Effects of supplementation with *Bifidobacterium infantis* in combination with bioactive milk components on gastrointestinal symptoms in children with autism

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**Background**

A feature of the evolution of lactation is the explicit development of a symbiotic relationship between microorganisms and the mammalian host that is essential for proper physiological function (1). Human milk guides the colonization and composition of the infant gut microbiota more than any other environmental factor and compelling evidence is emerging that a milk-oriented microbiome (MOM) guides the development of various systemic processes (immunity, metabolism, neurological networks) and sets the infant up for a lifetime of health, including optimal neurodevelopment (2). The extent of the effect of microbial composition and activity on human physiology has only begun to be explored with neurodevelopment positioned at the very edge of this investigation. Examination of neurodevelopmental disorders such as autism spectrum disorders (ASD) and their connection with gut microbial dysbiosis will provide insight into the explicit, but often elusive, gut-brain microbiota axis in human development. Children with ASDs, characterized by a range of cognitive and social deficits, are commonly plagued by gastrointestinal (GI) co-morbidities including chronic constipation and diarrhea (3). The severity of ASD symptoms is often correlated with the extent of GI symptomology, which has been correlated with the degree of microbial dysbiosis in the gut (4,5). Supplementation with the probiotic *Bifidobacterium infantis*, whose growth is promoted by milk components (6), has been shown be beneficial in adults with irritable bowel syndrome (IBS) (7) and preterm infants with necrotizing enterocolitis (NEC) (8). This study was designed to explore characteristics of microbial dysbiosis that may contribute to the severity of GI and behavioral symptoms in children with ASD, and to test whether supplementation with the probiotic *B. infantis* with bioactive milk components (bovine colostrum product – BCP) can ameliorate this microbial dysbiosis.

**Objectives**

**Am 1:** To assess tolerability of probiotic and prebiotic administration in children with ASDs

**Hypothesis:** Both probiotic and prebiotic will be well tolerated with limited side effect profile.

**Am 2:** To observe the biological effects of probiotics and prebiotics on gut microbiota in children with ASDs with GI symptoms

**Hypothesis:** Synbiotic administration will result in increased commensal *Bifidobacteria* and decreased pathogenic bacteria compared to probiotic alone.

**Am 3:** To observe the biological effects of probiotics and prebiotics on GI symptom frequency and severity

**Hypothesis:** GI symptoms (constipation, diarrhea) will improve in frequency and severity in relation to changes in stool microbiota.

**Research Design**

Our primary goal is to administer a symbiotic, which is a probiotic (B. infantis) in combination with a prebiotic (BCP-bovine colostrum product), to study its effect on stool microbial composition and GI symptoms in a subset of children with ASD. Therefore, we performed a randomized, double-blind, controlled clinical trial with a crossover study design. The trial period includes 5 weeks of colostrum use, 5 weeks of probiotic use, and a 2 week washout period in between. Stool, urine, and plasma were collected at baseline, at the end of the first arm, after the two week washout period, and at the end of the study. In addition, GI symptom changes and behavioral measures were reported based on clinician assessment, parent questionnaires and stool log. Exclusion criteria included presence of a diagnosed GI disease (celiac disease, etc) and recent antibiotic or steroid use.

**Subject Demographics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>Total Enrolled</td>
<td>11</td>
</tr>
<tr>
<td>Male-female</td>
<td>6/5</td>
</tr>
<tr>
<td>Age Range (years)</td>
<td>3-9</td>
</tr>
<tr>
<td>Constipation/ Diarrhea/ Mixed</td>
<td>3/4/4</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7</td>
</tr>
<tr>
<td>Gas/bloating</td>
<td>10</td>
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</tbody>
</table>

**Gastrointestinal Symptom Scoring:**

- **Frequency**
  - 1 = <2x/ wk
  - 3 = 3-5x/ wk
  - 5 = >5x/ wk

- **Consistency**
  - 1 = Very Hard
  - 3 = Normal
  - 5 = Watery

**Results**

**Preliminary Findings:**

- GI symptom alterations were robust and readily assessed by parents in some but not all children studied, especially in children with autism spectrum disorders.
- Willingness to try new foods is an important bias in all studies of foods in these children
- Increased weight gain was observed in some children who had previously experienced difficulty with weight gain and had low initial BMI and thus BMI is an important dependent variable
- Most common side effects included gassiness and lethargy

**Conclusions**

The administration of probiotic and prebiotic supplements were well tolerated by children in this first phase of the study analysis of outcome measures. The study supplement forms were well tolerated in all children with ASDs with gassiness and lethargy being the most commonly reported side effects. As anticipated for this group of subjects, some children were reluctant to regular consumption of the supplement due to palatability issues. The reporting of outcomes by parents was highly compliant although all parents reported some level of improvement in GI symptoms, both constipation and diarrhea, both in frequency and consistency of stools. Furthermore, the parents of children who have finished the study reported that they would like to continue to provide the supplement to their child. The removal of bias in all studies using children with ASD is a dominant design variable. As this and subsequent studies move forward, the opportunity to address gastrointestinal functions using novel milk products is compelling. With parental bias and mechanistic outcome measures in place, GI health can now be addressed in human subjects scientifically.

**Future Research**

It is important to note that this study is currently ongoing. We plan to perform the following analyses on our blood, urine and stool samples:

- Next generation sequencing of stool microbiota to look for population shifts
- Plasma cytokine analysis to look for changes in inflammatory markers
- Metabolic analysis of plasma, urine, and stool to look for changes in microbial metabolism
- Measurement of focal and plasma markers for GI barrier integrity, including zonulin and LPS

This pilot study provides preliminary evidence for a larger study concerning the gut microbiota response to symbiotics in children with ASD and GI symptoms. However, sensory studies to address the palatability of the supplement to facilitate administration of the symbiotics in this cohort are warranted. Additional potential biases in parental reporting necessitate the development of quantitative measures of behavioral change when studying the gut microbiota-brain axis.

**Impact to the Dairy Industry**

Among families with children facing behavioral and intestinal issues, a common procedure is a gluten/casein free diet. There are data to support gluten as a damaging component and yet casein has no evidence to support its avoidance in these children. This study is designed to test whether milk components may actually enhance GI function in children with GI and behavioral symptoms. Positive outcomes in these experiments will pave the way for larger studies that may ultimately result in the development of alternative, non-pharmacological treatment options that include bioactive milk components for children with ASDs and GI symptoms. The overall opportunity is to position dairy components as positive to gastrointestinal health in the broadest sense.

**References**


**Acknowledgments**

The project described was supported by a MIND pilot grant, the Jastro Shields Graduate Research Award, and the National Center for Advancing Translational Sciences, National Institutes of Health, through grant number UL1 TR000133. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

I would also like to thank the International Milk Genomics Consortium for my Student Travel Award, which made my attendance at the 12th International Symposium possible.