ABSTRACT

Studies have shown that β-glucans extracted from the cell wall of cereals, algae, and yeasts have been associated with improved immune function. However, it is unknown whether algae β-glucan supplementation affects the performance, blood metabolites, or cell counts of immune cells in dairy calves. The objective of this randomized clinical trial was to evaluate whether supplementation of β-glucans to milk replacer in dairy calves fed 6 L/d improved growth performance and fecal status and altered the blood metabolite profile. In this trial, we enrolled Holstein calves (n = 34) at birth (body weight 36.38 ± 1.33 kg; mean ± standard deviation) to receive, from 1 d of age, either 2 g/d algae β-glucans mixed into 6 L/d of milk replacer (22.4% crude protein and 16.2% fat) or an unsupplemented milk replacer (control). The calves were blocked in pairs according to birth weight, sex, and date of birth (up to 5 d difference). Calves were housed individually, and calf starter (24.7% crude protein and 13.9% neutral detergent fiber) was offered ad libitum based on orts of the previous day until 56 d of age (end of the trial). Body weight was measured weekly, and health checks and daily fecal consistency were evaluated daily in every calf by the same observer. Calves with 2 consecutive days of loose feces that sifted through bedding were considered diarrhea positive. We used a linear mixed effects model to evaluate the effects of β-glucan supplementation fed during the preweaning period on performance (average daily gain), final weight, feed efficiency (FE), white blood cell count, and selected blood metabolites, repeated by time. A generalized linear mixed effects model was also run to evaluate the likelihood of a diarrhea bout in the first 28 d of life, controlling for the calf as the subject with a logistic distribution. We included age, serum total protein at 48 h, and birth weight as covariates. At 56 d, β-glucan-supplemented calves weighed more than control calves (56.3 vs. 51.5 kg). Treatment had no effect on total starter intake, but there was a treatment by age interaction for FE, with greater FE for β-glucan-supplemented calves in wk 3 and 5 of age. There was only a tendency for average daily gain to be greater in supplemented calves than in control calves for the duration of the study. Furthermore, control calves had 14.66 [95% confidence interval (95% CI): 9.87–21.77] times greater odds of having a diarrhea bout than β-glucan-supplemented calves. Control calves had 12.70 (95% CI: 8.82–18.28) times greater odds of having an additional day with an abnormal fecal score compared with β-glucan-supplemented calves, suggesting that supplementation ameliorated diarrhea severity. We found no association of treatment with concentrations of serum total protein, albumin, creatinine, or glucose during the preweaning period. Our findings suggest that dietary supplementation of 2 g/d of algae β-glucans to milk replacer improved fecal status and may affect growth, as evidenced by a higher weaning weight, compared with control calves. Future studies should explore the effect of algae β-glucans on lower-gut physiology and digestibility in dairy calves.

Key words: calf, diarrhea, immunity, Euglena gracilis

INTRODUCTION

β-Glucans are polysaccharides commonly derived from pathogenic bacteria, algae, and yeast (Akramiene et al., 2007), which have specific glycosidic linkages, such as β-(1,3), β-(1,4), or β-(1,6) (Han et al., 2020). β-Glucans, mainly those rich in β-(1,3) linkages, have been observed to be immune modulators in livestock (Broadway et al., 2015); these β-glucans are found in the cells of potentially pathogenic organisms (Horst et al., 2019).

There are 2 common sources of bioavailable β-glucans: one is algae such as Euglena gracilis; these algae were shown to have highly water soluble portions in an in
vitro study (Phillips et al., 2019). Recently, supplementation of algae β-glucans to calves promoted greater abundance of the *Alloprevotella* and *Holdemanella* genera in feces, suggesting that feeding algae β-glucans to calves may positively alter the ratio of commensal bacteria in the lower gut (Virgínio Junior et al., 2021). However, to our knowledge, no study has investigated the effect of algae-derived β-glucans on calf health and performance, and this warrants investigation. The other, more common source of β-glucans is yeast (Magalhães et al., 2008; Broadway et al., 2015), and many researchers have observed the positive effects of its supplementation on calf health and productivity. For example, Zhou et al. (2009) showed that feeding yeast-derived β-glucans to dairy calves for 56 d in the solid diet (at 0.075 g/kg per day) was associated with lower counts (cfu) of pathogenic bacteria (e.g., *Escherichia coli*) in the lower gut and a higher ratio of commensal bacteria (e.g., *Lactobacillus*). This suggests that yeast-derived β-glucans fed during the first few weeks of life can improve the microbiota and gut stability of dairy calves. This is important because, as reviewed by Virgínio Júnior and Bittar (2021), an unstable microbiota associated with dysbiosis in preweaning calves is related to gastrointestinal disorders and lower nutrient absorption and, consequently, lesser animal performance. Xiao et al. (2016) observed that feeding yeast-derived β-glucans to calves (1 g/d) in the milk was associated with improved gut and ruminal development at 56 d of age, including increased villus height:crypt depth ratio of the small intestine and enhanced length and width of papillae in the ruminal epithelium. Ma et al. (2015) showed similar findings: feeding yeast-derived β-glucans to calves (0.075 g/kg) improved apparent nutrient digestibility, promoted intestinal development, and enhanced immunity by increasing the concentration of immunoglobulins and stimulating alkaline phosphatase in the serum of dairy calves. These studies suggest that yeast-derived β-glucans likely have positive effects on gut health, immunity, and ruminal development in calves.

Yeast-derived β-glucans have also been associated with improved immune response and productivity in cattle, rats, mice, and swine (Luo et al., 2019; de Vries et al., 2020). The supplementation of β-glucans to ruminants has improved growth performance (Angelakis, 2017) and metabolic profile (Chiofalo et al., 2004). Researchers have also observed that feeding yeast-derived β-glucans downregulated oxidation processes and activated genes associated with immune system activation (e.g., tumor necrosis factor-α and IL-1β) in an in vitro study, suggesting germicidal effects (Kim et al., 2006). This may explain why yeast-derived β-glucans have immunomodulatory effects in many species; feeding β-glucans was associated with the reduced proliferation of *Staphylococcus aureus* bacteria in rats (Liang et al., 1998), a lower risk of swine influenza virus in pigs (Jung et al., 2004), a reduced parasite load of *Plasmodium berghei* in mice (Holbrook et al., 1981), and lower levels of paracoccidioidomycosis in mice (Meira et al., 1996). The literature suggests that feeding yeast-derived β-glucans to ruminants, rats, mice, and swine alters immune response and may improve performance.

Feeding yeast-derived β-glucans may also improve calf productivity. Feeding yeast-derived β-glucans (e.g., at 4 g/d) in milk to preweaning calves was associated with higher ADG, increased feed intake, and improved feed conversion efficiency at 21 d of age (Ghosh and Mehla, 2012). Kim et al. (2020) also observed benefits to weaned calves when feeding hydrolyzed β-glucans (e.g., 0.2% in solid feed) to dairy calves, including improved BW gains, lowered circulating cortisol, and improved immune parameters in the blood.

Although the benefits of yeast-derived β-glucans are well established, it is unknown whether feeding algae-derived β-glucans to calves in the liquid diet improves immunity and performance. Considering the differences in β-glucan sources, their main glycosidic linkages, branching frequency, solubility, and dosage, research is warranted to examine the effects of supplementation of algae-derived β-glucans and its effects on performance, health, and metabolism in calves. *Euglena gracilis* is an alga that contains more than 50% 1,3-β-glucan, which is bioavailable without extraction because these algal cells are highly digestible. This study aimed to evaluate the effects of algae-derived β-glucans fed in milk replacer (MR) on calf performance (ADG, BW, and feed efficiency, FE), fecal status (odds of a diarrhea bout), cell counts (erythrocytes, leukocytes, neutrophils, lymphocytes, and monocytes), and selected blood metabolites (glucose, serum total protein, albumin, and creatinine) in preweaning Holstein dairy calves. We hypothesized that feeding algae β-glucans to calves would improve performance, health, and immune responses during the preweaning period.

**MATERIALS AND METHODS**

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 São Paulo (Piracicaba, Brazil). The Animal Research Ethics Committee of the Luiz de Queiroz College of Agriculture/University of São Paulo approved all animal procedures in this study (protocol no. 2019–11). The average temperature was 22.1°C (3.6–36.8°C), average humidity was 69% (14.8–99.4%), and average rainfall was 3.2 mm/d.
Experimental Design and Treatments

A total of 34 newborn (10 females and 24 males) Holstein dairy calves were enrolled in this randomized clinical trial (mean ± SD; BW = 36.38 ± 1.3 kg). All calves were separated from their dams at birth, transferred to the experimental facility, weighed, and bottle-fed a volume corresponding to 10% of birth weight (Godden, 2008) of high-quality nonpooled colostrum, previously measured for quality (>22% Brix) and stored in a freezer, within the first 6 h of life (Godden, 2008). A blood sample was collected from the jugular vein at 48 h after colostrum feeding to ensure transfer of passive immunity status by evaluating serum total protein (STP). All calves enrolled in this study exhibited transfer of passive immunity using a threshold of 5.5 g/dL (Deelen et al., 2014), and STP averaged (mean ± SD) 6.30 ± 0.20 g/dL. The calves were blocked in pairs by date of birth, birth BW, and sex, resulting in 17 blocks, and were randomly assigned to receive either algae-derived β-glucans (mixed in the 2 MR feedings at a rate of 2 g/d; Aleta, Kemin Industries Inc.), or a MR control (no supplementation). Treatment blinding was not possible because the algae-derived β-glucans were mixed with the MR just before feeding.

Housing, Management, and Feeding

All calves were housed in individual wood shelters (1.35 m high, 1 m wide, and 1.45 m deep) and tethered by a chain. Buckets with water and calf starter were available in each shelter. The shelters were distributed in a trimmed grassy field and moved daily. Calves were bucket-fed 6 L/d of a commercial milk replacer (14% solids, 22.46% CP, 16.20% fat; Sprayfo Azul, Sloten do Brazil Ltd.) split into 2 feedings (0700 and 1700 h) for the 56-d preweaning period. Feed refusals were measured to calculate feed intake per day. A precision scale (AUY220, Shimadzu Corp.) was used to weigh the amount of β-glucans per feeding. Every day, 2 g (1 g/meal) of Aleta (>50% 1,3-β-glucan) was added and mixed into the diluted MR (3 L/meal) of the treatment group before feeding.

Water and a commercial solid feed calf starter (24.6% CP and 13.9% NDF; Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda.) were available for ad libitum intake throughout the study. The solid feed was offered every morning, just after MR feeding. Calves were offered solid feed such that at least 200 g/d of orts remained per day to ensure ad libitum intake. Calves were enrolled in this study during the preweaning period, from d 1 to 56 of age. Weaning occurred gradually after the trial, from d 57 to 59.

Feed Analysis

Samples of MR and starter were collected weekly for analysis (Table 1). Dry matter 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 h (AOAC International, 2012; method 942.05). Ether extract was determined using petroleum ether (AOAC International, 2012; 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), using an N analyzer (FP-528; Leco). 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 calf starter and MR were estimated 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 calf starter and MR were estimated according to the following equation: NFC (%) = 100% − (% NDF + % CP + % fat + % ash), according to Mertens (1997). Dry matter of MR and calf starter were used for total DMI and calculation of the gain: feed ratio.

Body Measurements

Animals were weighed at birth and weekly until the end of the trial (8 wk of age), before the morning milk feeding on a mechanical scale (ICS-300; Coimma Ltd.). Body measurements were collected every other week and included heart girth (Bovitec), withers height, and hip width (Carci).

Health Exams

Health exams and interventions were performed daily on all calves by the same trained veterinarian after the morning feeding to assess for bovine respiratory disease (BRD), diarrhea, and umbilical infection. Signs of BRD were scored daily on each calf according to McGuirk and Peek (2014). Briefly, abnormal nasal

<table>
<thead>
<tr>
<th>Item</th>
<th>Calf starter¹</th>
<th>Milk replacer²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>89.3</td>
<td>96.1</td>
</tr>
<tr>
<td>Ash</td>
<td>9.6</td>
<td>8.8</td>
</tr>
<tr>
<td>CP</td>
<td>24.7</td>
<td>22.4</td>
</tr>
<tr>
<td>Crude fat</td>
<td>5.2</td>
<td>16.2</td>
</tr>
<tr>
<td>NDF</td>
<td>13.9</td>
<td>0.06</td>
</tr>
<tr>
<td>ADF</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>NFC</td>
<td>46.6</td>
<td>52.2</td>
</tr>
</tbody>
</table>

¹Commercial calf starter (Ração Bezerra AgMilk Agroceres Multimix Nutrição Animal Ltda.).
²Milk replacer (Sprayfo Azul, Sloten do Brazil Ltd.) fed to both treatments diluted to 14% of solids.
discharge, coughing, ear tilt, eye discharge, and an elevated rectal temperature (TS-101 Colors Techline digital, Techline São Paulo) were recorded. The presence of at least 2 categories of abnormal scores was required for a diagnosis of BRD (McGuirk and Peek, 2014). Umbilical health was checked but no infections were detected. Diarrhea was diagnosed based on fecal consistency. All calves were rectally stimulated to defecate, and fecal consistency was scored on a scale of 0 to 3, where 0 = normal consistency, 1 = semiformal or pasty, 2 = loose feces, and 3 = watery feces (Renaud et al., 2020). A fecal score ≥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 with diarrhea until the fecal consistency was ≤1. Antimicrobial therapy was administered only when the animal showed fever or depression symptoms, such as recumbence, and decreased or refused milk intake. The same veterinarian made all diagnoses and administered all interventions. All calves with a positive diarrhea bout received antimicrobial intervention on the day of initial diagnosis; sulfamethoxazole and trimethoprim were administered intramuscularly with a dosage calculated by BW (1 mL/15 kg; Trissulfim, Ourofino Animal Health) according to the herd veterinarian protocol. For BRD, florfenicol + flunixin meglumine was administered intramuscularly with the dosage calculated by BW (1 mL/15 kg florfenicol; Florkem, Ceva Sante Animale; 1 mL/45 kg flunixin meglumine; Flumax, J.A. Saúde Animal) according to the herd veterinarian protocol. Medications used, dosage, and duration of treatments were recorded for individual calves.

Metabolites

The remaining blood samples were centrifuged at 2,000 × g for 20 min at 4°C to obtain plasma and serum. Selected blood metabolites were analyzed on an Automatic Biochemistry System (model SBA-200; CELM) using commercial kits (Labtest Diagnóstica S.A.). The selected metabolites, albumin, creatinine, STP, and glucose, were chosen to evaluate the effects of β-glucans on blood metabolites in calves.

Cell Count

The techniques used for the blood cell count followed the recommendations of Bain et al. (2016). Blood samples from the K3EDTA tube (0.02 mL) were diluted with 4 mL of Gower solution (12.5 g of sodium sulfate and 33.3 mL of glacial acetic acid in 100 mL of distilled water) for white cell preservation. The dilution was pipetted into the Neubauer chamber and observed under a microscope (400×, Bioval) to determine the total count of erythrocytes in 1 μL. For the leukocyte count, blood samples (0.02 mL) were diluted with 0.4 mL of Turk solution (2 mL of acetic acid, 1 mL of gentian violet, 100 mL of distilled water) pipetted into the Neubauer chamber and observed under a microscope (400×, Bioval). Samples were analyzed for white blood cell count when calves were 2, 4, and 8 wk of age to determine total and differential counts of erythrocytes, leukocytes, lymphocytes, monocytes, and segmented neutrophils.

Statistical Analysis

A power analysis for ADG (Proc Power) was calculated using SAS version 9.4 (SAS Institute Inc.). We anticipated ADG for control calves in this study to be 0.28 kg/d with a variation of 0.03 kg/d based on growth for other control calves at this research station (Reis et al., 2021). We anticipated the treated calves to have an ADG benefit from β-glucans, with an expected minimum mean growth to detect a difference of 0.24 kg/d. Thus, based on 90% power, and an α of 0.05, we required 9 calves per treatment to detect a difference in mean ADG. Based on the availability of animals, 17 calves per treatment were enrolled.

All other statistical procedures were run in SAS 9.4 (SAS Institute Inc.). The experimental design used was a randomized block, considering birth date, birth weight, and sex as blocking factors. All data were tested for normal distribution by Shapiro-Wilk test, for homogeneity of the variances using the Levene
test, and by visually assessing residuals from linear mixed effects models (where applicable). The effects of β-glucan supplementation on calf performance and blood metabolites were analyzed using linear mixed effects models (Proc Mixed) in SAS 9.4 (SAS Institute Inc.) using measures over time (weeks) as a repeated measure according to the following model:

\[ Y_{ijk} = \mu + T_i + B_j + e_{ij} + W_k + (TW)_{ik} + E_{ijk}, \]

where \( Y_{ijk} \) = response variable, \( \mu \) = general average, \( T_i \) = fixed effect of treatment (control or β-glucans), \( B_j \) = random block effect, \( e_{ij} \) = residual error A; \( W_k \) = fixed age effect (days of life); \( (TW)_{ik} \) = fixed effect of the diet × age interaction; and \( E_{ijk} \) = 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 response variables, the means were obtained through the LSMEANS command. The treatment means were compared using the Tukey-Kramer adjustment test. Significance was declared at \( P \leq 0.05 \) and a tendency at 0.05 < \( P \leq 0.08 \).

We were interested in whether supplementation of β-glucans in the diet was associated with the likelihood of a calf having an abnormal fecal score during the first 28 d of life. Each day for each calf was characterized as a binary outcome variable of abnormal fecal score ≥2 to determine whether the study treatment could improve a calf’s daily fecal score. We used a generalized linear mixed effects model (Proc Glimmix) using a logistic distribution, with the calf as the subject, and tested for the effects of age, birth weight, and STP at 48 h of age as covariates; odds ratios are reported. We also assessed whether supplementation of β-glucans to the diet was associated with the likelihood of a diarrhea bout, with the binary outcome variable in the model being a calf experiencing ≥2 consecutive days of abnormal fecal score or not using the same model structure to that above.

**RESULTS**

**Performance**

All performance parameters were affected by age (\( P < 0.06; \) Table 2), although only FE showed a treatment × age interaction (control FE: 0.24 ± 0.03; β-glucan FE: 0.29 ± 0.03; \( P = 0.04; \) Table 2; Figure 1). Specifically, a higher FE for β-glucan-supplemented calves was observed in wk 3 and 5 of age. We also found a tendency for a treatment × age interaction (\( P = 0.06; \) Table 2) for ADG in wk 3 and 5 of age. Despite better FE in β-glucan-supplemented calves, supplementation was not associated with starter DMI or body measurements (Table 2; \( P > 0.10 \)). However, β-glucan-supplemented calves were heavier at 56 d (\( P = 0.05; \) Table 2).

**Health**

For disease, 2 control calves died due to pneumonia at 23 and 27 d of age, but no other cases of BRD were observed. Therefore, all calves were included in our analysis of the association of treatment with likelihood of abnormal fecal score and likelihood of a diarrhea

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Table 2. Evaluation of the effect of β-glucan supplementation on calf starter intake and performance (LSM ± SEM) of preweaning calves compared with unsupplemented controls (17 calves per treatment)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control  β-Glucan²</td>
<td>SEM</td>
</tr>
<tr>
<td>Total DMI³ (g/d)</td>
<td>1,090.8</td>
<td>1,144.8</td>
</tr>
<tr>
<td>Starter DMI (g/d)</td>
<td>251.86</td>
<td>311.13</td>
</tr>
<tr>
<td>ADG (kg)</td>
<td>0.276</td>
<td>0.328</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.238</td>
<td>0.290</td>
</tr>
<tr>
<td>BW (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial, 0 d</td>
<td>36.17</td>
<td>36.60</td>
</tr>
<tr>
<td>Final, 56 d</td>
<td>51.53</td>
<td>56.35</td>
</tr>
<tr>
<td>Body measurement (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withers height</td>
<td>81.24</td>
<td>80.80</td>
</tr>
<tr>
<td>Heart girth</td>
<td>80.97</td>
<td>81.24</td>
</tr>
<tr>
<td>Hip width</td>
<td>20.63</td>
<td>20.97</td>
</tr>
</tbody>
</table>

1T = treatment effect; A = age effect; T × A = interaction of treatment × age effect.
2β-Glucan = 2 g/d of algae-derived β-glucan fed with 6 L of milk replacer.
3Starter and milk replacer DMI.
bout for the first 28 d of life. In total, 88.2% (15/17) of β-glucan-supplemented calves had a bout of diarrhea, whereas 100% (17/17) of control calves had at least one bout of diarrhea. However, we did not power this study for the likelihood of treatment to affect diarrhea incidence in the calves. Instead, we determined whether treatment was associated with the likelihood of an abnormal fecal score in the diarrhea risk period or the first 28 d, and whether treatment was associated with the likelihood of a diarrhea bout to determine whether disease severity was associated with treatment. We defined a diarrhea bout as a calf who spent at least 2 consecutive days with an abnormal fecal score (≥2).

For control calves, 56.7% (270/476) of calf-days were spent with an abnormal fecal score, whereas for β-glucan-supplemented calves, 9.5% (45/476) of calf-days were spent with an abnormal fecal score. At 14.5 d of age, a control calf had greater odds (odds ratio 12.70; 95% CI: 8.82–18.28; \( P < 0.001 \)) for having a day with an abnormal fecal score compared with β-glucan-supplemented calves. Birth weight and STP were not associated with odds of abnormal fecal score and were not included in the model (\( P > 0.10 \)).

Furthermore, the duration of diarrhea or presence of a diarrhea bout was associated with treatment. Control calves spent 54.5% of time on study (259/476 consecutive days) with a diarrhea bout compared with β-glucan-supplemented calves, which spent 8% of time on study (39/476 consecutive days) with a diarrhea bout. At 14.5 d of age and a STP of 6.30, a control calf had greater odds (odds ratio 14.66; 95% CI: 9.87–21.77; \( P < 0.001 \)) of having a diarrheal bout compared with a β-glucan-supplemented calf. Birth weight was not associated with odds of diarrhea bout and was not included in the model (\( P > 0.10 \)).

### Blood Metabolites

Supplementation of β-glucans had no effect on the following blood parameters: glucose, albumin, creatinine, and STP (Table 3) during the preweaning phase, although all blood parameters were affected by age (\( P < 0.02 \)).

#### Cell Count

No differences were observed in the number (count/μL) of erythrocytes (control group: 7.1 × 10⁶ ± 0.27; β-glucan group: 6.9 × 10⁴ ± 0.27), leukocytes (control group: 7.4 × 10³ ± 0.37; β-glucan group: 6.3 × 10³ ± 0.37), lymphocytes (control group: 42.52 × 10³ ± 2.65; β-glucan group: 44.73 × 10³ ± 2.65), segmented neutrophils (control group: 44.84 × 10³ ± 3.0; β-glucan group: 44.58 × 10³ ± 3.0), and monocytes (control group: 11.99 × 10³ ± 0.97; β-glucan group: 10.52 × 10³ ± 0.97) between groups during the preweaning period (Table 4).

### DISCUSSION

Total DMI and starter DMI were unaffected by β-glucan supplementation, despite the higher final BW compared with control calves, as well as a tendency for increased ADG. Furthermore, we found that β-glucan-
supplemented calves had improved fecal status but that metabolic and immune indicators remained unaffected. Thus, we suggest that supplementation of algae-sourced β-glucans to calves may have beneficial effects on intestinal health and resulted in heavier calves when offered in MR for 56 d, although this supplementation did not alter circulating immune parameters or blood metabolites.

We supplemented algae-derived β-glucans to calves on this study, whereas, to date, researchers have only evaluated the effect of yeast-derived β-glucans on calf performance (Nargheskani et al., 2010; Ghosh and Mehla et al., 2012; Ma et al., 2015). The administration of β-glucans to calves reinforces the functions of intraepithelial lymphocytes present in the gastrointestinal tract, increasing absorption (Tsukada et al., 2003). As a consequence, β-glucans supplemented to calves may optimize gastrointestinal function and feed efficiency compared with controls (Celi et al., 2017). For example, Nargheskani et al. (2010) reported that the addition of 4 g/meal of mannanoligosaccharide β-glucans in whole milk improved DMI and ADG compared with unsupplemented control calves. Similarly, Ghosh and Mehla (2012) observed improvements in starter intake, FE, and BW gain of calves supplemented with 4 g/d mannanoligosaccharide β-glucans at 21 d of age. Ma et al. (2015) observed that calves had higher FE when fed 0.075 g/kg of β-glucans compared with controls. Thus, the results of our study broadly agreed with those of others, and we observed improved FE in β-glucan-supplemented calves at wk 3 and 5 compared with controls. A positive effect of β-glucan supplementation on FE and BW has been observed in mice (Kurashige et al., 1997) and lambs (Haddad and Goussous, 2005; Gao et al., 2008). However, in the current study, withers height, hip width, and heart girth circumference were not associated with supplementation, even though they are directly correlated with calf weight and development (Gelsinger and Heinrichs, 2007). We hypothesize that we did not observe an association of supplementation with these parameters because the calves in this study experienced diarrhea and had a lesser rate of growth. However, the improved growth observed in the supplemented calves suggests a performance benefit to β-glucan supplementation in these calves.

Nearly all of the calves in this study experienced a diarrhea bout. Sickness behavior is a motivational state that has been observed to reduce starter intake in calves during disease (Cantor and Costa, 2022). Thus, it is possible that we did not observe an association of supplementation with DMI or starter intake because nearly every calf experienced diarrhea. However, there was a health benefit to calves who received algae-derived β-glucans, as these calves had a lower likelihood of spending an additional day with abnormal feces. The dysbiosis caused by diarrhea may have effects on the metabolism and performance of preweaning calves (Virgínio Júnior and Bittar, 2021). Consequently, this may positively affect digestion and nutrient absorption, partially explaining the improved FE observed in our calves during wk 3 and 5. Furthermore, β-glucans may enhance modulation of the gut microbiome and reinforce the intestinal barrier (Ma et al., 2015), which would amelioriate the severity of a diarrhea bout in calves. It is possible that diarrhea occurred in most calves in this study because the MR had vegetable protein sources that have been associated with reduced digestibility in calves (Bittar et al., 2018). However, in this study, we observed a positive effect of β-glucan supplementation on final BW compared with control calves, probably because supplemented calves had fewer days with abnormal feces. We suggest that algae β-glucan supplementation may have decreased the severity of diarrhea in our calves. Mitigating diarrhea is important because it negatively affects ADG in dairy calves and, potentially, long-term productivity (Abuelo et al., 2021). Future research should explore the effect of algae-sourced β-glucans on lower-gut physiology and digestibility in dairy calves.

Research has shown that β-glucan supplementation to calves results in an improved gut microbial balance; decreased counts of pathogenic E. coli and increased

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>FE (g/kg)</th>
<th>BW (kg)</th>
<th>SEM</th>
<th>T</th>
<th>A</th>
<th>T × A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (10^6/µL)</td>
<td>Control</td>
<td>7.1</td>
<td>6.9</td>
<td>0.27</td>
<td>0.39</td>
<td>&lt;0.01</td>
<td>0.23</td>
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<td></td>
<td>β-Glucan</td>
<td>7.4</td>
<td>6.3</td>
<td>0.37</td>
<td>0.45</td>
<td>&lt;0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>Leukocytes (10^6/µL)</td>
<td>Control</td>
<td>44.84</td>
<td>44.58</td>
<td>3.00</td>
<td>0.95</td>
<td>&lt;0.01</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>β-Glucan</td>
<td>42.52</td>
<td>44.73</td>
<td>2.65</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>0.44</td>
</tr>
<tr>
<td>Neutrophils (10^3/µL)</td>
<td>Control</td>
<td>11.99</td>
<td>10.52</td>
<td>0.97</td>
<td>0.19</td>
<td>0.74</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>β-Glucan</td>
<td>12.52</td>
<td>10.52</td>
<td>0.97</td>
<td>0.19</td>
<td>0.74</td>
<td>0.95</td>
</tr>
</tbody>
</table>

1T = treatment effect; A = age effect; T × A = interaction of treatment × age effect.
2β-Glucan = 2 g/d of algae-derived β-glucan fed with 6 L of milk replacer.
counts of commensal Lactobacillus have been observed in supplemented calves compared with controls (Zhou et al., 2009). It is also possible that calves had lowered odds of diarrhea because β-glucans are associated with improved gut development in calves (Ma et al., 2015; Xiao et al., 2016) and increased abundance of Alloprevotella (Virginio Junior et al., 2021), a genus associated with improved intestinal barriers and tighter epithelial junctions in the lower gut (Li et al., 2018). However, we can only hypothesize that this β-glucan mechanism played a role in the fecal status of our calves.

We can affirm that supplementing algae β-glucans to calf MR results in benefits to the fecal bacteriome. For example, Virginio Junior et al. (2021) evaluated the fecal bacterial community of a subset of animals from our study (7 per treatment) and detected a higher abundance of bacteria with anti-inflammatory capacity and improvement of the intestinal barrier. We observed that β-glucans ameliorated the odds of a diarrhea bout in the current study, suggesting that disease severity was positively influenced by treatment. Nargeskhani et al. (2010) reported that calves fed 4 g/meal of mannanoligosaccharide β-glucans had lower fecal scores than control calves. Similar to our results, Kim et al. (2019) observed that supplementation of algae-derived β-glucans to weaned pigs ameliorated diarrhea bouts after experimental exposure to pathogenic E. coli. Thus, we suggest that β-glucan supplementation improved the fecal status of our calves, which may be due to a better disease response, in broad agreement with the literature in several species. However, this needs further investigation because we can only state that treatment decreased the duration of diarrhea, rather than the occurrence of diarrhea.

In general, calves in this study experienced high disease pressure, and we do not know whether β-glucans reduce the duration of diarrhea in calves when disease pressure is low. Furthermore, despite the effects of supplementation on calf fecal score, we found no association of treatment with immune parameters in the calves. However, mean values of circulating lymphocytes, segmented neutrophils, and monocytes in our calves were within the reference intervals for healthy calves (Jezek et al., 2011). Our study has 2 main limitations. First, our performance results are for dairy calves with modest growth (ADG of 0.30 kg/d) and these calves were fed a mid-quality MR at a volume of 6 L/d. Second, calves were not followed after weaning, when we may have seen some benefits from supplementation with algae β-glucans.

Although we have discussed studies involving yeast β-glucan, this is an unfair comparison because yeast-derived β-glucans have different physicochemical characteristics than algae-derived β-glucans. According to Ma et al. (2021), the content of β-glucan in yeast is very low (1 to 7% DM), and they are insoluble or partially soluble in water. This differs greatly from algae-derived β-glucan, which is water-soluble and has high β-glucan content (20–50% DM; Barsanti et al., 2001). In addition to these structural differences, we caution that differences exist in dosing of β-glucans to calves among the aforementioned studies, as well as differences in purity, molecular mass, degree of branching, polymer charge, and chemical structure between these β-glucans (Han et al., 2020). However, because there are few studies of algae-derived β-glucans, a comparison is the only option. Thus, more work is needed to investigate whether β-glucans have long-term health benefits on calves. However, β-glucans were associated with ameliorating severity of diarrheal bouts in our calves. Future research should investigate the mechanisms underlying this amelioration following algae-derived β-glucan supplementation in calves.

CONCLUSIONS

Supplementing algae β-glucans to the MR of calves led to higher BW at 8 wk compared with unsupplemented controls. However, β-glucan supplementation did not alter metabolic or immune indicators. Furthermore, algae β-glucan supplementation ameliorated diarrhea bouts in our calves, including a lower likelihood of abnormal fecal score compared with controls in the first 28 d. Thus, algae-derived β-glucan supplementation to calves fed 6 L/d of MR improved calf performance and calf fecal status without altering metabolic indicators or immune parameters.

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REFERENCES


