Impacts of various milk replacer supplements on the health and performance of high-risk calves

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INTRODUCTION

High-risk calves have an increased risk for morbidity and mortality due to failure of passive transfer (FPT), high exposure to pathogens, and increased stressors in the first few days of life. Because of the immature anatomy of the gastrointestinal tract and naïve immune system calves are especially susceptible to environmental bacteria and viruses. The main cause of disease in neonatal calves is scours due to Escherichia coli or Salmonella infections, resulting in a 7.8% mortality rate for pre-weaned heifers on U.S. dairy farms with at least 56.5% of those deaths being attributable to gastrointestinal disease (NAHMS, 2007).

When high-risk calves are exposed to disease there are ways to decrease the risk of morbidity and mortality through supplementation of milk replacer additives. Probiotics and yeast cell wall fractions are increasing in supplementation strategies as studies begin to show advantageous impacts acting with similar health and performance results to antibiotics. Supplementing probiotics and mannanoligosaccharides (MOS) to calves has been shown to increase performance and health measures including increases in BW, ADG, and decreases in fecal scores (Heinrichs et al., 2003; Ghosh et al., 2012). Supplementing β-glucan (BG) was observed to increase pro-inflammatory cytokine production, increase leukocyte and lymphocyte activation, and moderate the impacts of inflammation during sepsis in multiple species (Novak et al., 2009; Eicher et al., 2010). Supplementation of these compounds has shown equivocal results, but each appears to work through separate mechanisms of action.

The objectives of this study were to determine the impacts of supplementing a blend of probiotics containing Lactobacillus casei, Enterococcus faecium, and Saccharomyces cerevisiae, β-glucan, a heat stable blend of Bacillus subtilis probiotic with mannanoligosaccharides, and IGF-1 molecules fractionated from colostrum on the performance and health of high-risk Holstein calves and to determine if any treatment had carry-over effects into the immediately post-weaned period.

MATERIALS AND METHODS

This study was a completely randomized design consisting of two 84-day periods with a total of 100 Holstein bull calves. Treatments were completely randomized and included a negative control group (CON), Mushroom β-glucan; ImmuOligo, Irvine, CA (BG) supplemented at 5mL/day, Immu-PRIME; Sterling Technologies, Brookings, SD (ImmPr) given at 0.75 grams per feeding for only the first 3 days of life, probiotic; PROVIDA Calf; MB Nutritional Sciences LLC, Lubbock, TX (PROVIDA) treatment of 2x10⁹ CFU/d of Lactobacillus casei, Enterococcus faecium, and Saccharomyces cerevisiae, and mannanoligosaccharide; CEREVIDA Calf MOS; MB Nutritional Sciences LLC, Lubbock, TX (MOS+Bs) treatment with 4x10⁹ CFU/d Bacillus subtilis and 3g/d of MOS. Upon arrival calves were weighed, randomly assigned a treatment and
administered a bolus of a full day’s treatment, with control calves receiving a sham bolus. Peripheral blood was drawn immediately to assess passive immune status via total serum protein (TSP) analysis on a refractometer. All calves were enrolled in the study within 24 h of birth. Calves were housed outdoors in individual calf hutches (2.13 x 1.09 m Agri-Plastics, Cortland, NY). Calves were fed 700 g of milk replacer containing 22% CP and 20% fat (Milk Specialties, Eden Prairie, MN) and texturized calf starter ad libitum at 22% CP (Purina Ampli Calf, Nestle Purina, St. Louis, MO). All calves were vaccinated on d 28 with Inforce 3 (Zoetis Inc, Parsippany-Troy Hills, NJ) and Bovi-shield (Zoetis Inc, Parsippany-Troy Hills, NJ). Calves were stepwise weaned starting d 53 only being fed milk in the morning until d 56 when they were moved into randomized group pens after the morning milk feeding. Treatments were ceased at weaning and calves were co-mingled in groups of 10 to 12 calves per pen with treatments equally represented within each pen. Measurements of BW were taken on d 0, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 84. Rectal temperatures were assessed on d 1, 4, 8, 11, 15, 18, and 22. Peripheral blood samples were taken on d 1, 3, 7, 14, 21, 42, 56, and 84. Blood was analyzed within 2 hours for a complete blood cell count on an IDEXX Procyte analyzer.

Statistical Analysis
All continuous, repeatedly measured data were analyzed as a repeated measure using the Mixed Procedure in SAS (SAS 9.4, Cary, NC). The model included fixed effects of treatment, time, and treatment x time. Initial BW and TSP were tested as covariates in the model and were retained in the final model if they were significant. Initial BW was a significant covariate for ADG and TSP for calf starter intake. Period was included as a random effect, and the subject of the repeated statement was calf nested within treatment. All appropriate covariance structures for unequal spacing and variance structures were analyzed and the most appropriate model was selected based on the lowest Bayesian Information Criterion. Differences of $P \leq 0.05$ were considered significant and a tendency was reported when $0.05 < P \leq 0.10$. Significant treatment x time interactions were further evaluated by sliced treatment differences at each sample time using a Duncan adjustment to control for the familywise error. All pairwise comparisons at each significant time were determined. Before analysis all data were trimmed using the Windsor method.

RESULTS AND DISCUSSION
This study investigated the impacts of four different nutritional supplement strategies on the health and performance of high-risk Holstein bull calves during both the pre-weaned period as well as carry over effects in the immediate post-weaned, comingled period. The data from the current study suggest that BG, MOS+Bs, and PROVIDA probiotics influenced the performance and some measures of health; however, the mechanisms of action appear to be different.

Body weight, TSP, starter intake, ADG, body measures, and blood metabolites are reported in Table 1. The TSP data was reported as average TSP per treatment and the percent of calves in each treatment with FPT (<5.2g/dL). There was a treatment difference for pre-weaned starter intakes from day 0 to day 28 ($P=0.016$). The BG supplemented calves consumed the most starter in the first month of life and CON calves consumed the least. Starter intakes did not differ for PROVIDA calves from any treatment except they were less than the BG calves from d 0 to d 28. The starter intake from d 29 through d 56 reflects the total pre-weaned starter intake as most of the pre-
weaned starter intake was consumed during this period. Total and post-weaned ADG did not differ between groups but a tendency for difference was detected for pre-weaned ADG ($P=0.081$; Table 1). The ADG in the pre-weaned period was greatest for the BG and PROVIDA calves and the CON calves gained the least. The ADG among the PROVIDA and BG calves during the pre-weaned period was a 3.92 and 2.80 kg improvement in pre-weaned BW gain, respectively. This is in an agreement with data from multiple studies that supplemented probiotic bacteria to young calves and observed increased ADG as well as BW (Abu-Tarboush et al., 1995; Mokhber et al., 2007; and Jatkauskas et al., 2010). The increase in ADG with probiotic supplementation may be due to a few mechanistic actions. Supplementation of 12 *Lactobacillus* strains decreased colonization of the GIT with 3 strains of *E. coli* and up to 5 strains of *Salmonella*. This is likely because some of the *Lactobacillus* species can adhere strongly to the small intestinal mucosa and epithelium and is suggested there is production of substances to decrease pathogenic growth (Jin et al., 2014). A study completed by Ewaschuk et al. (2012) recorded no difference in feed intake, ADG, or BW in BG supplemented pigs. However, in agreement with the current study, Dritz et al. (1995) supplemented a purified BG product to pigs and observed an increase in ADG as well as BW. The effect of feeding MOS to calves is equivocal with some studies reporting increased ADG, BW, and decreased fecal scores (Ghosh et al., 2012 and Berge et al., 2016) with other studies reporting no differences in ADG or final BW (Hill et al., 2008; Nargeskhani et al., 2010). In agreement with the pre-weaned calf starter intake data in this study, Heinrichs et al. (2003) reported that a MOS supplement increased starter intake at a younger age than a control group; however, there were no differences in calf performance at the end of the study. Similarly, Terre et al. (2007) observed no change in final BW but did report an increased pre-weaned starter intake for MOS supplemented calves.

The health of calves likely impacts the performance outcomes of many nutritional supplements. As reported by Gilliland et al. (1980), when calves were fed a probiotic *Lactobacillus* strain there was no difference observed between treatments due to the general good health of all the calves in the study. Similar findings in a *Bacillus sp.* based probiotic study where there were no differences in either calf starter intakes or ADG between a control and a probiotic supplemented group of healthy, unstressed calves (Riddell et al., 2008). The calves in the current study were high-risk bull calves from a commercial calf ranch, and despite intensive management of the calves there was still an overall 17% mortality in the study.

Calf health was evaluated daily over the course of the study. Average fecal scores were greatest during the 2nd week of life and were lowest among BG calves. However, there were no differences in the dry matter (DM) content of fecal samples among treatments throughout the entire study. Rectal temperatures were taken during the first 3 weeks of life, and contrary to the increased rectal temperatures in the MOS+Bs group, Kara et al. (2015) observed a decrease in temperature of dairy calves supplemented with MOS. The increase in rectal temperature on day 4 and 8 in the ImmPr calves may be related to the increased serum haptoglobin concentrations seen within the first week of life for ImmPr calves as well.

The greater calf starter intakes for PROVIDA calves during the second month of life coincided with a decrease in fecal scores. Fecal scores, fecal dry matter, rectal temperature, and hematology measures are all reported in Table 2. There was a treatment
x time interaction among fecal scores ($P<0.0001$). Fecal score differences are indicated in Figure 2 at week 2 ($P=0.036$), week 5 ($P=0.017$), week 6 ($P=0.001$), week 7 ($P=0.001$), and week 8 ($P=0.002$). During weeks 5, 6, and 7, the PROVIDA supplemented calves had the lowest fecal scores. Probiotics have been shown to decrease fecal scores during both the pre-weaned and post-weaned periods (Mokhber et al., 2007; Meale et al., 2017).

Fecal scores during week 8 were greatest for CON calves, which corresponded with the reduced starter intake among CON calves. There was a treatment x time interaction among rectal temperatures ($P=0.049$). Rectal temperature differences are indicated in Figure 3 and occur at d 4 ($P=0.010$) and d 8 ($P=0.006$). Table 2 also contains blood metabolite data. There was a treatment x time interaction for serum glucose concentration ($P=0.049$). The treatment x time differences in serum glucose are indicated in Figure 1.

The CON had decreased serum glucose concentrations on d 3 ($P=0.007$) and d 7 ($P=0.107$) when compared to all other treatment groups. Both CON and ImmPr calves had increased serum glucose concentrations at d 56 and d 84. The exact mechanisms leading to the greater glucose concentrations among these calves at these later time points is unclear; however, these 2 treatments had numerically lower calf starter intake when compared to the other 3 treatments. Therefore, the differences in serum glucose concentrations could be associated with nutrient availability or anatomical site of digestion. Serum haptoglobin was assessed as a measure of systemic inflammation and analyzed as AUC as well as concentration over time. Serum haptoglobin concentrations had a treatment x time interaction ($P=0.010$). Figure 4 shows the treatment interactions at d 7 ($P=0.036$), d 14 ($P=0.033$), d 42 ($P=0.019$), and d 56 ($P=0.006$). There was a tendency for a treatment interaction when haptoglobin was assessed using an area under the curve (AUC) approach ($P=0.075$), whereas BG, MOS+Bs, and PROVIDA all had reduced haptoglobin concentrations when compared to the CON and the ImmPr was not different than any other treatment. Haptoglobin AUC was greatest for CON calves and lowest for BG, MOS+Bs, and PROVIDA calves. Further, on d 7 the ImmPr and CON calves had the greatest serum haptoglobin concentrations, suggesting there may have been a greater exposure or lack of microbial control in the GIT of those calves in the first week of life. Some of the effect in the ImmPr calves may be associated with the greater proportion of FPT calves in that group at enrollment. Exposure and inability to combat bacterial or viral infection of the GIT could increase haptoglobin levels as a general marker inflammation. In agreement with the current study, Sandvik et al. (2007) that observed BG modulated inflammation in rats undergoing LPS-induced endotoxemia. Therefore, BG may reduce the intensity of the systemic inflammatory response through some mechanism. A study with pigs supplemented with BG reported a consistent decreased haptoglobin concentrations in the BG supplemented pigs compared to a control group from d 7 through d 28 (Dritz et al. 1995). Additionally, Liang et al. (2017) reported decreased serum haptoglobin concentrations among high-risk Jersey calves after an oral *Salmonella typhimurium* challenge if they were supplemented with the same blend of probiotic bacteria used in the current study. In contrast to the current study, Terre et al. (2007) observed no difference in serum haptoglobin concentrations between MOS supplemented calves and a negative control group.

The MOS+Bs calves had the lowest total leukocyte count, neutrophil count and percent, and the lowest lymphocyte count at all time points. Hematology data are reported in Table 3. Total leukocyte count did not have a treatment x time interaction
(P=0.360); however, there was a treatment difference (P<0.002). There was no treatment x time interaction for polymorphonuclear neutrophil (PMN) counts (P=0.224), but there was a treatment difference (P=0.003). Additionally, there was no treatment x time interaction for PMN percentage (P=0.290), but there was a treatment difference (P=0.005). Lymphocyte counts also differed with a treatment x time interaction (P=0.001). The differences in lymphocyte counts are illustrated in Figure 4 at d 3 (P=0.071), d 7 (P=0.043), d 14 (P=0.065), d 21 (P=0.054), d 42 (P=0.061), d 56 (P=0.003), and d 84 (P=0.010). There was no treatment x time interaction in the ratio of PMN to lymphocytes (P=0.189); however, the PMN:lymphocyte had a strong tendency for a treatment difference (P=0.051), whereas the BG treatment had the greatest PMN:lymphocyte. Neutrophil counts decrease over time in the first 6 to 8 weeks of life in dairy calves and may reflect maturation of the GIT immune system and/or exposure to enteric pathogens.

The GIT of young calves is colonized by a wide variety of bacteria that represent diverse phyla, and this colonization is dynamic during the first few months of life. A primary mode of action of MOS is to bind mainly gram-negative bacteria in the small intestine, which is assumed to decrease pathogenic exposure by those bacteria that may be in the environment. However, some of the beneficial microbes beginning to colonize the small intestine of neonatal calves are gram-negative. Many gram-negative bacteria express Type 1 Fimbriae, a type of adherence filament. Type 1 Fimbriae are mannose-specific filaments that induce agglutination and are expressed on many types of gram-negative bacteria, both pathogenic and commensal (Rendon et al., 2007; Lasaro et al., 2009). The high affinity of MOS for Type 1 Fimbriae of gram-negative bacteria may also be binding some of the beneficial gram-negative GIT microbes in addition to potentially pathogenic ones. This may be contributing to the reduced leukocyte counts in the MOS group. More research is needed to understand how MOS maybe affecting the microbial ecology of the GIT of calves early in life.

In contrast to the MOS+Bs treatment, the PROVIDA supplemented calves maintained the greatest lymphocyte counts throughout the entire study. The probiotic bacteria colonizing the GIT may be stimulating lymphocyte production similar to findings by Bai et al. (2004) on in vitro stimulation of intestinal epithelium by probiotics. In contrast, Fleige et al. (2009) observed a decrease in the lymphocyte population of calves fed probiotics. The ImmPr supplemented calves also had elevated lymphocyte counts on d 21 when compared to the MOS+Bs treatment. Blum et al. (2008) reported that supplementing IGF-1 to neonatal calves may stimulate lymphocyte development. The increased lymphocyte count in peripheral circulation among the ImmPr calves was demonstrated on d 21; therefore, the impacts of ImmPr on lymphocyte development are not understood.

The BG calves had increased neutrophil counts and neutrophil to lymphocyte ratios in peripheral circulation. Whether the BG stimulated granulopoiesis or reduced the marginating pool of neutrophils in circulation is not completely known. However, the latter appears plausible because the BG supplemented calves had the lowest L-selectin expression on the surface of peripheral blood neutrophils. Reduced L-selectin adhesion protein can increase the number of neutrophils measured in circulation because less neutrophils are loosely adhered to the vascular endothelium. Neutrophil L-selectin expression, phagocytosis and oxidative burst capacity data are reported in Table 3. There
was no treatment x time interaction for any data in this table ($P \geq 0.371$). The mean fluorescence intensity for neutrophil phagocytosis had a tendency for a treatment difference ($P=0.087$). Additionally, the neutrophil oxidative burst capacity had a treatment difference ($P=0.011$). The surface expression of L-selectin on neutrophils had a treatment difference ($P<0.0001$) and no treatment x time difference ($P=0.371$). The BG calves had the lowest L-selectin expression on neutrophils, the lowest numerical phagocytosis and oxidative burst capacity percentage, and the lowest phagocytosis and oxidative burst intensities. The exact reason for the attenuated neutrophil responses is unknown. Supplementing BG to lambs increased neutrophil phagocytosis and oxidative burst capacities when compared to a control (Wojcik, R., 2007). Further, supplementing rats with BG increased neutrophil functionality (Stier et al., 2014), and the potential killing activity as well as respiratory burst activity of phagocytes (Malaczewska et al., 2010). Harris et al. (2017) supplemented calves with a yeast cell wall extract that contained both MOS and BG and reported an increase in L-selectin expression in supplemented calves. Supplementing the PROVIDA probiotics also led to a similar low neutrophil oxidative burst MFI as the BG treatment. These data are in contrast to Indart et al. (2012) that observed an increase in neutrophil functionality when supplemented with probiotics. The CON and ImmPr calves had the greatest neutrophil oxidative burst capacity, implying the need for greater leukocyte function to fight off bacterial or viral infections.

**CONCLUSION**

Supplementing high-risk Holstein calves with either BG, MOS+Bs, or PROVIDA increases measures of performance and health, albeit by different mechanisms. Supplementing BG to high-risk calves likely decreases neutrophil oxidative burst and L-selectin expression, potentially moderates some systemic inflammation, and simultaneously stimulates starter intake in the first months of life. Feeding PROVIDA to high-risk calves increases starter intake and body weight gain, moderates a systemic inflammation response and stimulates lymphocytes. Supplementing MOS+Bs to high-risk calves decreases the lymphocyte and neutrophil populations while decreasing neutrophil functionality. However, MOS+Bs was shown to increase L-selectin expression on neutrophils, implying some stimulation may be occurring.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


Berge, A. C. (2016). A meta-analysis of the inclusion of Bio-Mos in milk or milk replacer fed to dairy calves on daily weight gain in the pre-weaning period. *Journal*


Table 1. The effect of treatments on performance measurements in high-risk Holstein dairy calves

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments(^1,^2)</th>
<th>Largest SEM</th>
<th>Fixed Effects</th>
<th>Trt</th>
<th>Time</th>
<th>Trt*Time</th>
<th>(P \leq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein, TSP(^3)</td>
<td>CON  5.50</td>
<td>ImmPr  5.24</td>
<td>BG  5.47</td>
<td>MOS+Bs  5.31</td>
<td>PROVIDA  5.28</td>
<td>.122</td>
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<tr>
<td>Failure of passive transfer, %</td>
<td>CON  25.0</td>
<td>ImmPr  50.0</td>
<td>BG  35.0</td>
<td>MOS+Bs  45.0</td>
<td>PROVIDA  40.0</td>
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<td>...</td>
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<tr>
<td>Initial body weight, kg</td>
<td>CON  41.7</td>
<td>ImmPr  39.1</td>
<td>BG  41.7</td>
<td>MOS+Bs  39.2</td>
<td>PROVIDA  40.1</td>
<td>1.238</td>
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<td>Weaned body weight, kg</td>
<td>CON  66.6</td>
<td>ImmPr  66.0</td>
<td>BG  68.4</td>
<td>MOS+Bs  66.4</td>
<td>PROVIDA  69.0</td>
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<tr>
<td>Final body weight, kg</td>
<td>CON  91.6</td>
<td>ImmPr  90.2</td>
<td>BG  91.5</td>
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<td>PROVIDA  94.2</td>
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<td>Preweaned starter intake, kg</td>
<td>CON  13.95</td>
<td>ImmPr  14.79</td>
<td>BG  17.10</td>
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<td>PROVIDA  18.50</td>
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<td>CON  0.59</td>
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<td>BG  0.62</td>
<td>MOS+Bs  0.61</td>
<td>PROVIDA  0.65</td>
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<tr>
<td>Urea Nitrogen, pg/mL</td>
<td>CON  103.2</td>
<td>ImmPr  105.2</td>
<td>BG  104.2</td>
<td>MOS+Bs  103.4</td>
<td>PROVIDA  104.5</td>
<td>1.663</td>
<td>0.603</td>
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</table>

\(^1\) Treatments included a control group which were fed a base diet of milk replacer and calf starter; ImmPr which were fed 1.5 g/d ImmPr first 3 d only; BG which were fed 1 g/d β-Glucan; MOS+Bs which were fed 3 g/d Mannanoligosaccharides + 4 x 10^9 CFU/d Bacillus subtilis; PRO which were fed a blend of 2 x 10^9 CFU/d Lactobacillus casei and Enterococcus faecium + 2 x 10^9 CFU/d Saccharomyces cerevisiae.

\(^2\) Differing superscripts within a row indicate a difference between means (\(P < 0.05\)).

\(^3\) The Largest SEM for total serum protein was reported in this table as the largest standard deviation because TSP was calculated only as an average per treatment.
Table 2. The effect of treatments on health measurements in high-risk Holstein dairy calves

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>ImmPr</th>
<th>BG</th>
<th>MOS+Bs</th>
<th>PROVIDA</th>
<th>Treatments1,2</th>
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<td>Time</td>
<td>Trt*Time</td>
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<td>76.7a</td>
<td>69.2b</td>
<td>83.7c</td>
<td>76.1a</td>
<td>4.771</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Neutrophil phagocytosis &amp; oxidative burst, %</td>
<td>45.1</td>
<td>44.9</td>
<td>42.7</td>
<td>43.6</td>
<td>44.2</td>
<td>3.559</td>
<td>0.485</td>
<td>&lt;0.0001</td>
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<td>9.7ab</td>
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<td>10.6a</td>
<td>10.2a</td>
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<td>174.1</td>
<td>177.6</td>
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<td>187.1</td>
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<td>0.198</td>
<td>&lt;0.0001</td>
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<td>71.5a</td>
<td>65.6b</td>
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<td>108.7</td>
<td>107.2</td>
<td>109.5</td>
<td>109.5</td>
<td>1.945</td>
<td>0.611</td>
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Table 3. Effects of treatment on hematology measurements in high-risk Holstein dairy calves

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1Treatments included a control group which were fed a base diet of milk replacer and calf starter; ImmPr which were fed 1.5 g/d ImmPr first 3 d only; BG which were fed 1 g/d β-Glucan; MOS+Bs which were fed 3 g/d Mannanoligosaccharides + 4 x 10^9 CFU/d Bacillus subtilis; PRO which were fed a blend of 2 x 10^9 CFU/d Lactobacillus casei and Enterococcus faecium + 2 x 10^9 CFU/d Saccharomyces cerevisiae.

2Differing superscripts within a row indicate a difference between means (P < 0.05).
Figure 1. Serum glucose concentrations were measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction (P=0.049). The CON treatment had lower glucose concentrations than all other treatments on d 3 (P≤0.006) and was reduced on d 7 when compared to PROVIDA (P=0.009). Largest SEM per time point are expressed as mg/dL and include 3.727, 2.271, 3.051, 2.571, 2.739, 3.131, 3.149, and 2.933 for d 1, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of (P≤0.05) and a # indicates a tendency for a treatment difference (0.05<P≤0.10) when treatments were sliced by time.

Figure 2. The fecal scores were averaged by week per treatment for weeks 1, 2, 3, 4, 5, 6, 7, and 8. There was a treatment x time interaction (P<0.0001). The BG calves had lower fecal scores during week 2 (P≤0.087). The PROVIDA calves had decreased fecal scores when compared to other treatments during weeks 5, 6, 7, and 8 (P≤0.027). Largest SEM per time point are expressed as fecal score weekly average and include 0.096, 0.076, 0.080, 0.094, 0.072, 0.076, and 0.066 for d 1, 4, 8, 11, 15, 18, and 22, respectively. An * indicates a treatment difference of (P≤0.05) when treatments were sliced by time.

Figure 3. Rectal temperature was measured on d 1, 4, 8, 11, 15, 18, and 22. There was a treatment x time interaction (P≤0.049). The CON treatment had decreased rectal temperatures when compared to other treatments; CON vs. BG (P≤0.091) on d 4 and d 8; CON vs. ImmPr (P≤0.021) on d 4 and d 8; and CON vs. MOS+Bs (P≤0.064) on d 4 and d 8. The ImmPr treatment had increased rectal temperatures when compared to other treatments; ImmPr vs. CON (P≤0.021) on d 4 and d 8; ImmPr vs. BG (P=0.085) on d 4; and ImmPr vs. PROVIDA (P≤0.012) on d 4 and d 8. Largest SEM per time point are expressed as degrees Celsius and include 0.096, 0.076, 0.080, 0.094, 0.072, 0.076, and 0.066 for d 1, 4, 8, 11, 15, 18, and 22, respectively. An * indicates a treatment difference of (P≤0.05) when treatments were sliced by time.
Figure 4. Serum haptoglobin concentration was measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction ($P \leq 0.010$). On d 7 ImmPr had greater haptoglobin concentrations than BG, MOS+Bs, and PROVIDA ($P \leq 0.015$). On d 14, BG had decreased haptoglobin when compared to Con, ImmPr, and PROVIDA ($P \leq 0.068$). The CON treatment had greater concentrations on d 42 than ImmPr, BG, and PROVIDA ($P \leq 0.016$). On d 56 ImmPr had the greatest haptoglobin concentrations ($P \leq 0.035$). Largest SEM per time point are expressed as $\mu g/mL$ and include 31.202, 24.013, 61.286, 19.575, 29.605, 16.917, and 45.241 for d 1, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) when treatments were sliced by time.

Figure 5. Lymphocyte count was measured on d 0, 3, 7, 14, 21, 42, 56, and 84. There was a treatment x time interaction ($P \leq 0.003$). The MOS+Bs treatment had decreased lymphocytes when compared to other treatments; MOS+Bs vs. CON ($P \leq 0.079$) on d 42, and 84; MOS+Bs vs. ImmPr ($P \leq 0.068$) on d 3, 7, 14, 21, 42, and 56; MOS+Bs vs. BG ($P \leq 0.05$) on d 3, 7, 14, and 42; MOS+Bs vs. PROVIDA ($P \leq 0.042$) on d 3, 14, 42, 56, and 84. Largest SEM per time point are expressed as $10^6/mL$ and include 0.183, 0.229, 0.237, 0.263, 0.244, 0.230, 0.214, and 0.231 for d 0, 3, 7, 14, 21, 42, 56, and 84, respectively. An * indicates a treatment difference of ($P \leq 0.05$) and a # indicates a tendency for a treatment difference ($0.05 < P \leq 0.10$) when treatments were sliced by time.