)91825-5.

Huuskonen, A., H. Khalili, J. Kiljala, E. Joki-Tokola, and J. Nousiainen. 2005. Effects of vegetable fats versus lard in milk replacers on feed intake, digestibility, and growth in finnish ayrshire bull calves. J. Dairy Sci. 88: 3575-3581. https://doi.org/10.3168/jds.S0022-0302 (05)73042-3.

Jones, C., and J. Heinrichs. 2017. Feeding the newborn dairy calf. Pennsylvania State

University Cooperative Extension.

Jones, D.B.; J. L. Nelssen, and R. H. Hines. 1989. Effects of lecithin and lysolecithin additions on growth performance and nutrient digestibility in weanling pigs. Kansas Agricultural Experiment Station Research Reports. https://doi.org/10.4148/2378-5977.6295

Kastelic, O.G. Bentley, and P.H. Phillips. 1950. Studies on growth and survival of calves fed semi-synthetic milks from birth. J. Dairy Sci. 33: 725-736. http**://**10.3168/jds.S0022-0302(50)91962-X.

Kertz, A.F., T.M. Hill, J.D. Quigley, A.J. Heinrichs, and J.K. Drackley. 2017. A 100-Year Review: Calf nutrition and management. J. Dairy Sci. 100:10151-10172. https://doi.org/10.3168/jds.2017-13062.

Khan, M. A., D. M. Weary, and M.A. G. von Keyserlingk. 2011. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 94:1071–1081. https://doi.org/10.3168/jds.2010-3733.

Khan, M.A, A. Bach, D.M. Weary and M.A. G. von Keyserlingk. 2016. Invited review: Transitioning from milk to solid feed in dairy heifers. J. Dairy Sci. 99:885–902. https://doi.org/10.3168/jds.2015-9975.

Lee, C., D. L. Morris, J. E. Copelin, J. M. Hettick, and I. H. Kwon. 2019. Effects of lysophospholipids on short-term production, nitrogen utilization, and rumen fermentation and bacterial population in lactating dairy cows. J. Dairy Sci. 102:3110-3120. https://doi.org/10.3168/jds.2018-15777.

Lee, H.J., M.A. Khan, W.S. Lee, H.S. Kim, K.S. Ki, S.J. Kang, T.Y. Hur, M.S. Khan, and Y.J. Choi. 2008.Growth, blood metabolites, and health of holstein calves fed milk replacer containing different amounts of energy and protein. Asian-Australas J. Anim. Sci. 21:198-203. https://doi.org/10.5713/ajas.2008.70261.

Lundbak, J. A., S. A. Collingwood, H. I. Ingolfsson, R. Kapoor, and O.S. Andersen. 2010. Lipid bilayer regulation of membrane protein function: Gramicidin channels as molecular force probes. J. R. Soc. Interface. 7:373–395. https://doi.org/10.1098/rsif.2009.0443.

Maingret, F., A. J. Patel, F. Lesage, M. Lazdunski, and E. Honor′e, 2000.Lysophospholipids open the two-pore domain mechanogated K (+) channels TREK-1 and TRAAK. J. Biol. Chem.275:10128–10133. https://doi.org/10.1074/jbc.275.14.10128.

McFadden, J. W. 2019. Dietary lecithin supplementation in dairy cattle. Department of Animal Science Cornell University.

Mcguirk, S. and S. F. Peek 2014. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. Anim. Health Res. Reviews. 15:1-3. https://doi.org/10.1017/S1466252314000267.

Mertens, D.R. 1997.Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci.80:1463-1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.

Pandey A.K., P. Kumar, and M.J. Saxena. 2019. Feed Additives in Animal Health. Nutraceuticals in Veterinary Medicine. Springer, Cham. https://doi.org/10.1007/978-3-030-04624-8_23.

Papadopoulos, G. A., K. Müller, D. Schertling, and M. di Benedetto. 2014. Supplementation of lysolecithin in combination with a multi-non-starch polysaccharides enzyme improves the gain-to-feedduring the post-weaning period in piglets. Acta Agric. Scand. A Anim. Sci. 64: 130–136. https://doi.org/10.1080/09064702.2014.978360.

Papadopoulos, G. A., T. Poutahidis, S. Chalvatzi, M. Di Benedetto, A. Hardas, V. Tsiouris, and P.D. Fortomaris.2018. Effects of lysolecithin supplementation in low-

energy diets on growth performance, nutrient digestibility, viscosity, and intestinal morphology of broilers. Br. Poultry Sci. 59: 232–239. https://doi.org/10.1080/00071668.2018.1423676.

Pogliani F.C. and E.H. Birgel Júnior. 2007. Reference values of the lipid profile of Holstein cattle bred in state of São Paulo. Braz. J. Vet. Res. Anim. Sci. 44: 373-383. https://doi.org/10.11606/issn.1678-4456.bjvras.2007.26621.

Raju, M. V. L. N., P.P. Chakrabarti, S.V. Rama Rao, B.V.S.K. Rao, A.K. Panda, V. Sujatha, J.R.C. Reddy, B.L.A. Prabhavathi Devi, G. Shyam Sunder and R.B.N. Prasad. 2017.Effects of dietary inclusion of lysolecithin from rice bran oil on performance, serum biochemical profile, organ weights, immune response, and nutrient digestibility in broiler chicken. Indian J. Vet. Anim. Sci. 51: 700-705. https://doi.org/10.18805/ijar.v0iOF.7804.

Raven, A. M., and Robinson, K.L. 1964. Factors affecting the nutritive value of fat for calves. I.—the mode of incorporation into milk diets. J. Sci. Food Agric., 15:214-219. https://doi.org/10.1002/jsfa.2740150403.

Rico, D.E., Y. Ying, and K.J. Harvatine. 2017. Short communication: Effects of lysolecithin on milk fat synthesis and milk fatty acid profile of cows fed diets differing in fiber and unsaturated fatty acid concentration. J. Dairy Sci. 100:9042-9047. https://doi.org/10.3168/jds.2017-12976.

Roy, A., S. Haldar, S. Mondal, and T.K. Ghosh. 2010. Effects of supplemental exogenous emulsifier on performance, nutrient metabolism, and serum lipid profile in broiler chickens. Vet. Med. Int. 262604. https://doi.org/10.4061/2010/262604.

SAS Institute. 2014. SAS Users Guide. SAS Institute Inc., Cary, NC.

Seegraber, F. J. and J. L. Morrill. 1986. Effect of Protein Source in Calf Milk Replacers on Morphology and Absorptive Ability of Small Intestine1. J. Dairy Sci. 69(2):460-

469. https://doi.org/10.3168/jds.S0022-0302(86)80424-6

Silva Júnior. A. 2009. Chemical and philosophic interactions of acidifiers, probiotics, enzymes and lysophospholipids in the piglest digestion Interações químico-fisiológicas entre acidificantes. probióticos. enzimas e lisofosfolipídios na digestão de leitões. Braz. J. Anim. Sci.38:238-245. https://doi.org/10.1590/S1516-35982009001300024.

Siyal FA, M.E. A. El-hack, M. Alagawany, C. Wang, X. Wan, J. He, M. Wang, L. Zhang, X. Zhong, T. Wang and K. Dhama. 2017.Effect of soy lecithin on growth performance, nutrient digestibility, and hepatic antioxidant parameters of broiler chickens. Int. J. Pharmacol. 13:396-402. https://doi.org/10.3923/ijp.2017.396.402.

Sun, H. Y., and I. H. Kim, 2019. Evaluation of an emulsifier blend on growth performance, nutrient digestibility, blood lipid profiles, and fecal microbial in growing pigs fed low energy density diet. Livestock Sci. 227: 55–59. https://doi.org/10.1016/j.livsci.2019.06.016.

Sun, H. Y., Yoon, S. B., and Kim I. H.2019. Growth performance, nutrient digestibility, blood lipid profile and faecal Escherichia coli and Lactobacillus counts on growing pigs fed with de-oiled lecithin emulsifier. Italian J. Anim. Sci. 18:1111-1116. https://doi.org/10.1080/1828051X.2019.1620140.

Thornsberry R.M., D. Wood, A.F. Kertz, and D. Hutcheson. 2016. Alternative ingredients in calf milk replacer – a review for bovine practitioners. The Bovine Practitioner. 50: 1-24.

Toullec, R. and P. Guilloteau. 1989. Research into digestive physiology of the milk-fed calf. In: Van Weerdon E.J. Huisman J. Nutrition and Digestive Physiology in Monogastric Farm Animals. Pages 37-55. Wageningen, Netherlands.

Toullec, R., M. Thfiriez, and P. Thivend.1980. Milk replacers for calves and lambs.

World Anim. Rev. 33: 32-42.

Treede, I., A. Braun, R. Sparla, M. Kuhnel, T. Giese, J.R. Turner, E. Anes, H. Kulaksiz, J. Fullekrug, W. Stremmel, G. Griffiths, and R. Ehehalt. 2007. Anti-inflammatory effects of phosphatidylcholine J. Biol. Chem. 282: 27155–27164. https://doi: 10.1074/jbc.M704408200.

Wiles, P. G., I. K. Gray, and R. C. Kissling. 1998. Routine analysis of protein by Kjeldahl and Dumas methods: Review and interlabora- tory study using dairy products. J. AOAC Int. 81:620–632.

Witchanun J., P. Theerawit, P Kanokporn., R. Choawit, K. Pudcharaporn, and B. Chaiyapoom. 2019. The effects of supplementing lysophosphatidylcholine in diet on production performance, egg quality and intestinal morphology of laying hens. Int. J. Poultry Sci. 18: 238-243. https://doi.org/10.3923/ijps.2019.238.243.

Xing J.J., E. Van Heugten, D.F. Li, K.J. Touchette, J.A. Coalson, R.L. Odgaard, and J. Odle. 2004. Effects of emulsification, fat encapsulation, and pelleting on weanling pig performance and nutrient digestibility. J. Anim. Sci. 82:2601–2609. https://doi.org/10.2527/2004.8292601x.

Zangeneh, S., M. Torki, H. Lotfollahian, and A. Abdolmohammadi. 2018. Effects of dietary supplemental lysophospholipids and vitamin C on performance, antioxidant enzymes, lipid peroxidation, thyroid hormones and serum metabolites of broiler chickens reared under thermoneutral and high ambient temperature. J. Anim. Physiol. Anim. Nutr. 2:1521-1532. http://doi.org/10.1111/jpn.12935.

Zhang, B., L. Haitao, D. Zhao, Y. Guo, and A. Barri. 2011. Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids, and apparent metabolizable energy content. Anim. Feed Sci. Technol. 163: 177-184. http://doi.org/10.1016/j.anifeedsci.2010.10.004.

## 9. An evaluation of the effects of β-glucans on performance and metabolic profile dynamics in Holstein dairy calves


### ABSTRACT

β-Glucans are sugars extracted from the cell walls of bacteria, fungi, yeasts, algae, lichens, and plants, such as oats and barley used to improve immunity and animal performance. The objective of this study was to evaluate whether supplementation of 2 g/d β -glucans, or a negative control (nothing added), divided into two feedings of 6 L/d conventional (vegetable fat) milk replacer was associated with performance, metabolic profile dynamics and immune responsiveness in dairy calves. Neonatal Holstein calves (n=34) at birth, weighing 36.38 ± 1.33 kg; mean ± SD) were blocked in pairs (17 blocks) by date of birth, birth BW, and gender. Calf pairs were randomly assigned to receive either: β -glucans mixed in the two milk replacer feedings at a rate of 2g/d (Aleta®, Kemin Industries, Inc., USA), or a milk replacer control, (nothing added). All calves received colostrum no later than 6 hours after birth. Calves were housed individually in wood hutches, received 6L of milk replacer and grain was fed ad libitum until 56 d of age. Body weights were measured weekly, and fecal consistency was scored daily. A linear mixed model evaluated the effects of β-glucans on performance (average daily gain (ADG), final weight, feed efficiency (FE), immune status (white blood cell count), and blood metabolites (glucose, albumin, creatinine and total serum protein), repeated by time, with calf as the subject and pair as a random effect. A logistic model evaluated the odds of improving a calf's fecal score with β-glucans supplementation, controlling for block, ID and gender as fixed effects. While there was no association of β -glucans with starter intake ($P > 0.06$), there was a treatment by age interaction for FE ($P = 0.04$). Indeed, FE

(P = 0.04) and ADG (P =0.06) tended to be greater for β-glucans calves in the third and fifth week of age. Moreover, final weights were higher for β-glucans calves (β-glucans 56.3 ±2.17 kg; control 51.5 ± 2.17 kg; LSM ± SEM; *P* = 0.05). Moreover, control calves were more likely to have a diarrhea bout than β-glucans calves OR 22.46 (95% CI: 15.14-33.32; *P* <0.0001). There were no blood metabolite differences, or association with β-glucans on the serum concentrations of total protein, albumin, creatinine and glucose (*P* >0.06) during the preweaning phase. Similarly, immune parameters such as white blood cell count were similar between treatments (*P* > 0.06). Our findings suggest that dietary supplementation of β-glucans improved final BW through a tendency to improve FE and ADG at three and five weeks of age and improved fecal status of calves offered 6 L/d preweaning, without negating blood metabolites, or immune status. Future research should explore β-glucans digestibility and immune responsiveness in calves.

**Keywords:** calf, diarrhea, immune, *Euglena gracilis*

## 9.1 INTRODUCTION

Diarrhea is one of the leading causes of mortality and morbidity in dairy calves (Urie et al., 2018). Specifically, diarrhea is one of the most economically costly diseases occurring during the first weeks of life of the dairy calf, with life long lasting effects (Kaneene and Hurd, 1990). Moreover, diarrhea preweaning was associated with reduced growth in calves (Winderyer et al., 2014), a lower likelihood of becoming pregnant as a heifer (Zanton and Henrichs, 2005), and a higher risk of culling before completing the first lactation (Besecker et al., 2008). Therefore, it is imperative to explore methods to ameliorate disease risk in calves, specifically as judicious antibiotic use becomes more scrutinized (FAO,2019).

One possibility to ameliorate diarrhea bouts in calves is by feeding nutraceuticals, which are nutritional interventions thought to have positive effects on physiological status and health outcomes (Nasri et al., 2014). For example, β-glucans are immune modulators (Hulbert and Moisá, 2016 and Broadway et al., 2015) which could potentially ameliorate diarrhea bouts in calves by providing immune support. Specifically, β-glucans are polysaccharides, which are glucose polymers commonly derived from pathogenic bacteria and fungi (Akrameine et al., 2007). Most of the commercially available β-glucans are derived from yeast, while there is limited research on algae-derived β-glucans in calves. β-glucans can be sourced from *Euglena gracilis*, a unique alga which contains more β-glucans bioavailable without extraction and highly digestible (Phillips et al., 2019). Thus, β-glucans can be sourced from yeast products, but yeast contains less β-glucans and requires extraction because of its indigestible cell wall (Rahar et al., 2011).

β-glucans are perceived as a threat when ingested and have activated the immune system in biomedical issues study (Kim et al., 2006). Specifically, research has shown that feeding ß-glucans to beef and dairy cattle activated macrophages, a key component of the innate immune system (Krehbiel and Zhang, 2016). This immunomodulatory effect of β-glucans has been shown to reduce the proliferation of bacteria in rats (Liang et al., 1998), reducing the risk of viral infections in swine (Jung et al., 2004), reducing the parasite load of *Plasmodium berghei* in mices (Holbrook; Cook; Parker, 1981) and pathogenic fungi in mices (Meira et al., 1996). Moreover, the supplementation of β-glucans to productive animals reduced the proliferation of bacteria, preventing colonization and increasing energy intake (Angelakis et al., 2017), which may impact on animals' performance and metabolism.

Some studies showed that calves supplemented with β-glucans yeast at 0.075g/kg presented an improved composition of the intestinal microflora, with decreased numbers

of pathogenic *E. coli* and increased numbers of commensal *Lactobacillus* (Zhou et al., 2009). This mechanism may be important to young calves due to the unstable microbiota during the first weeks of life which affects the digestion and absorption of nutrients (Celi et al., 2016).

Similarly, mannanoligosaccharide β-glucans fed at 4g/d to calves increase daily body weight gain, feed intake, and feed conversion efficiency at 21 days of age (Gosh and Mehla, 2012). In a different study, 1g/d of yeast β-glucans fed to calves was associated with improved gut development at 56 days of age including: increased villus height-to-crypt depth ratio of the small intestine and enhanced the papilla length and width of the ruminal epithelium (Xiao et al., 2016). This research suggests β-glucans can enhance gut development in calves. Kim et al. (2011) reported that calves supplemented with β-glucans had increased rumen pH and nutrient digestibility. However, research is limited on whether or not β-glucans can ameliorate diarrhea bouts in calves preweaning, or improves the immune response in weaned calves.

Since gut health was improved in other studies, β-glucans may improve a calf's ability to respond to diarrhea, and stabilize a calf's response to a stressful event. Since β-glucans modulate the host's gut microbiota and immune system in dairy calves (Ma et al., 2014), it may be beneficial for prevention of immune attenuation caused by various stressors and strengthening of immune competence of neonatal calves. Thus, there is evidence that probiotics such as β-glucans may modulate feeding behavior, showing improved performance in livestock animals (Anadon et al., 2019).

This study aimed to evaluate the effects of milk replacer supplemented with alga β-glucans on health, performance and blood metabolites of Holstein dairy calves. It was hypothesized that alga β-glucans supplementation since the second day of life would

strengthen the calf's immunocompetence assisting in health and performance improvement.

## 9.2 MATERIALS AND METHODS

The Animal Research Ethics Committee of the Luiz de Queiroz College of Agriculture/University of São Paulo approved all procedures involving animals in this study (protocol nº 2019-11).

### *9.2.1 Experimental Design and Treatments*

This study was conducted from June 2018 to January 2019 at the Experimental Calf Facility of the Animal Science Department at “Luiz de Queiroz” College of Agriculture, University of Sao Paulo, Piracicaba. The average temperature during the study period was 22.3°C (max. of 29.4 °C and min. of 16.2°C); the relative humidity was 72.5%, and the average rainfall was 80.5 mm/mo.

Thirty-four newborn (10 females and 24 males) Holstein dairy calves (BW = 36.38 ± 1.3 kg; mean ± SD) were separated from their dams at birth, transferred to the experimental facility, weighed and fed a high-quality colostrum (> 22 % Brix) by bottle within the first 6 h of life, a volume corresponding to 10% of birth weight (Godden et al., 2009). A blood sample was collected from the jugular vein, 48h after colostrum feeding, to ensure passive transfer through evaluation of total serum protein (TSP). All calves had passive transfer using a threshold of 5.2 g/dL (Deelen et al., 2014) and TSP averaged 6.30 ± 0.20 g/dL. The calves were blocked in pairs by date of birth, birth BW, and gender, resulting in 17 blocks, and were randomly assigned to receive either: alga ß-glucans: (mixed in the two milk replacer feedings at a rate of 2g/d Aleta®, Kemin Industries, Inc., USA), or a milk replacer control (nothing added).

### *9.2.2 Housing, Management and Feeding*

All calves were housed in individual wood shelters (1.35m in height, 1m in width and 1.45 m in depth) with buckets for feed and water, and wood shelters were distributed in a trimmed grassy field. The animals were bucket-fed with 6 L of a commercial MR (Sprayfo Azul, 14% solids, 22.46% CP, 16.20% fat, Sloten do Brazil Ltd., Santos, SP, Brazil), split into two meals (0700 h and 1700 h).

A precision scale was used to weigh the amount of ß-glucan per feeding. Every day, 2 g of ß-glucan (1 g/meal) was added and sufficiently mixed to the diluted MR (3 L/meal) just before feeding. Water and a commercial calf starter (24.6% CP and 13.9% NDF; Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda., Rio Claro, SP, Brazil) were available for ad libitum intake throughout the 56-d study. The starter was offered every morning, just after milk feeding, and was available until the following morning, when orts were weighted for daily intake calculations. Calves were enrolled in this study for the preweaning period, d 1 to d 56, prior to weaning. Calves were gradually weaned after the trial ended.

***9.2.3Feed Analysis***

Samples of MR and starter were collected weekly for analysis. DM was measured by drying at 100°C in a forced-air oven for 24 h, and ash by furnace incineration at 550 ° C for 4 hours (AOAC International, 2002; method 942.05). Ether extract (EE) was determined using petroleum ether (AOAC International, 2002; method 920.39), with acidification with glacial acetic acid for the MR samples. Crude protein was analyzed according to the Dumas method (Wiles et al., 1998), with an N analyzer (FP-528; LECO, St. Joseph, MI, USA). Determination of free-ash NDF was done according to Van Soest et al. (1991) and ADF, according to Goering and Van Soest (1970), using sodium sulfite and thermostable amylase. The NFC of the starter and MR were estimated according to the following equation: NFC (%) = 100% - (% NDF + % CP + % fat + % ash), according

to Mertens (1997). Dry matter of MR was used for total DMI and to calculate gain to feed ratio (Table 1).

**Table 1. Chemical composition of calf starter and milk replacer fed to calves throughout the 56d trial.**

| Item | Calf starter[1] | Milk replacer [2] |
|---|---|---|
| DM % | 89.3 | 96.1 |
| Ash, % DM | 9.6 | 8.8 |
| CP, % DM | 24.7 | 22.4 |
| EE, % DM | 5.2 | 16.20 |
| NDF, % DM | 13.89 | 0.06 |
| ADF, % DM | 5.5 | - |
| NFC, % DM | 46.6 | 52.2 |

[1] Commercial calf starter (Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda., Rio Claro, SP, Brazil).

[2] Milk replacer (Sprayfo Azul, Sloten do Brazil Ltd., Santos, SP, Brazil) was fed to both treatments diluted to 14.5% of solids.

### *9.2.4 Body measurements*

Animals were weighed once weekly before the morning liquid diet feeding, on a mechanical scale (ICS-300; Coimma Ltd., Dracena, SP, Brazil) until the final weight after weaning at 56 d of age. Body measures were collected every other week and included hearth girth (Bovitec, Sao Paulo, SP, Brazil), wither height, and hip-width (Carci, Sao Paulo, SP, Brazil).

### *9.2.5 Incidence of health disorders and treatments*

Health exams and interventions were performed daily on all calves by the same veterinarian to assess for Bovine Respiratory Disease, diarrhea, and navel ill according to the UW Calf Health Scoring chart. In brief, the following symptoms (e.g. 0 normal to 3 abnormal) were collected daily on each calf: presence of abnormal nasal discharge, coughing, ear tilt, eye discharge, a rectal temperature recorded by a digital thermometer (TS-101 Colors Techline digital, Techline Sao Paulo, SP, Brazil), and navel status, (McGuirk and Peek, 2014). Fecal consistency was scored on a scale of 0 to 3, where fecal score of 0 = normal consistency, 1 = semiformed or pasty, 2 = loose feces, and 3 = watery feces (McGuirk, 2008). A fecal score $\geq 2$ was considered a diarrhea bout when it occurred for more than 2 consecutive days (McGuirk, 2008). Oral rehydration solution (2 L/d of warm water with 50 g of dextrose, 20 g of sodium bicarbonate, and 10 g of sodium chloride) was offered between milk feedings, for every calf that presented diarrhea until the fecal consistency was $\leq 1$. Antibiotic therapy was administered only when the animal showed fever or/and depression symptoms, such recumbence and decreased or refused milk intake. A veterinarian made all the diagnoses and medicine interventions. Medication (antibiotics, anti-inflammatory, and other products) was administered only when the animal presented fever or/and symptoms of depression, such as decreased or refused milk intake and recumbence. Medication used, dosage, and duration of treatments were recorded for individual calves.

### *9.2.6 Blood sampling and analysis*

Blood samples were collected weekly, 2 h after the morning milk feeding via jugular vein puncture with evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The samples were collected in three different tubes containing either sodium fluoride as an anti-glycolytic and potassium EDTA as an anticoagulant to obtain plasma; K3 EDTA to obtain plasma; or a clot activator to obtain serum. An aliquot of blood from the K3 EDTA tube was used for capillary hematocrit using a microhematocrit centrifuge (Model SPIN

1000, MICROSPIN). Blood samples from K3 EDTA tube (0.02ml) were diluted with 4mL of Gower solution (12.5g sodium sulfate and 33.3 mL glacial acetic acid in 100mL on distilled water) for white cells preservation. The dilution was pippeted into the Neubauer chamber and observed under a microscope (400X, Bioval, PR, Brazil) for the total count of erythrocytes in μL. For the leukocytes count, blood samples (0.02 mL) were diluted with 0.4 mL of Turk solution (2mL of acetic acid, 1mL of gentian violet, 100 mL distilled water) pipetted into the Neubauer chamber and observed under a microscope (400X, Bioval, PR, Brazil). White blood cells samples were analyzed on week 2, 4, 8 to determine total and differential erythrocytes, leukocytes, lymphocytes, monocytes count and segmented neutrophil.

The remaining samples were centrifuged at 2,000 x g for 20 minutes at 4 ° C to obtain plasma and serum. The determination of selected blood metabolites was performed on an Automatic Biochemistry System - Model SBA - 200 (CELM, Barueri, SP, Brazil) using commercial kits (LABTEST Diagnóstica S.A., Lagoa Santa, MG, Brazil). The selected metabolites, protein [albumin (Ref. 19), creatinine (Ref. 35), TSP (Ref. 99)], carbohydrates [glucose (Ref. 85)], and urea (Ref. 104), were chosen to understand the effects of ß-glucan in the intermediate metabolism.

### *9.2.7Statistical Analysis*

A power analysis (R Studio) was conducted for health outcome measures (diarrhea). This power analysis showed that a total of 28 calves (n = 14 per treatment) was required at 80% power to detect an expected difference of 50% in diarrhea incidence between the treatment and control group. We powered this study at half-widths of 0.05, and an expected herd incidence of diarrhea at 80%. We included an additional 3 calves per treatment in case of loss to follow up. Thus, 17 calves per treatment were enrolled.

The experimental design used was a randomized block, considering birth date, birth weight, and gender as blocking factors. All data were tested for normal distribution by Shapiro-Wilk test and homogeneity of the variances using the Levene test. Performance, health and blood metabolites were analyzed as time-repeated measures using the MIXED procedure of SAS statistical package (version 9.4, SAS Institute Inc., Cary, NC) according to the following model:

$Yijk = \mu + Ti + Bj + eij + Wk + (TW)ik + Eijk,$

Where, Yijk = response variable; μ = general average; Ti = fixed effect of treatment (control or ß-glucans); Bj = random block effect; eij = residual error A; Wk = fixed age effect (days of life); (TW)ik = fixed effect of the diet × age interaction; Eijk =residual error B. The covariance matrices "compound symmetry, heterogeneous compound symmetry, autoregressive, autoregressive heterogeneous, unstructured, banded, ante-dependence, variance components, toeplitz, and heterogeneous toeplitz" were tested and defined according to the lowest value obtained for "Akaike's Information Criterion Corrected" (AICC) and the subject of the repeated measures used was animal (treatment).. For all the response variables, the means were obtained through the LSMEANS command. The effect of treatments was performed by test F in the analysis of variance. Significance was declared when $P \leq 0.05$ and a tendency when $0.05 \leq P \leq 0.08$. For data that were pooled to create a single measure, such as veterinary treatments/calf, PROC MIXED of the statistical package SAS (version 9.4, SAS Institute Inc., Cary, NC) was used according to the following model. The model included treatment as a fixed effect and block as a random effect.

$Yijk = \mu + Ti + Bj + Eij,$

Where Yijk = response variable; μ = general average; Ti = fixed effect of treatment (control or ß-glucans); Bj = random block effect; and Eij = residual error.

The effect of ß-glucans to ameliorate a diarrhea bout for the first 28 d was calculated with a logistic model (Proc Logistic) with gender, and block as fixed effects. Age was explored in the model, but not significant and removed. The effect of ß-glucans on odds of reducing the duration of days spent with an abnormal fecal score was calculated with a logistic model (Proc Logistic) with gender, and block as fixed effects.

## 9.3 RESULTS

All performance parameters were affected by age (P <0.06; Table 2), although only feed efficiency was affected by treatment and age interaction (control 0.24 ± 0.03; β-glucans 0.29 ± 0.03; P=0.04; Table 2; Figure 1), with higher FE for β-glucans supplemented animals only in the third and fifth week of age. We also found a tendency by treatment and age (P= 0.06; Table 2) for average daily gain in the third and fifth week of age. Despite better FE in β-glucans calves, supplementation was not associated with starter DMI (P <0.06; Table 2). Moreover, β-glucans calves weighed more at fifth, seventh and eighth (final) week (Table 2; Figure 2) as well as increased the final hip width (P= 0.05; Table 2).

**Table 2. Feed intake and performance of calves supplemented or not with β-glucans on milk replacer.**

| Item | Treatment | | | P value [2] | | |
|---|---|---|---|---|---|---|
| | Control | β-glucans[1] | SEM | T | A | T×A |
| Total DMI, g/d | 1090.9 | 1144.8 | 30.84 | 0.22 | <0.001 | 0.61 |
| Starter DMI, g/d | 251.8 | 311.1 | 31.11 | 0.17 | < 0.01 | 0.40 |
| ADG, kg | 0.276 | 0.328 | 0.04 | 0.10 | < 0.01 | 0.06 |
| Feed Efficiency | 0.24 | 0.29 | 0.03 | 0.08 | 0.05 | 0.04 |
| Initial BW, 0 d | 36.2 | 36.6 | 1.33 | 0.52 | - | - |
| Final BW, 56 d | 51.5 | 56.3 | 2.17 | 0.05 | - | - |
| Body measures, cm | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Birth withers height | 78.00 | 77.11 | 1.04 | 0.37 | - | - |
| Final withers height, 8 weeks | 83.95 | 85.20 | 1.41 | 0.49 | - | - |
| Average withers height | 81.2 | 80.8 | 1.00 | 0.62 | < 0.01 | 0.55 |
| Birth heart girth | 76.68 | 76.20 | 1.01 | 0.70 | - | - |
| Final heart girth, 8 weeks | 86.36 | 87.30 | 1.46 | 0.64 | - | - |
| Average heart girth | 81.0 | 81.2 | 1.01 | 0.81 | < 0.01 | 0.42 |
| Birth hip-width | 19.51 | 18.99 | 0.46 | 0.28 | - | - |
| Final hip-width, 8 weeks | 22.15 | 23.60 | 0.57 | 0.05 | - | - |
| Average hip-width | 20.6 | 21.0 | 0.38 | 0.33 | < 0.01 | 0.32 |

[1] β-glucans = 2g of β-glucans fed with 6L of the milk replacer.
[2] T= treatment effect; A= age effect; T x A= interaction treatment x age effect.

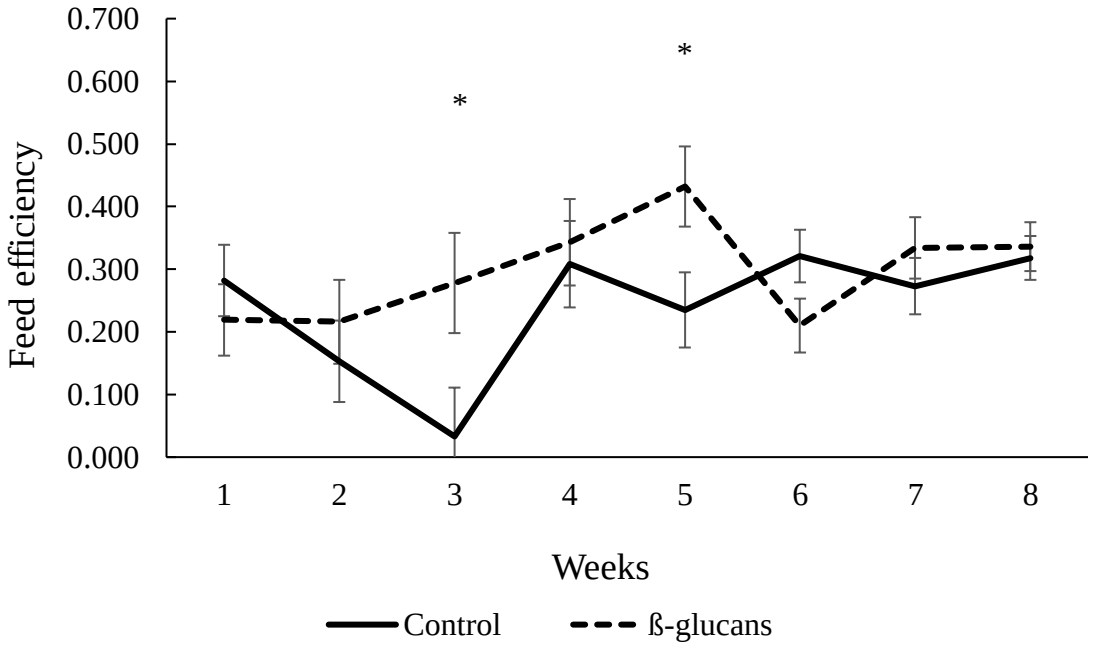


Figure 1. Feed efficiency of calves supplemented with or without β-glucans on milk replacer (2g of β-glucans fed with 6L of the MR), according to weeks of age. *Denotes difference with P<0.06.

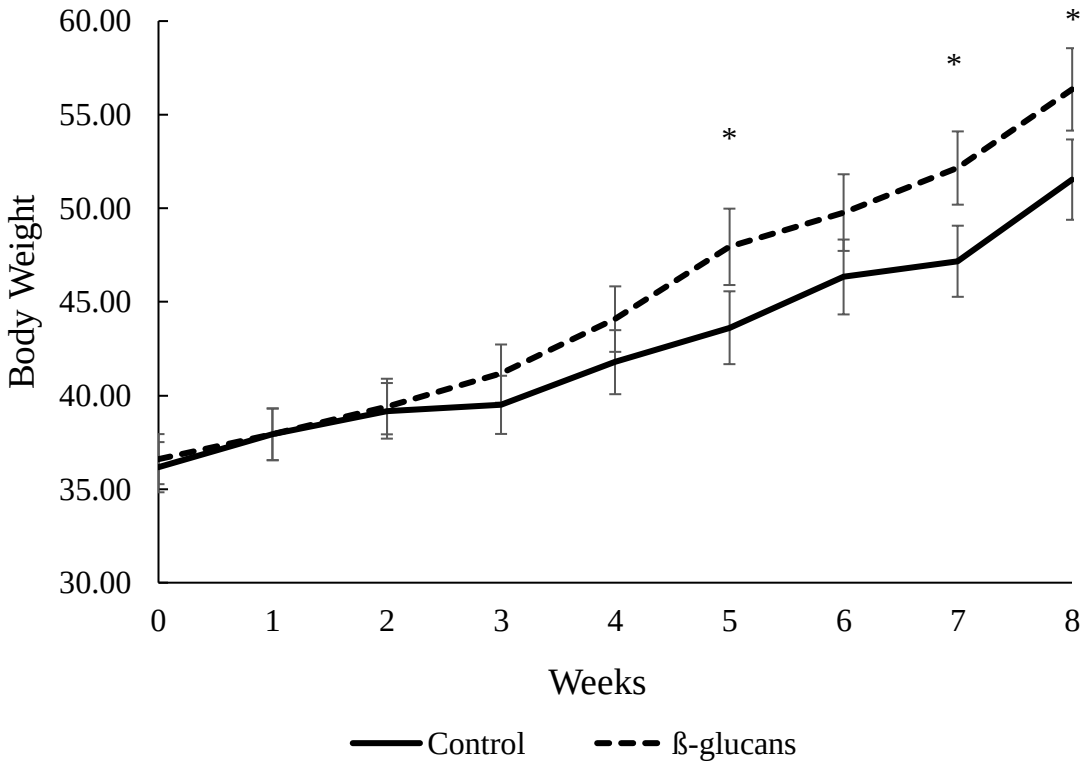


Figure 2. Body weight of calves supplemented with or without β-glucans on milk replacer (2g of β-glucans fed with 6L of the MR), according to weeks of age. *Denotes difference with P<0.06.

For diarrhea, control calves were more likely to have a diarrhea bout than β-glucans calves OR 22.46 (95% CI: 15.14-33.32; *P* <0.0001). Moreover, the days spent with an abnormal fecal score (diarrhea) was more likely for control calves compared to β-glucans OR 12.68 (95% CI: 8.84-18.19; *P* <0.0001).

Supplementation of ß-glucans had no effect on the following blood parameters: glucose, albumin, creatinine and total serum protein (Table 3; P > 0.06) during the preweaned phase. Although all blood parameters were affected by age (*P* <0.02). No differences were observed in the number of erythrocytes (control 7.1 $x10^6 \pm$ 0.27; β-glucans $6.9x10^3 \pm$ 0.27), leukocytes (control 7.4 $x10^3 \pm$ 0.37; β-glucans 6.3 $x10^3 \pm$ 0.37), lymphocytes (control 42.52 $x10^3 \pm$ 2.65; β-glucans 44.73 $x10^3 \pm$ 2.65), segmented

neutrophil (control 44.84 x10³ ± 3.0; β-glucans 44.58 x10³± 3.0; Table 3 and monocytes (control 11.99 x10³ ± 0.97; β-glucans 10.52 x10³ ± 0.97; Table 3) in both treatments during the preweaned period. However, the number of erythrocytes, leukocytes, lymphocytes and segmented neutrophil counts were affected by age (P > 0.01; Table 3).

**Table 3.** Blood parameters of calves supplemented or not with β-glucans during the preweaning phase.

| Item | Treatment | | | P value[2] | | |
|---|---|---|---|---|---|---|
| | Control | β-glucans[1] | SEM | T | A | T×A |
| Glucose, mg/dL | 85.85 | 86.32 | 1.97 | 0.87 | 0.01 | 0.35 |
| Total Serum Protein, g/dL | 5.42 | 5.44 | 0.10 | 0.87 | < 0.01 | 0.47 |
| Albumin, g/dL | 2.97 | 2.96 | 0.04 | 0.83 | 0.01 | 0.80 |
| Creatinine, mg/dL | 1.31 | 1.36 | 0.04 | 0.37 | < 0.01 | 0.79 |
| Erythrocytes, $10^6$ µL | 7.1 | 6.9 | 0.27 | 0.39 | <0.01 | 0.23 |
| Leukocytes, $10^6$ µL | 7.4 | 6.3 | 0.37 | 0.45 | <0.01 | 0.85 |
| Neutrophils, $10^3$ µL | 44.84 | 44.58 | 3.00 | 0.95 | <0.01 | 0.38 |
| Lympocytes, $10^3$µL | 42.52 | 44.73 | 2.65 | 0.56 | <0.01 | 0.44 |
| Monocytes, $10^3$ µL | 11.99 | 10.52 | 0.97 | 0.19 | 0.74 | 0.95 |

[1] β-glucans = 2g of β-glucans fed with 6L of the milk replacer.
[2] T= treatment effect; A= age effect; T x A= interaction treatment x age.

## 9.4 DISCUSSION

In the current study, total DMI and starter DMI were unaffected by ß-glucans supplementation, despite improved FE and better final weights than control calves. Supplemented calves showed a tendency to improve ADG compared to those not fed. Most of the β-glucans studies in calves are derived from yeast, while there is limited research on algae-derived β-glucans as fed in the present study. Nargeskhani et al. (2010) reported that the addition of 4g/ meal of mannanoligosaccharide β-glucans in the whole

milk improved DMI and ADG compared with control calves. In agreement, Gosh and Mehla (2012) observed improvements in starter intake, FE and BW gain of calves supplemented with 4g/d mannanoligosaccharide β-glucans at 21 days of age. Potential improvements on performance of young calves fed yeast ß-glucans might reflect increased starter intake, enhanced immune function, or reduced incidence of diseases (Magalhães et al., 2008). The administration of oral β-glucans reinforces the functions of intraepithelial lymphocytes present in the gastrointestinal tract increasing absorption (Tsukada et al.,2003), as a consequence, the supplementation may optimize gastrointestinal functionality and feed efficiency (Celi et al., 2017). Ma et al. (2014) reported that FE of calves supplemented with 0.075g/kg of yeast β-glucans was significantly improved when compared to those not fed. Those results are consistent with this study where supplementation with alga β-glucans improved FE at third and fifth week. Supporting FE improvement, supplemented calves showed a tendency to higher ADG at the same weeks of age. These results may be due to the physiological process of digestion and absorption that can contribute to improvement of feed efficiency as seen when mices were fed with ß-glucans (Kurashige et al., 1997). Previous studies have also shown improved performance when fed yeast ß-glucans as compared to controls in broilers and lambs (Haddad and Goussous, 2005; Gao et al., 2008). In this present study, dairy calves supplemented with alga ß-glucans in the milk replacer had a heavier BW at the fifth, seventh and eighth week when compared with the control calves. Brewer et al. (2014) did not record calf starter intake, FE or final BW but their data indicated that calves fed yeast had a greater BW gain on a percentage basis over the study period when compared with the control calves. On the other hand, Quigley et al. (1992) observed no differences in starter intake, BW gain, or FE in calves fed yeast culture for 12 wk and suggested that the high incidence of health problems and low starter intake might have

masked response to treatments. Although alga ß-glucans improved growth performance, withers height and heart girth were unaffected by supplementation, even though these measures are directly correlated with calf weight and development (Heinrichs et al., 2007). However, the supplementation positively affected hip width, demonstrating that the animals that presented greater weight also developed more during the preweaning phase (Heinrichs et al., 2003).

Products derived from *Saccharomyces cerevisiae* as ß-glucans were reported to improve enteric health and increase leukocyte function (Magalhães et al., 2008; Brewer et al., 2014, Harris et al., 2017) and macrophages activation in calves (Wojcik, 2014). The biological activity of β-glucans plays an important role in the activation of innate and adaptive immune systems. Calves are very susceptible to disease in the first weeks of life (Krehbiel and Zhang, 2016), and specific adaptive functions develop as calves age (Gelsinger and Heinrichs, 2017). Since calves are immune naive during the first weeks of life it is possible that β-glucans supplementation assisted immune modulation in our calves. This may be consistent with our results for FE improvement and a tendency to increase ADG at the third and fifth week of age. Besides that, our results for FE and ADG may be correlated with nutrient digestibility which may explain the tendency for higher ADG and the higher final weight. Research has shown that supplementation of β-glucans to calf diets resulted in an improved composition of the intestinal microflora, with decreased numbers of pathogenic E. coli and increased numbers of commensal Lactobacillus (Zhou et al., 2009). It is also possible that calves had lowered odds of diarrhea since β-glucans improve gut development. Previous studies have shown that the height of the intestinal villi and the villous heights to crypt depth ratio were improved in calves fed β-glucans (Zhou et al., 2009), and these variables were associated with a more efficient nutrient digestion in calves (Ma et al., 2014). However, unfortunately, we did

not measure gut digestibility changes as a function of β-glucans supplementation. Thus, we can only hypothesize that this β-glucans mechanism -played a role in the fecal health of our calves.

Indeed, we observed that β-glucans ameliorated the odds of a diarrhea bout. Nargeskhani et al. (2010) reported that calves fed 4g/ meal of mannanoligosaccharide β-glucans had lower fecal score than control treatment. Like our results, Kim et al. (2019) incorporating dietary supplementation of algae-derived β-glucans reduced incidence of diarrhea in weaned pigs infected with a pathogenic *E. coli*. Multiple pathogens are known or postulated to cause or contribute to calf diarrhea development as reviewed by (Cho and Yoon, 2014) such as bacteria, viruses, and protozoa (Foster et al., 2009). The immunomodulatory effect of β-glucans was effective in parasites in mices (Holbrook; Cook; Parker, 1981); fungi in mices (Meira et al., 1996); bacteria in rats (Liang et al., 1998) and virus in swine (Jung et al., 2004) infections. Furthermore, the β-glucans mechanisms have been shown to modulate immune response in vitro, with reduction in inflammatory response and oxidative stress (Jensen et al., 2007). This can be important in diseases in which inflammatory response exacerbates the deleterious effects of the illness such as in chronic processes or in infections associated with gut pathogens (Magalhaes et al., 2008). These effects are expected to improve gut health and might explain the benefits to fecal health scores and diarrhea observed in the current study when calves were fed β-glucans. However, this needs further investigation since calf diarrhea is a multifactorial disease and we did measure only fecal scores.

Selected blood parameters were within the normal range for dairy calves during the preweaning phase (Pogliani and Birgel Junior, 2007). Concentration of glucose in plasma was not affected by treatment, possibly because of a lack of effect on overall nutrient intake, even though we have postulated that digestion and absorption were

improved. Quigley et al. (1992) found no difference on glucose concentration in Jersey calves supplemented with β-glucans yeast in the first 12 wk of age. β-glucans supplementation also did not affect creatinine levels, and concentrations were in a safe range (Hammon et al., 2002), suggesting normal renal and liver functions in these calves. No treatment effects were observed for total serum protein and albumin. This observation is consistent with Nargeskhani et al. (2010) who reported similar total protein and albumin concentration in calves supplemented with mannanoligosaccharide β-glucans.

Mean values for the number of lymphocytes, segmented neutrophils and monocytes were not affected by treatment. However, mean values of these cells were within the reference intervals for preweaning health calves (Jezek et al., 2011). According to Brun-Hansen et al. (2006) the number of neutrophils decreased until the age of 6–8 weeks, whereas numbers of lymphocytes and monocytes increased according to the calf's age (Brun-Hansen et al., 2006). Which is consistent with our findings that the values of different blood variables in calves are changing with the age.

In summary, algae β-glucans improved calf FE, and final weights without negating starter intake. Similarly, we observed alga β-glucans to ameliorate odds of abnormal fecal scores during the first 56 days of life. We hypothesize this mechanism is multifactorial by reducing inflammation in the lower GIT and improving fat digestion for better lower GIT function. Moreover, β-glucans can be supplemented to calves with no effect on blood glucose, liver and kidney function. Calves white blood cells were unaffected by treatment. Supplementation of β-glucans added on 6 L/d of milk replacer may improve calf performance and fecal health.

## 9.5 CONCLUSION

Results of the present study indicated that milk replacer supplemented with algae β-

glucans seems to offer benefits to calves through improved feed efficiency and final BW in calves. Specifically, β- glucans tended to improve average daily gain through some weeks of calf's age. Furthermore, compared with control calves, the supplementation also reduces abnormal feces by reducing the risk of diarrhea during the first 56 d of life.

## ACKNOWLEDGEMENTS

The authors appreciate the partial financial support from Kemin South America. The authors acknowledge the assistance of the undergraduate students in the Experimental Calf Station "Evilasio de Camargo" (College of Agriculture "Luiz de Queiroz," University of Sao Paulo). The authors also appreciate the financial support provided by the Coordination for the Improvement of Higher Education Personnel (CAPES) as a scholarship for the first author.

## 9.6 REFERENCES

Akramiene, D., A. Kondrotas, J. Didziapetriene, and E. Kevelaitis. 2007. Effects of beta-glucans on the immune system. Medicina (Kaunas) 43(8):597-606.

Agarwal, N.; Kamra, D.N.; Chaudhary, L.C.; Agarwal, L.; Sahoo, A.; Pathak,N.N.2002.Microbial status and rumen enzyme profile of crossbred calves on different microbial feed additives. Letters in Applied Microbiol. 34: 29336.

Anadón, A., I. Ares, M. R.,Martínez-Larrañaga, and M. A Martínez. 2019. Prebiotics and Probiotics in Feed and Animal Health. Nutr Vet Med, 261–285. d https://doi.org/10.1007/978-3-030-04624-8_19.

Angelakis, E. 2017. Weight gain by gut microbiota manipulation in productive animals, Microb Pathog. 106: 162-163.

Baumann H, Gauldie J. The Acute Phase Response. Immunol Today. 1994;15(2):74-80. https://doi.org/10.1016/0167-5699(94)90137-6.

Brewer, M. T., K. L. Anderson, I. Yoon, M.F. Scott and S.A. Carlson. 2014. Amelioration of salmonellosis in pre-weaned dairy calves fed Saccharomyces cerevisiae fermentation products in feed and milk replacer. Vet Microbiol. 172(1-2): 248–255. https://doi.org/10.1016/j.vetmic.2014.05.026.

Broadway, P., J. Carroll and N. Sanchez. 2015. Live Yeast and Yeast Cell Wall Supplements Enhance Immune Function and Performance in Food-Producing Livestock: A Review .Microorganisms. 3(3):417427. https://doi.org/10.3390/microorganisms3030417.

Brun-Hansen, H. C., A. H.Kampen and A. Lund. 2006. Hematologic values in calves during the first 6 months of life. Veterinary Clinical Pathology. 35(2):182–187. https://doi.org/10.1111/j.1939-165x.2006.tb00111.x

Hawkins, A., K. Burdine, D. Amaral-Philips and J. H. Costa . 2019. An economic analysis of the costs associated with pre-weaning management strategies for dairy heifers. Animals 9(7):471. https://doi.org/10.3390/ani9070471.

Celi,P. A.J. Cowieson, F. Fru-Nji, R.E. Steinert, A.-M. Kluenter, V. Verlhac. 2017.Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production, Anim Feed Sci Tech. 234: 88-100. https://doi.org/10.1016/j.anifeedsci.2017.09.012.

Celi, P.; A.J. Cowieson, F. Fru-nji, A.M. Kluenter and V. Verlhac. 2016. The growing importance of defining gut "health" in animal nutrition and health. J Anim Sci.Vol. 94(5).

Cho Y.I. and K.J. Yoon. 2014.. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J Vet Sci. 15(1):1-17. https://doi.org/110.4142/jvs.2014.15.1.1.

Foster, D. M. and G.W. Smith. 2009. Pathophysiology of Diarrhea in Calves. Veterinary Clinics of North America: Food Animal Practice. 25(1): 13–36. https://doi.org/10.1016/j.cvfa.2008.10.013

Craig Gifford, C., S. Burns and M. Ward. 2018.Minimizing Weaning Stress on Calves. College of agricultural, consumer and environmental sciences. Cooperative Extension Service.

Davis, C.L. and J. K. DRACKLEY. 1998.. The development, nutrition and management of the young calf.

Deelen, S., T. Ollivett, D. Haines, and K. Leslie. 2014. Evaluation of a brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97: 3838-44. https://doi.org/10.3168/jds.2014-7939.

Durst, P., E. Ferry and D.Thompson. 2019. Veterinary Feed Directive – Year One in Review.Michigan State University Extension.

FAO. 2019 Environmental performance of feed additives in livestock supply chains. Livestock Environmental Assessment and Performance.

Fomenky, B. E., J. Chiquette, N. Bissonnette, G. Talbot, P.Y. Chouinard and E. M. Ibeagha-Awemu. 2017. Impact of Saccharomyces cerevisiae boulardii CNCMI-1079 and Lactobacillus acidophilus BT1386 on total lactobacilli population in the gastrointestinal tract and colon histomorphology of Holstein dairy calves. Anim Feed Sci and Tech 234:151–161. https://doi.org/10.1016/j.anifeedsci.2017.08.019

Haddad, S. G. and S. N. Goussous. 2005. Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. Anim Feed Sci and Tech. 118(3-4): 343–348. https://doi.org/10.1016/j.anifeedsci.2004.10.003

Gao, J., Zhang, H. J., Yu, S. H., Wu, S. G., Yoon, I., Quigley, J., … Qi, G. H. (2008). Effects of Yeast Culture in Broiler Diets on Performance and Immunomodulatory Functions. Poultry Science, 87(7), 1377–1384. https://doi.org/10.3382/ps.2007-00418

Godden, S. M., D. M Haines, and D. Hagman. 2009. Improving passive transfer of immunoglobulins in calves. I: dose effect of feeding a commercial colostrum replacer. J Dairy Sci. 92: 1750–1757. https://doi.org/10.3168/jds.2008-1846.

Hammon, H.M. and J.W. Blum. 2002. Feeding different amounts of colostrum or only milk replacer modifies receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domest. Anim. Endocrinol. 22:155-168. https://doi.org/10.1016/S0739-7240(02)00122-4.

Harris, T. L., Y. Liang, K. P. Sharon, M. D. Sellers , I. Yoon, M. F. Scott and M.A Ballou. 2017. Influence of Saccharomyces cerevisiae fermentation products, SmartCare in milk replacer and Original XPC in calf starter, on the performance and health of preweaned Holstein calves challenged with Salmonella enterica serotype Typhimurium. J Dairy Sci. 100(9): 7154–7164. https://doi.org/10.3168/jds.2016-12509.

Gelsinger, S.L., A.J. Heinrichs. 2017. A short review: the immune system of the dairy calf and the importance of colostrum IGG. J Dairy Vet Anim Res., 5(3):104–107.

Ghosh, S., and R.K. Mehla. 2012. Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. Trop Anim Health Prod. 44:617-622.

Heinrichs, A. J., H. N. Erb, G. W. Rogers, J. B. Cooper, and C. M. Jones. 2007.Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. Prev. Vet. Med. 78: 333–338. https://doi.org/10.1016/j.prevetmed.2006.11.002.

Holbrook, T.W., J.A. Cook and B.W. Parker. 1981a: Glucan-enhanced immunogenicity of killed erythrocyte stages of Plasmodium berghei. Infect. Immun. 32(2): 542 – 546.

Hulbert, L. E. and S.J. Moisá. 2016. Stress, immunity, and the management of calves 1. J Dairy Sci. 99(4): 3199–3216. https://doi.org/0.3168/jds.2015-10198

Jung, K.; Y. Ha , S. K. Ha, D.U. Han , D.W. Kim, K. W. Moon and C. Chae. 2004.Antiviral effect of Saccharomyces cerevisiae β-glucan to swine in uenza by increased production of interferon-γ and nitric oxide. J. Vet Med 51(2): 72-76.

Kaneene, J.B. and H.S. Hurd. 1990. The National Animal Health Monitoring System in Michigan. III. Cost estimates of selected dairy cattle diseases. Prey. Vet. Med., 8: 127-140.

Kim, S. Y., H.J. Song, Y.Y Lee, K.H. CHO and Y. K. Roh. 2006. Biomedical issues of dietary ber β- glucan.J Korean Med Sci. 21 (10):781-789.

Kim, M.H., J.K. Seo, C.H. Yun, S.J. Kang, J.Y. Ko, and J.K. Ha. 2011. Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. Animal. 5:953-960.

Kim K., A. Ehrlich, V.P erng, J. A. Chase, H. Raybould, X. Li, E. R. Atwill, R. Whelan, A. Sokale, Y. Liu. 2019. Algae-derived β-glucan enhanced gut health and immune responses of weaned pigs experimentally infected with a pathogenic E. coli. Anim Feed Sci and Tech. 248:114-125. https://doi.org/10.1016/j.anifeedsci.2018.12.004.

Krehbiel, C.; Zhang, G. 2016. Modulation of Gut Health in Beef and Dairy Cattle. Oklahoma State University.

Krehbiel, C.R.; S.R. Rust, G. Zhang and S.E. Gililand. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J Anim Sci. 81(2):120-132.

Kurashige, S., Y. Akuzawa and F. Endo. 1997. Immunipharmacol. Immunotoxicol, 19:175.

Liang, J., D. Melican, L. Cafro, G. Palace , L. Fisette , R. Armstro and M.L. Patchen.1998. Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int J Immunopharmac. 20(11): 595-614.

Jensen, G. S., A. N. Hart and A. G. Schauss. 2007. An antiinflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes in vitro. Nutrition Research. 27(6): 327–335. https://doi.org/10.1016/j.nutres.2007.04.008

López-Valencia,G.;Zapata-Ramírez,O.;Núñez-González,L.;Núñez-Benítez,V.;Landeros-López,H.;Lópezsoto,M.;Barreras,A.;González,V.;Estradaângulo,A.;Zinn,R.;Plascencia, A. 2017. Effective use of probiotic-glyconutriet combination as an adjuvant to antibiotic therapy for diarrhea in rearing dairy calves. Turk J Vet Anim Sci.

Ma J, A.M. Shah, Y. Shao, Z.Wang, H. Zou and Kun Kang.2020.Dietary supplementation of yeast cell wall improves the gastrointestinal development of weaned calves. Anim Nutr. 6(3) 231–378 . https://doi.org/10.1016/j.aninu.2020.06.003.

Ma, T., Y. Tu, N. Zhang, K. Deng,Z. Zhou, Q. Yun and Q. Diao. 2014.Effects of dietary yeast β-glucan on nutrient digestibility and serum profiles in pre-ruminant Holstein calves. J Int Agri. 14: 749-757.

Magalhães, V. J. A., F. Susca, F. S. Lima, A. F. Branco, I. Yoon and J. E. P. Santos. 2008. Effect of Feeding Yeast Culture on Performance, Health, and Immunocompetence of Dairy Calves. J Dairy Sci. 91(4): 1497–1509. https://doi.org/10.3168/jds.2007-0582

Mcguirk, S. and S. F. Peek 2014. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. Anim. Health Res. Reviews. 15:1-3. https://doi.org/10.1017/S1466252314000267.

Meira, D. A.; Pereira, P. C. M.; Marcondes- Machado, J.; Mendes, R. P.; Barraviera, B.; Pellegrino, J. R. J.; Rezkallah-Iwasso, M. T.; Peraçoli, M. T. S.; Castilho,L. M.; Thomzaini, I.; Silva, C. L.; Foss, N. T.; Curri, P. R. 1996. The use of glucan as immunostimulant in the treatment of paracoccidioidomycosis. Amer J Trop Med Hyg. 55(5):496-503.

Mertens, D.R. 1997.Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci.80:1463-1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.

Nargeskhani A.,N. Dabiri ,S. Esmaeilkhanian , A. M. Mohammad and M. Bojarpour. 2010. Effects of Mannanoligosaccharide–β Glucan or Antibiotics on Health and Performance of Dairy Calves. Animal Nutr and Feed Tech. 10(1):29-36.

Phillips F.C., G.S. Jensen,L. Showman,R. Tonda, G. Horst,R. Levine . 2019. Particulate and solubilized β-glucan and non-β-glucan fractions of *Euglena gracilis* induce pro-and anti-inflammatory innate immune cell responses and exhibit antioxidant properties. *J Inflamm Res*. 12:49-64. https://doi.org/10.2147/JIR.S191824.

Pogliani F.C. and E.H. Birgel Júnior. 2007. Reference values of the lipid profile of Holstein cattle bred in state of São Paulo. Braz. J. Vet. Res. Anim. Sci. 44: 373-383. https://doi.org/10.11606/issn.1678-4456.bjvras.2007.26621.

Quigley, J. D., L. B. Wallis, H. H. Dowlen and R. N. Heitmann .1992. Sodium Bicarbonate and Yeast Culture Effects on Ruminal Fermentation, Growth, and

Intake in Dairy Calves. J Dairy Sci. 75(12): 3531–3538. https://doi.org/10.3168/jds.s0022-0302(92)78129-6.

Rahar S, G. Swami, N. Nagpal , M.A. Nagpal MA and G.S. Singh. 2011.Preparation, characterization, and biological properties of β-glucans. J Adv Pharm Technol Res. 2(2):94-103. https://doi.org/10.4103/2231-4040.82953

Shen Y.B., X.S. Piao, S.W. Kim, L. Wang, P. Liu I. Yoon and Y.G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs J. Anim. Sci., 87 2009, pp. 2614-2624. https://doi.org/10.2527/jas.2008-1512

Tsukada, C. , H. Yokoyama, C. MIYAJI, Y. ISHIMOTO, H. KAWAMURA and T. ABO. 2003. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of β- glucan. Cellular Immunology, 221(1):1-5.

Urie, N. J., J. E. Lombard, C. B. Shivley, C. A. Kopral, A. E. Adams, T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J Dairy Sci.

Wiles, P. G., I. K. Gray, and R. C. Kissling. 1998. Routine analysis of protein by Kjeldahl and Dumas methods: Review and interlabora- tory study using dairy products. J. AOAC Int. 81:620–632.

Windeyer Mc, K.E. Leslie, S.M. Godden, D.C. Hodgins, K.D. Lissemore and S.J. LeBlanc. 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev Vet Med. 113(2):231-40. https://doi.org/10.1016/j.prevetmed.2013.10.019.

Wojcik, R. 2014. The effect of Leiber Beta-S (1,3- 1,6-β-D-glucan) on the phagocytic activity and oxidative metabolism of peripheral blood granulocytes and monocytes in calves. Acta vet. Brno 83:347-354

Xiao, J. X., G. M. Alugongo, R.Chung, S.Z. Dong , S.L. Li, I. Yoon and Z. J. Cao. 2016. Effects of Saccharomyces cerevisiae fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community. J Dairy Sci. 99(7):5401–5412. https://doi.org/10.3168/jds.2015-10563 .

Zanton, G. I., and A. J. Heinrichs. 2005. Meta-Analysis to Assess Effect of Prepubertal Average Daily Gain of Holstein Heifers on First-Lactation Production. J Dairy Sci. 88(11):3860–3867. https://doi.org/10.3168/jds.s0022-0302(05)73071-x .

Zhang, H., Y. Wang, Y. Chang, H. Luo, L. F. Brito, Y. Dong, R. Shi, Y. Wang, G. Dong, L. Liu. 2019 . Mortality-Culling Rates of Dairy Calves and Replacement Heifers and Its Risk Factors in Holstein Cattle. Anim. 9(10): 730. https://doi.org/10.3390/ani9100730 .

Zhou, Y., Diao, Q., TU, Y. and Yun Q. 2009 Effects of yeast β-glucan on gastrointestinal development in early-weaning calves. Chinese J of Anim Nutr. 21:846-852.

Zhou, Y., Diao, Q., Tu, Y. and Yun Q. 2009. Effects of yeast β-glucan on gastrointestinal development in early-weaning calves. Chinese Journal of Animal Nutrition 21:846-852.