EFFECTS OF PROBIOTICS ON MEMORY, ADHD, AND ANXIETY IN CHILDREN

by

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Thesis
submitted in partial fulfillment of the requirements for the Degree of Master of Science (Clinical Psychology)

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ii
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Date
Table of Contents

List of Tables ........................................................................................................ vi

List of Figures ....................................................................................................... vii

List of Appendices ................................................................................................ viii

Abstract ................................................................................................................. ix

List of Definition of Abbreviations ....................................................................... x

Acknowledgements ................................................................................................. xi

Effects of Probiotics on Memory, ADHD, and Anxiety in Children....................... 1

   The Gut Microbiome ......................................................................................... 1

   The Microbiome-Gut-Brain Axis .................................................................... 3

   Probiotics: Definition, Viability, and Safety .................................................... 6

   Probiotics and Cognitive Function ................................................................ 7

   Probiotics and Anxiety ..................................................................................... 10

   Probiotics and Cortisol ................................................................................... 12

   Present Study and Hypotheses ....................................................................... 12

Method .................................................................................................................. 14

   Participants ..................................................................................................... 14

   Materials ......................................................................................................... 16

   Procedure ....................................................................................................... 22

Results .................................................................................................................... 23
Discussion .............................................................................................................. 31

Probiotics and Anxiety ................................................................................................. 33

Probiotics and ADHD ................................................................................................. 35

Stimulants, Diet, and Probiotics .................................................................................... 38

References ................................................................................................................... 42

Appendices .................................................................................................................. 51
List of Tables

1. Age and Sex of Participants by Treatment Group.............................................. 15
2. Clinical Status of Participants............................................................................... 17
3. Means and Standard Deviations for Outcome Measures................................. 25
List of Figures

1. Comparison of Results for Full Sample and Non-Medicated Sub-sample on ADHD Symptoms……………………………………………………………………………………………………31

2. Comparison of Results for Full Sample and Non-Medicated Sub-sample on Memory Scores……………………………………………………………………………………………………32
List of Appendices

A. Newspaper Advertisement ................................................................. 51
B. Brochure .......................................................................................... 52
C. Poster ................................................................................................ 53
D. Information Sheet ............................................................................ 54
E. Eligibility Criteria ............................................................................. 67
F. Online Consent Form ........................................................................ 68
G. Screening Questionnaire ................................................................. 70
H. Informed Consent Form ................................................................. 78
Abstract

There is increasing interest in how the gut microbiome affects cognitive, emotional, and behavioural function via the gut-brain axis. Attention Deficit Hyperactivity Disorder and Anxiety are among the most prevalent psychological disorders experienced by children. Probiotic supplementation is beneficial for gut health and may subsequently impact central nervous system function. The current double-blind placebo controlled trial examined the effects of probiotic supplementation on symptoms of ADHD, Anxiety, and Memory in 48 children. Probiotics were expected to outperform a placebo in reducing symptoms and improving memory function. Although these hypotheses were generally not supported in the main results, exploratory analyses demonstrated a pattern of improvement in ADHD symptoms and memory in a subsample (n = 36) of participants who were not taking stimulant medication. This unexpected finding suggests that probiotic supplementation may be a beneficial first-line treatment for ADHD prior to the introduction of stimulant medication.
List of Definition of Abbreviations

ADHD ..........................................................Attention Deficit Hyperactivity Disorder
ASD .............................................................Autism Spectrum Disorder
CBCL ...........................................................Child Behaviour Checklist
CNS .............................................................Central Nervous System
DBD .............................................................Disruptive Behaviour Disorders Rating Scale
ENS .............................................................Enteric Nervous System
GI .................................................................Gastrointestinal
HPA .............................................................Hypothalamic Pituitary Adrenal
IBS .............................................................Irritable Bowel Syndrome
PAL .............................................................Paired Associate Learning
SCARED ..............................Screen for Child Anxiety Related Emotional Disorders
UC .............................................................Ulcerative Colitis
Acknowledgements

It would be impossible to thank all of the people who have helped and supported me through the completion of two degrees at Acadia. It has been an amazing experience and I have learned and grown in ways that I could not have anticipated when I first decided to return to school five years ago. Many thanks to Susan Potter for letting me join her team of probiotics researchers. It has been rewarding and challenging work. Thanks also to my classmates: Adria, Liz, Natasa, and Tammy. It was great learning alongside such dedicated and passionate people. I am also grateful to the members of my thesis committee for spending their valuable time reviewing this work.

This research would not have been possible without the financial support of the Nova Scotia Innovation and Research program, the Nova Scotia Health Research Foundation, and the Canadian Institutes of Health Research.

Finally, I am grateful to my family. Without the unwavering love and support of my parents I would not have gotten through the challenges of the last five years. And without my son I would never have had the energy and determination to follow through on this dream. His endless curiosity and enthusiasm is a source of constant inspiration. Thank you, Aries, I don’t know where I’d be without you.
Effects of Probiotics on Memory, ADHD, and Anxiety in Children

There are multiple pathways of communication, both afferent and efferent, between the gastrointestinal (GI) tract and central nervous system. This bi-directional neurochemical communication system has been termed the gut-brain axis (Cryan & Dinan, 2012). Centrally, activity in the hypothalamic pituitary adrenal (HPA) axis, which regulates cortisol levels and responds to environmental stress, influences function in the GI tract. Conversely, the enteric nervous system (ENS) and immune system influence the central nervous system (CNS) via the vagus nerve (Kennedy et al., 2012). Ascending and descending signals are also relayed between the enteric nervous system and the CNS via the sympathetic nervous system. By these routes, the CNS can alter motility and secretion in the GI tract while visceral sensations from the ENS reach the higher brain regions (Mayer, 2013). This complex system of messaging has been reviewed in detail by Emeran Mayer (2013). Investigation of these pathways has intensified over the past two decades and researchers are now focusing their attention on an area that appears to be amenable to alteration in the treatment of a growing number of disorders: the gut microbiome.

The Gut Microbiome

In 2012, there were four times as many studies published on the microbiome as there were in 2005 (Petschow et al., 2013). In human adults, the 1,000 or more microbial species that inhabit the GI tract (Collins, Surette, & Bercik, 2012) account for more than 90% of the cells in the body (Petschow et al., 2013). This microbial community is known collectively as the microbiome. There are multiple factors known to influence the composition of the microbiome, including: mode and timing of birth, host genetics, diet,
infection, and antibiotic use (Grenham, Clarke, Cryan, & Dinan, 2011; Petschow et al., 2013). Researchers had long believed that the human gut is virtually sterile at birth and that rapid colonization begins immediately postnatally. However, some researchers have isolated bacteria from meconium indicating that colonization of the gut microbiome may begin in the womb (Gritz & Bhandari, 2015). During the first year of life, there is significant inter- and intrapersonal variation in microbiome composition (Grenham et al., 2011). By one to three years, the microbiome takes on an adult-like profile. This same general pattern of development is also seen in rodents, which allows researchers to utilize germ free animals (animals which are surgically delivered and raised in sterile conditions to circumvent the normal bacterial colonization of the gut that occurs at birth) and animals which have had their microbiome altered (through the introduction of antibiotics or infection) as models to study the role of the microbiome in health and disease (Grenham et al., 2011).

There are approximately 540,000 microbial genes in the human gut and while a majority of people share a core 55% of these micro-organisms, many others are present in fewer than 20% of people (Petschow et al., 2013). This wide inter-individual variation has led researchers to examine the correlates between atypical microbiota and patterns of disease. The links between digestive disorders and the microbiome have attracted considerable attention from researchers. The gut microbiota of people with irritable bowel syndrome (IBS) have been shown to lack diversity and stability compared to healthy people (Kennedy, Cryan, Dinan, & Clarke, 2014). A similar lack of biodiversity was observed in children with ulcerative colitis (UC) compared to healthy children. Further, among children with UC, nonresponders to steroid treatment had even less
diversity than responders (Michail et al., 2011). Microbiome disturbances have also been observed in obesity. Specifically, obese mice and obese humans alike have been shown to have fewer Bacteroidetes and more Firmicutes, the two predominant bacterial phylotypes in the human gut, than healthy controls (Grenham et al., 2011).

*The Microbiome-Gut-Brain Axis*

The role of the microbiome in digestive illness makes intuitive sense but, increasingly, there is interest in how the microbiome affects cognitive, emotional, and behavioural function. Accordingly, the term microbiome-gut-brain axis has been adopted by many researchers (Cryan & Dinan, 2012; Grenhan et al., 2013). It has been clearly demonstrated that CNS activity can impact the microbiome. Bailey et al. (2011) studied the impact of daily exposure to stress on the microbiome in mice. When an aggressive mouse was introduced to the environment of non-aggressive mice for two hours, there was an immediate decrease in the diversity of the microbiome and an increase in circulation of the cytokine IL-6, which is seen in cases of chronic inflammation. An inverse relationship between microbiome abundance and cytokine circulation (i.e., immune response) was also found for three different genera of microbiota (Bailey et al., 2011). These results are consistent with earlier findings by O’Mahony and colleagues (2009) who demonstrated that maternal separation led to alterations in the gut microbiome and increased levels of inflammatory cytokines in rat pups. External stressors have been shown to produce similar effects in multiple studies (Cryan & Dinan, 2012), suggesting a clear descending influence from the HPA axis to the gut microbiome.

Two other lines of research that support the inclusion of gut flora as a key systematic player in the gut-brain axis are the observed links between microbiome
composition and CNS disorders, and the connection between digestive disorders and cognitive and emotional function. Cryan and Dinan (2012) report that multiple small studies have shown microbiome disturbances in children with autism spectrum disorder (ASD) compared to healthy controls. They rightfully suggest that these findings require further investigation, as children with ASD also tend to take more antibiotics and follow restricted diets, which may be responsible for the observed abnormalities. Previous research compared children with ASD to healthy siblings and found that, although antibiotic use was similar for both groups, 90% of those with ASD had digestive disturbance while only 25% of siblings did (Parracho, Bingham, Gibson, & McCartney, 2005), suggesting that antibiotics are not the sole contributor to microbiome abnormalities in this population.

As mentioned, abnormalities have been observed in the microbiota of people with digestive disorders. Links between digestive disorders with cognitive and emotional dysfunction have been examined in a number of ways. When digestive inflammation was induced in mice through the intracolonic injection of zymosan, a glucan found on the surface of fungi, anxiety-like behaviours were observed, as were pain biomarkers in the brain (Zhang et al., 2014). The introduction of an effective pain-inhibitor did not lead to any reduction in anxiety-like behaviour, suggesting that these behaviours were related to alterations in the gut rather than the resulting pain. Similarly, Gareau and colleagues (2011) similarly induced colitis in mice and assessed its impact on cognitive function. When exposed to stress in the form of a water avoidance situation, colitis-induced mice were shown to have considerable memory deficits compared to controls. Similar links between disordered digestion and brain function have been observed in humans. In a
study comparing university students who fulfilled the criteria for IBS to those who did not, 40% of the IBS-positive group also reported clinical levels of chronic stress and anxiety compared to only 20% in the non-IBS group. The severity of the participants’ anxiety symptoms was associated with the severity of their IBS symptoms (Gulewitsch, Enck, Schwille-Kiuntke, Weimer, & Schlarb, 2011). Heightened levels of anxiety have also been observed in people experiencing IBS symptoms up to ten years after a GI infection (Schwille-Kiuntke et al., 2011).

Kennedy and colleagues (2014) conducted a thorough assessment of cognitive function in people with IBS and found subtle deficits in performance on a test of visuospatial episodic memory, which is known to engage the hippocampus. Higher numbers of errors on this test were also significantly associated with lower levels of cortisol. The authors noted that this relationship was consistent with past research showing that HPA axis dysfunction can cause cognitive deficits on tasks that are reliant on the hippocampus (Kennedy et al., 2014). Cognitive inflexibility has also been observed in people with IBS on the Wisconsin Card Sorting Test, which requires repeated re-evaluation of success criteria based on error feedback (Aizawa et al., 2012). This inflexibility was associated with fMRI imaging showing lower levels of activity in the hippocampal and frontal regions compared to the control group (Aizawa et al., 2012). It is important to note that the cognitive deficits seen in IBS (and inflammatory bowel disease) do not extend to intelligence, as people with these disorders perform at the same level as controls on intelligence testing (Berrill et al., 2013; Kennedy et al., 2014). The altered cognitive function in people with a disorder that features abnormal microbiome composition supports a role for gut flora in optimal brain function.
Probiotics: Definition, Viability, and Safety

Probiotics are defined as living organisms that improve the health of the host when administered in sufficient quantity (Schrezenmeir & de Vrese, 2001). The health benefits of introducing live micro-organisms to the GI tract are being explored by researchers. Such probiotic therapy has been shown to improve a wide range of physical symptoms in people with IBS in clinical trials using placebo control groups (Didari, Mozaffari, Nikfar, & Abdollahi, 2015). In contrast to earlier definitions of these substances as promoters of new micro-organism growth (Holzapfel, Haberer, Geisen, Bjorkroth, & Schillinger, 2001), the beneficial nature of these substances is now viewed as their defining feature, along with a tendency to promote microbial balance (Schrezenmeir & de Vrese, 2001). Although probiotics are considered beneficial, there are numerous factors that affect their clinical efficacy.

Schrezenmeir and de Vrese (2001) point out that a probiotic must survive passage through the GI tract in order to fulfill this definition. Bezkorovainy (2001) reports that only 20-40% of ingested probiotics remain viable (i.e., survive) upon reaching the lower GI tract. This reduction is due primarily to the high levels of acidity and bile in the stomach (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012). However, recent advances in microencapsulating probiotics in a protective polymer matrix has allowed for the creation of probiotic products that survive transit through the stomach without breaking down. Cook et al. (2012) outline a variety of materials that have been used to create these polymer matrices, including plant sources (e.g., alginate), fats (e.g., palm oil), and whey protein. Another factor affecting the successful passage of probiotics through the stomach is timing relative to food consumption. Tompkins, Mainville, and
Arcand (2011) used a model of the human digestive system to assess whether the buffering effect of being ingested after eating would impact the amount of viable probiotics that survived. They found that if probiotics are taken prior to or with a meal, they survive at a much greater rate than if they are taken 30 minutes after food. Therefore, clinical trials of probiotics should take care to strictly control for timing around meals as failing to do so may lead to significant variation in effectiveness.

The safety of probiotics is supported by a long history of inclusion in both natural and processed food (Ishibashi & Yamazaki, 2001). No major safety concerns were found in a meta-analysis of 74 studies including a total of 15,885 children under the age of 18 (van den Nieuwboer et al., 2015). The population of participants included healthy children and children with intestinal disorders, inflammatory or allergic conditions, infections, obesity, and children with compromised immune systems. Study durations were generally less than three months although a small minority of studies were longer. Van den Nieuwboer and colleagues (2015) concluded that probiotic use was not associated with increased health risks in children, although they also expressed concerns that many of the studies reviewed made no mention of adverse events and could not be included in the meta-analysis.

**Probiotics and Cognitive Function**

Probiotics have been suggested as a plausible intervention to improve symptoms of Attention Deficit Hyperactivity Disorder (ADHD; Pelsser, Buitelaar, & Savelkoul, 2008) and enhance memory (Misra & Medhi, 2013). ADHD is characterized by increased impulsivity, hyperactivity, and an inability to maintain attention (American Psychiatric Association, 2013). It is the most common childhood neurobiological disorder, occurring
in about 5% of children (American Psychiatric Association, 2013; ATTENTION-DEFICIT, 2011). In order to meet the criteria for the disorder, children must exhibit impairment prior to the age of 12 years, in multiple settings (e.g., both at home and at school) for greater than six months (American Psychiatric Association, 2013). A proper evaluation would include information gathered from multiple sources, including parents, teachers, other care-givers, and/or mental health clinicians involved in the child’s care (ATTENTION-DEFICIT, 2011). A diagnosis of hyperactive/impulsive, inattentive, or combined presentation may be applied, depending on which symptom pattern predominates (APA, 2013). A meta-analysis of memory function in children with ADHD concluded that this population experiences working memory deficits in comparison with non-affected controls (Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005). Many biological factors have been suggested as contributors to the deficits observed in this disorder, including non-allergic hypersensitivity to food (Pelsser et al., 2008) and HPA axis dysfunction (Ma, Chen, Chen, Liu, & Wang, 2011). Blunted cortisol response to stress (a proxy measure of HPA axis function) has been observed in children with ADHD and associated with disinhibited behaviour (Ma et al., 2011). If it is correct that food sensitivity or HPA axis dysfunction are mechanisms of action in the etiology of ADHD then the introduction of probiotics to the microbiome may ameliorate cognitive deficits in ADHD.

There is evidence suggesting that diet can alter cognitive function and that probiotics can correct diet-induced cognitive deficits. Ohland and colleagues (2013) assessed the effect of Lactobacillus helveticus, a known probiotic, on memory in mice fed a western-style diet (18% protein, 49% refined carbohydrates, 33% fat). Mice with a
low-grade inflammatory condition that were fed a western-style diet for 21 days were shown to perform worse on a Barnes maze task than mice on a western diet supplemented with L. helveticus.

Davari, Talaei, Alaei, and Salami (2013) demonstrated that a mixture of Lactibacillus and Bifidobacterium probiotics could improve learning and memory in diabetic and healthy rats. In a study comparing the performance of diabetic rats to control rats on a Morris water maze task, probiotics administered for 56 days were shown to significantly improve memory in both groups. Indeed, diabetic rats given probiotics were not shown to be significantly different from healthy controls (Davari et al., 2013).

Memory enhancing effects of ingested bacteria have also been observed in healthy mice. Supplementation with Mycobacterium vaccae in healthy mice was shown to double the speed with which they completed a maze task compared to untreated controls (Matthews & Jenks, 2013). These findings support a role for probiotics as a valuable supplement for improving cognitive function but, until recently, direct evidence for the effectiveness of probiotics in the treatment of ADHD has been lacking.

A 2003 study by Harding, Judah, and Gant suggested that a combination of dietary supplements could improve performance on a Continuous Performance Task in a small sample of children with ADHD to the same degree as treatment with Ritalin. A combination of probiotics was among the supplements provided but it is impossible to ascertain what role they played in the observed improvements. The participants in this study were also not randomly assigned to treatment condition, further limiting the usefulness of this research in drawing any conclusions regarding probiotic supplementation for ADHD. More promising, however, are recent results from Pärty,
Kalliomäki, Wacklin, Salminen, and Isolauri (2015), who randomly assigned 75 newborns to receive a probiotic or placebo for the first 6 months of life. The researchers followed up at various time-points and found that by 13 years of age, 17% of participants in the placebo condition had developed either ADHD or Asperger syndrome while none of the participants in the probiotic condition had developed a disorder. Furthermore, the children who developed disorders also had lower levels of Bifidobacterium species in fecal samples taken during the first six months of life. It is possible that probiotics can help prevent the onset of ADHD but, as yet, no research has been performed to show that symptoms and cognitive function can be improved in extant cases. In comparison, the evidence for the efficacy of probiotics in improving emotional functioning is considerably stronger.

**Probiotics and Anxiety**

Probiotics have demonstrated efficacy in reducing anxiety-like behaviours in mouse models, and improving emotional well-being in humans. Anxiety disorders are characterized by either an exaggerated fear response to a situation or stimuli (as in panic attacks) or heightened tension and vigilance regarding anticipated outcomes along with dysfunctional activation of the autonomic nervous system (American Psychiatric Association, 2013). Anxiety disorders are among the most prevalent of mental health concerns (American Psychiatric Association, 2013). As in ADHD, working memory is often poorer in children with anxiety compared to non-anxious controls (Darke, 1988).

Matthews and Jenks (2013) observed a reduction in four anxiety-related behaviours in healthy mice that were given Mycobacterium vaccae, a bacteria commonly found in soil, prior to completing a maze task. Furthermore, after treatment was
withdrawn, the level of anxiety exhibited in both groups was nearly equalized (Matthews & Jenks, 2013). Savignac, Tramulla, Kiely, Dinan, and Cryan (2015) demonstrated the effect of a probiotic mix including Bifidobacterium longum on BALB/c mice, an innately anxious strain. Probiotic supplementation was shown to both reduce anxious behaviour and improve cognitive function. The authors noted that this was the first study to demonstrate cognitive enhancement in healthy animals, as past research has generally involved some form of infecting process or stress inducement. The fact that the mouse strain used for the study is innately anxious also provides support for the use of probiotics in anxiety disorders.

Messaoudi et al. (2011) examined the effects of a proprietary probiotic formulation combining L. helveticus R0052 and B. longum R0175 (produced by Institut Rosell-Lallemand, France) on anxiety and emotional disturbance in both rats and humans. The rat study compared three treatment conditions: probiotic supplementation, diazepam, and placebo. Following two weeks of treatment, behaviours were observed and measured after exposure to an electric shock. Anxious responding in the probiotic group was significantly lower than the placebo group, though not as low as in the diazepam group. Similar benefits were also observed in humans using the same probiotic formulation. Messaoudi et al. randomly assigned healthy participants to take either the combined L. helveticus R0052 and B. longum R0175, or a placebo for 30 days. There were significant improvements in the global severity index of the Hopkins Symptom Checklist-90 and the anxiety subscale of the Hospital Anxiety and Depression Scale.
**Probiotics and Cortisol**

Often referred to as the “stress hormone,” cortisol is a common measure used in research examining factors related to stress and anxiety (Messaoudi et al., 2011). Cortisol is the major hormone secreted by the adrenal glands when the HPA axis is activated and cortisol levels (e.g., in saliva, blood, etc.) provide a measure of HPA axis function (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Higher levels of cortisol in waking saliva samples are found among individuals with active anxiety disorders (Vreeburg et al., 2010). Conversely, lower levels of cortisol have been observed in children with ADHD (Ma et al., 2011). These findings indicate a common biological factor in both disorders – a dysregulated HPA axis. It is plausible, then, that an intervention aimed at improving the function of the microbiome-gut-brain axis would bring about positive benefits in both disorders.

Probiotic supplementation has been shown to affect cortisol levels in studies with both animals and humans. In a study examining the effect of probiotics on stress, it was shown that fish treated with probiotics exhibited lower levels of cortisol than controls when exposed to stress in the form of an acidic pH (Rollo et al., 2006). Significant reductions in cortisol levels were also observed in healthy humans after receiving L. helveticus R0052 and B. longum R0175 for 30 days (Messaoudi et al., 2011). These results suggest that probiotics may be an effective agent in regulating HPA axis function.

**Present Study and Hypotheses**

Stimulant medications prescribed to treat ADHD can cause a range of side effects including loss of appetite, weight loss, difficulty falling asleep, headaches, moodiness, restlessness, and abdominal pain (Sahin, Yuce, Alacam, Karabekiroglu, Say, & Salis,
2014). If effective, probiotic supplementation would offer a safe and tolerable treatment option with the added benefit of possibly improving other areas of function, such as digestion. Although there is pre-clinical evidence and theoretical grounds for viewing probiotics as a potential treatment option, as yet no human trials with children affected by ADHD and/or anxiety have been completed. Medications aimed at the treatment of anxiety (e.g., fluoxetine, sertraline, and fluvoxamine) are comparatively better-tolerated than ADHD medications. However, in Canada, their use in children is generally restricted to the treatment of severe cases of anxiety where there is significant functional impairment (Korczak, 2013). The data supporting the anxiolytic effects of probiotics in adults is limited, and there have been no studies exploring the affects of probiotics on anxiety in children. Positive results would represent an important step toward large-scale clinical trials which might conclusively demonstrate the efficacy of this form of treatment.

The present study is a double-blind, placebo-controlled clinical trial aimed at assessing the effects of probiotic supplementation on memory and symptoms of anxiety and ADHD in a population-based sample of children and adolescents. As with Messaoudi et al. (2011), the probiotic formulation tested was Lallemand’s proprietary combination of L. helveticus R0052 and B. longum R0175. Based on the combined evidence from research with healthy and disordered animals and recent trials in humans, the following hypotheses were tested in the current study:

1. Post-treatment levels of overall ADHD symptom scores would be lower in children given probiotics than those given a placebo treatment, after controlling for pre-treatment ADHD symptom levels.
2. Post-treatment levels of parent-reported anxiety symptoms would be lower in children given probiotics than those given a placebo treatment, after controlling for pre-treatment parent-reported symptom levels.

3. Post-treatment levels of child-reported anxiety symptoms would be lower in children given probiotics than those given a placebo treatment, after controlling for pre-treatment anxiety symptom levels.

4. Post-treatment verbal memory scores would be higher among children given probiotics than those given a placebo, after controlling for pre-treatment verbal memory scores.

5. Post-treatment visual memory scores would be higher among children given probiotics than those given a placebo, after controlling for pre-treatment visual memory scores.

**Method**

**Participants**

Fifty-two children between the ages of six and fifteen years exhibiting symptoms of anxiety and/or ADHD were recruited from the Annapolis Valley and Halifax areas of Nova Scotia. Of these, four were found to have sub-clinical symptom levels related to anxiety and ADHD and were removed from the analyses. Although scores on the eligibility questionnaire qualified these participants for the study, scores on the baseline Child Behaviour Checklist DSM scales completed at the first visit were within the normal range. Age and sex distribution for the remaining 48 participants in the probiotic and placebo conditions are summarised in Table 1. Parents/guardians of participants were
Table 1
Age and sex of the participants taking the probiotic supplement or placebo (Mean values and standard deviations with minimum-maximum values)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>18</td>
<td>9.6</td>
<td>2.3</td>
<td>6—15</td>
</tr>
<tr>
<td>Girls</td>
<td>7</td>
<td>10.3</td>
<td>2.7</td>
<td>7—14</td>
</tr>
<tr>
<td>Both sexes</td>
<td>25</td>
<td>9.8</td>
<td>2.4</td>
<td>6—15</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>17</td>
<td>9.5</td>
<td>2.3</td>
<td>6—14</td>
</tr>
<tr>
<td>Girls</td>
<td>6</td>
<td>10.2</td>
<td>2.1</td>
<td>8—13</td>
</tr>
<tr>
<td>Both sexes</td>
<td>23</td>
<td>9.7</td>
<td>2.2</td>
<td>6—14</td>
</tr>
</tbody>
</table>
required to have internet access and a functioning email address to complete
questionnaires and maintain contact with the researchers throughout the study.
Potential confounds and safety concerns were addressed through the following
exclusionary criteria: currently taking probiotics or antibiotics; dairy intolerance;
HIV/AIDS; undergoing chemotherapy; having cancer, Crohn’s disease, ulcerative colitis,
acute pancreatitis, or other immune-compromised condition (e.g., lymphoma, patients
undergoing long-term corticosteroid treatment); and a soy allergy. Participants taking
medication prescribed to treat either anxiety or ADHD medication were permitted to
enrol if the medication and dosage were consistent for two months prior to and
throughout the duration of the study. Inclusion criteria related to initial anxiety/ADHD
symptoms are detailed in the Materials section. Participants were deemed to have
symptoms in the clinical range if T-scores on the Child Behaviour Checklist DSM Scales
for Anxiety and/or ADHD exceeded the borderline cut-off. The clinical status of
participants in regards to ADHD and Anxiety problems for each of the study conditions is
presented in Table 2. Distribution of clinical status was equivalent across the two
treatment groups. Baseline group similarity was also confirmed by conducting paired
sample t-tests on CBCL T-scores for Anxiety and ADHD. Anxiety T-scores were similar
for the probiotics ($M = 65.1, SE = 9.4$) and placebo ($M = 63.6, SE = 9.9$) groups, $p = .589$.
ADHD T-scores were also similar for the probiotics ($M = 66.7, SE = 8.4$) and placebo ($M
= 68.3, SE = 7.2$) groups, $p = .661$.

Materials

Recruitment Material

The present study represents the first phase of a larger (13 week) research project.
Table 2
*Clinical status of participants taking the probiotic supplement, placebo, and overall as assessed by a Child Behaviour Checklist DSM Problem score exceeding the borderline cut-off.*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Probiotic (n=25)</th>
<th>Placebo (n=23)</th>
<th>Overall (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD Problems</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Anxiety Problems</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>ADHD &amp; Anxiety Problems</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>
Only those materials and measures that are relevant to this phase will be discussed here. Participant recruitment efforts included the use of online advertisements, newspaper ads, brochures, and posters. Online advertisements including a link to the study’s website were posted on Kijiji and Facebook, while newspaper ads were placed in local papers (Appendix A). Brochures were distributed to the offices of family physicians, psychologists, and other health service providers for display in waiting rooms (Appendix B). Advertisement posters were posted in public venues throughout Halifax and the Annapolis Valley with pull-tabs containing the study’s website URL (Appendix C). The study was also reported on in local print news media.

**Screening Material**

Upon accessing the study website, parents or guardians of potential participants were able to view a detailed information sheet about the study (Appendix D) and examine the study’s eligibility criteria (Appendix E). Individuals interested in having their child take part in the study completed the online consent form (Appendix F) and were then able to complete the online screening questionnaire (Appendix G). This questionnaire included items pertaining to the frequency and severity of the child’s ADHD and anxiety symptoms, as well as questions relating to the child’s general health, medical history, and diet. The children’s scores on this questionnaire determined if they met the criteria for the anxiety group (a score of 10 or higher on the anxiety questions, calculated by summing the scores of the endorsed items) or the ADHD group (an average score of 2.5 or higher on either the hyperactive/impulsive or inattentive symptoms, calculated by summing the scores of the items endorsed and dividing by the number of items, plus an average score of at least 1.5 on the other symptom cluster).
Baseline and Outcome Measures

Although a formal diagnosis was not required or performed prior to study participation the Child Behaviour Checklist (CBCL, Achenbach & Rescorla, 2001) was utilized to obtain baseline clinical characteristics of participants and confirm the screening measure of anxiety and ADHD symptoms completed prior to the first study visit. The CBCL is a 113 item diagnostic measure designed to identify the presence and severity of psychopathology based on parental report. The measure has been normed and validated for children aged 6 to 18 years. Parents respond to each item based on the extent to which it describes their child on a three point scale (0 = “not true”, 1 = “somewhat or sometimes true”, 2 = “very true or often true”). The present study utilized T-scores from the DSM-oriented scales related to ADHD and Anxiety to determine if symptom levels were homogenous across the two treatment conditions.

The ADHD subscale of the Child Disruptive Behaviour Disorders Rating Scale (DBD) is an 18 item, parental report measure designed to be used as a diagnostic tool for ADHD in children (Friedman-Weineth, Doctoroff, Harvey, & Goldstein, 2009). Each item corresponds to one of two subscales representing the two key symptom clusters in ADHD (Inattention and Hyperactivity-Impulsivity) and respondents are asked to respond to each of the symptoms on a four-point Likert scale (0 = “not at all”, 1 = “just a little”, 2 = “pretty much”, 3 = “very much”). Total scores range from 0 to 54 with higher scores indicating greater symptom severity. The DBD has acceptable reliability, internal consistency, factor structure, convergent/divergent, and discriminant validity (Friedman-Weineth et al., 2009).
The *Screen for Child Anxiety Related Emotional Disorders* (SCARED) is a child and parent self-report measure used to identify DSM-IV classified anxiety disorders in children. The 41-item version of the measure was used in the current study (Birmaher et al., 1999). Each item asks the respondent to indicate whether anxiety symptoms are present on a three-point scale (0=not true or hardly ever true, 1=somewhat true or sometimes true, 2=very true or often true). Total scores range from 0 to 123 with higher scores indicating higher levels of anxiety. Each item corresponds with one of five factors related to child anxiety disorders: panic/somatic, generalized anxiety, separation anxiety, social phobia, and school phobia. The measure has been shown to possess good internal and discriminant validity (between anxiety and disruptive disorders) and meta-analysis has demonstrated that the SCARED possesses discriminant validity cross-culturally, and reliably assesses the presence of anxiety in children (Hale, Crocetti, Raaijmakers, & Meeus, 2010). Parents completed the parent-version of the SCARED in relation to the participant’s symptoms, while participants completed the child-version of the SCARED.

Verbal memory was assessed using a verbal paired-associate learning (PAL) task created for the current study, similar to the *Word Pairs* test from the Children’s Memory Scale (Cohen, 1997). These new tests were created due to a lack of availability of a verbal learning test with 4 different versions. The test begins with a list of word pairs being read aloud to the child, followed by three learning trials in which the examiner says one of the words and the child attempts to name its paired associate. Following the three learning trials, the child is asked to recall all of the word pairs without prompting. A point is given for each correct answer. There are 14 word pairs for children aged nine years and above and ten word pairs for children aged six to eight years. A total score out of 56 was
calculated for participants in the older age range and out of 40 for the younger age range. In order to arrive at scores comparable across age groups, scores were converted into percentages. In order to facilitate re-testing across all four visits of the larger research project, four PAL word pair lists were created with words matched for number of syllables, emotional valence, concreteness, and familiarity (Toglia & Battig, 1978). Versions of the test were counterbalanced across the four visits of the larger research project using the balanced Latin Square method.

A Visual Memory Test, also created for this study, was used to assess participants’ ability to remember and recreate simple shapes. This test is a visual analog of the verbal PAL test in that it features pairs of shapes that are displayed together during a learning phase and then shown individually for three trials, during which participants are asked to draw the associated shape. Following the three trials, participants were asked to draw as many pairs of shapes as they could recall with no prompts provided. Children aged six to eight were shown six pairs of shapes and children older than eight were shown eight pairs of shapes. One point was given for each correct answer. A total score out of 32 was calculated for participants in the older age range and out of 24 for the younger age range, and percentages were calculated for comparison across age groups. As with the verbal memory test, four versions of each test were created and counterbalanced across study visits.

Investigational Product

The probiotics used for the study were supplied by Lallemand Health Solutions in Montreal. Commercially marketed as Probiotic Stick, the packets of fruit-flavoured powder contain probiotic strains of Lactobacillus helveticus R0052 and Bifidobacterium
longum R0175, microencapsulated to improve survival rates when exposed to high stomach acidity and bile salts. The placebo substance, comprised of the nonmedicinal ingredients contained in the probiotic powder but without the probiotics (i.e., fruit flavor, xylitol, maltodextrin, and malic acid), was also supplied by Lallemand. The probiotic and placebo were packaged in individual foil packets, unmarked except for a tiny batch number printed on each packet. Both researchers and participants were blind as to which batch numbers were probiotics and which were placebo. This information was known only to the Clinical Director at Lallemand Health Solutions. To prevent research assistants from seeing the batch numbers, the packets were placed in paper bags, stapled shut and labelled with participant code numbers. The bags were made up by individuals who did not meet with participants. Equal numbers of each batch code were bagged and stored in the lab refrigerator to maintain roughly equal numbers in each experimental condition. Random assignment to condition was achieved when the research assistant randomly took any coded bag from the refrigerator.

**Procedure**

The study protocol was reviewed by Acadia University’s Research Ethics Board and the study was registered on clinicaltrials.gov prior to starting enrollment. Following completion of the online screening questionnaire, participants who met the study criteria were scheduled for an initial visit to take place at either Acadia University or the Halifax location (an office in a private psychology practice). Informed consent for their continuation in the study (Appendix H) was obtained and any questions regarding the study were answered. Following informed consent, parents were given a questionnaire package including the CBCL, SCARED (Parent), and DBD to complete in a nearby
room. Child participants were shown where their parents would be to reduce anxious reactions related to separation. Memory testing was then conducted with the child participant starting with the verbal memory test followed by the visual memory test. Children then completed a computerized continuous performance task (CPT) which was intended for inclusion as part of the larger research study but yielded highly flawed data due to technical difficulties with the reaction time software. As a result, these data were not included in the present analyses. Children completed the SCARED questionnaire at the end of the study visit. Following testing, the participant’s parent was brought back to the testing room and a supply of 28 powder packets was provided to the participant to be taken daily for the next 28 days.

During the first week of the study, the researcher contacted participants by email on the third day to ensure that participants/their guardians did not have any concerns and/or questions. Following the initial week of each phase, participants were contacted weekly to monitor progress and address any concerns. Confidentiality was maintained by assigning each participant a code number based on the last four digits of their phone number and the first letter of their last name when they began the study. All study measures were matched with the participant’s data via their code number. At the second study visit, the primary outcome tests and measures were administered again following the same procedure as at the first visit. Compensation of $50 was provided to study participants at the end of the full 13-week research program.

**Results**

Descriptive statistics for the baseline CBCL T-scores for Anxiety and ADHD Problems at baseline are presented in Table 3, as well as the baseline and post-
intervention scores on the anxiety (SCARED child and parent), ADHD (DBD), verbal memory (PAL), and visual memory outcome measures. Each of the study outcome measures was analyzed separately to determine if post-intervention scores differed between the probiotic and placebo conditions after controlling for pre-test scores. One-way analysis of covariance (ANCOVA) was utilized due to its superior power compared to change from baseline methods in randomized trial designs (van Breukelen, 2013).

In order to correct for family-wise error rate while also reducing the risk of Type II errors, an alpha value of .01 was used as a cut-off for statistical significance (Perneger, 1998). All means reported in this section are adjusted.

1. Effect of Probiotics vs Placebo on ADHD Symptoms.

A one-way ANCOVA was run to determine the effect of the probiotic versus placebo on the participants’ post-intervention DBD Total scores after controlling for pre-intervention DBD Total scores. Post-intervention DBD scores were normally distributed for both conditions as assessed by Shapiro-Wilk’s test (p > .05) and visual inspection of histograms. There were no outliers as assessed by box plots. There was a linear relationship between pre- and post-intervention DBD Total scores for each condition, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, $F(1,44) = .074, p = .786$, and there was homogeneity of variances as assessed by Levene’s test ($p = .494$). Thus, the assumptions were met for ANCOVA.

Contrary to the hypothesis, there were no significant differences between the Probiotic and Placebo conditions on post-intervention DBD Total scores after controlling for pre-intervention scores, $F(1,45) = .018, p = .90$, partial $\eta^2 = .000$. However, the results
Table 3
*Means and standard deviations for outcome measures and CBCL problem scales.*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Probiotic (n=25)</th>
<th>Placebo (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>DBD Pre</td>
<td>50.0</td>
<td>13.0</td>
</tr>
<tr>
<td>DBD Post</td>
<td>45.4</td>
<td>13.4</td>
</tr>
<tr>
<td>DBD Change</td>
<td>4.6</td>
<td>8.9</td>
</tr>
<tr>
<td>SCARED Parent Pre</td>
<td>23.1</td>
<td>14.9</td>
</tr>
<tr>
<td>SCARED Parent Post</td>
<td>20.7</td>
<td>14.9</td>
</tr>
<tr>
<td>SCARED Parent Change</td>
<td>2.4</td>
<td>6.9</td>
</tr>
<tr>
<td>SCARED Child Pre</td>
<td>22.8</td>
<td>16.0</td>
</tr>
<tr>
<td>SCARED Child Post</td>
<td>20.2</td>
<td>15.9</td>
</tr>
<tr>
<td>SCARED Child Change</td>
<td>2.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Verbal Memory Pre</td>
<td>46.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Verbal Memory Post</td>
<td>47.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Verbal Memory Change</td>
<td>-1.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Visual Memory Pre</td>
<td>47.0</td>
<td>22.3</td>
</tr>
<tr>
<td>Visual Memory Post</td>
<td>52.3</td>
<td>23.8</td>
</tr>
<tr>
<td>Visual Memory Change</td>
<td>-5.4</td>
<td>18.1</td>
</tr>
</tbody>
</table>
were in the hypothesized direction with slightly lower post-intervention scores among children who received probiotics \((M = 45.5, SE = 1.9)\) than placebo \((M = 45.9, SE = 2.0)\) after controlling for pre-intervention scores.

Analyses were conducted to determine possible differences in sub-scores of the DBD. Two one-way ANCOVAs were run to compare the effect of trial group on DBD Hyperactivity (DBD-H) and Inattention (DBD-I) sub-scores. There were no significant differences in post-intervention scores on the DBD-H for those receiving probiotics \((M = 21.4, SE = .9)\) and placebo \((M = 21.9, SE = 1.0)\) after controlling for pre-intervention scores, \(F(1,45) = .170, p = .682\), partial \(\eta^2 = .004\). Post-intervention scores on the DBD-I were similar for those receiving probiotics \((M = 24.1, SE = 1.1)\) and placebo \((M = 24.0, SE = 1.2)\) after controlling for pre-intervention scores, \(F(1,45) = .011, p = .92\).


A one-way ANCOVA was run to determine the effect of the probiotic and placebo trials on post-intervention SCARED Parent scores after controlling for pre-intervention SCARED Parent scores. Post-intervention SCARED Parent scores were normally distributed for both conditions as assessed by Shapiro-Wilk’s test \((p > .05)\) and visual inspection of histograms. There were no outliers as assessed by box plots. There was a linear relationship between pre- and post-intervention SCARED Parent scores for each condition, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, \(F(1,44) = .003, p = .954\), and there was homogeneity of variances as assessed by Levene’s test \((p = .779)\). Thus, the conditions for ANCOVA were met.
Contrary to the hypothesis, the post-intervention scores did not differ between probiotic and placebo conditions, \( F(1,45) = .289, p = .59, \) partial \( \eta^2 = .006 \). However, the results were in the hypothesized direction with slightly lower SCARED Parent scores among children who received probiotics \( (M = 18.8, SE = 1.4) \) than those who received placebo \( (M = 19.9, SE = 1.5) \), after controlling for pre-intervention scores.


A one-way ANCOVA was run to determine the effect of the probiotic and placebo trials on post-intervention SCARED Child scores after controlling for pre-intervention SCARED Child scores. Post-intervention SCARED Child scores were not normally distributed for both conditions as assessed by Shapiro-Wilk’s test \( (p < .05) \) and visual inspection of histograms. However, the ANCOVA is robust to violations of this assumption when sample sizes are similar. Inspection of boxplots revealed potential outliers but standardized residuals were assessed and no outliers were deemed too extreme to warrant removal. There was a linear relationship between pre- and post-intervention SCARED Child scores for each condition, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, \( F(1,44) = .000, p = .992 \), and there was homogeneity of variances as assessed by Levene’s test \( (p = .275) \).

The probiotic and placebo groups did not differ on SCARED Child scores, \( F(1,45) = 1.212, p = .277, \) partial \( \eta^2 = .026 \). The results failed to support the hypothesis; in fact, post-intervention child-rated anxiety scores were somewhat higher among those receiving probiotics \( (M = 20.4, SE = 1.8) \) than placebo \( (M = 17.5, SE = 1.9) \) after controlling for pre-intervention scores.
4. Effects of Probiotics vs Placebo on Verbal Memory

A one-way ANCOVA was run to determine the effects of the probiotic and placebo on post-intervention verbal memory scores after controlling for pre-intervention verbal memory scores. Post-intervention verbal memory scores were normally distributed for both conditions as assessed by Shapiro-Wilk’s test ($p > .05$) and visual inspection of histograms. There were no outliers as assessed by box plots. There was a linear relationship between pre- and post-intervention verbal memory scores for each condition, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, $F(1,44) = .964, p = .332$. There was homogeneity of variances as assessed by Levene’s test ($p = .372$). Thus, the assumptions for ANCOVA were met.

The results failed to support the hypothesis that verbal memory scores would be significantly higher in the probiotic condition post-intervention, $F(1,45) = .747, p = .392$, partial $\eta^2 = .016$. However, the results were in the right direction, with children who received probiotics scoring somewhat higher ($M = 44.0, SE = 3.3$) than those who received placebo ($M = 39.9, SE = 3.4$), after controlling for pre-intervention scores.

5. Effects of Probiotics vs Placebo on Visual Memory

A one-way ANCOVA was run to determine the effects of the probiotics and placebo on post-intervention visual memory scores after controlling for pre-intervention visual memory scores. Post-intervention visual memory scores were normally distributed for both conditions as assessed by Shapiro-Wilk’s test ($p > .05$) and visual inspection of histograms. There were no outliers as assessed by box plots. There was a linear relationship between pre- and post-intervention visual memory scores for each condition,
as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant, $F(1,44) = 1.002, p = .322$, and there was homogeneity of variances as assessed by Levene’s test ($p = .995$). Thus the assumptions for ANCOVA were met.

As hypothesized, visual memory scores were significantly higher post-intervention among children who received the probiotics ($M = 53.3, SE = 3.6$) than those who received the placebo ($M = 34.2, SE = 3.8$) after controlling for pre-intervention scores, $F(1,45) = 13.401, p = .001$, partial $\eta^2 = .229$.

_Ancillary Analyses: Non-medicated Participants_

The possibility that the presence of medicated participants in the sample had introduced a confound was explored. Of the 48 participants included in the main analyses, 12 were being treated for their ADHD symptoms with stimulant medications (e.g., lisdexamfetamine, methylphenidate). Of these 12, nine had been randomly selected to receive the probiotic supplement while only three were assigned to the placebo group. Due to this imbalance in the distribution of medicated participants between groups, the post-hoc hypothesis that stimulant treatment may have weakened the effect of the probiotic supplement was explored by removing all medicated cases from the samples and re-running the analyses related to ADHD symptoms and memory function. Due to the exploratory nature of these analyses, results are presented as observed with no adjustments for group error rate so findings are only suggestive and should be interpreted with caution.

Separate ANCOVAs were conducted for DBD Total, DBD-H, and DBD-I scores in the non-medicated sub-sample ($n = 36$, probiotic $n = 16$, placebo $n = 20$). Post-
intervention scores on the DBD Total were lower for those receiving probiotics (M = 41.1, SE = 2.1) than placebo (M = 45.9, SE = 1.8) after controlling for pre-intervention scores, F(1,33) = 2.905, p = .10, partial η² = .081. Post-intervention scores on the DBD-H were lower for those receiving probiotics (M = 19.3, SE = 1.0) than placebo (M = 22.1, SE = 0.9) after controlling for pre-intervention scores, F(1,33) = 4.519, p = .04, partial η² = .12. Post-intervention scores on the DBD-I were slightly lower for those receiving probiotics (M =21.8, SE = 1.3) than placebo (M = 23.8, SE = 1.1) after controlling for pre-intervention scores but this was not a statistically significant difference, F(1,33) = 1.459, p = .24, partial η² = .04. The adjusted means of ADHD symptom measures for all participants (N = 48) are contrasted with the non-medicated sub-sample (n = 36) in Figure 1.

Separate ANCOVAs were conducted for verbal and visual memory scores in the non-medicated sub-sample (n = 36). Post-intervention scores on the verbal memory test were higher for those receiving probiotics (M = 48.6, SE = 3.8) than placebo (M = 37.3, SE = 3.3) after controlling for pre-intervention scores, F(1,33) = 4.928, p = .03, partial η² = .13. Post-intervention scores on the visual memory test were higher for those receiving probiotics (M = 59.1, SE = 4.6) than placebo (M = 35.5, SE = 4.1) after controlling for pre-intervention scores, F(1,33) = 14.798, p = .001, partial η² = .31. The adjusted means of the memory measures for all participants (N = 48) are contrasted with the non-medicated sub-sample (n = 36) in Figure 2. Although these are exploratory analyses, a clear trend emerged with all ADHD symptom and memory measures improving in favour of the probiotic treatment group when medicated participants were removed from the sample.
Figure 1: Adjusted means of post-treatment DBD scores for full sample (N = 48) and non-medicated sub-sample (n = 36) for probiotic and placebo groups. (* p < .05)
Figure 2: Adjusted means of post-treatment verbal and visual memory test scores for full sample (N = 48) and non-medicated sub-sample (n = 36) for probiotic and placebo groups. (* p < .05; ** p < .01)
Discussion

The present study investigated the effects of a daily probiotic supplement on memory, symptoms of anxiety, and symptoms of ADHD in children and adolescents. Most of the hypothesized effects, apart from those on visual memory, were not supported in the initial analyses. However, exploratory analyses of a sub-sample of the participants excluding those taking stimulant medications revealed effects in the expected direction for ADHD symptom measures, verbal memory, and visual memory. These findings are somewhat surprising and suggest areas for future research to determine the specific conditions under which probiotic supplementation might play an important role in the treatment of ADHD.

Probiotics and Anxiety

The hypothesized effects of probiotic supplementation on parental and child reports of anxiety were not supported. Post-treatment anxiety levels for those taking the probiotic were slightly lower according to parental report and slightly higher according to child report, but neither were statistically significant. These result are similar to those observed in a recent study with adults that failed to demonstrate anti-anxiolytic effects after eight weeks of treatment with the same probiotic formulation (Romijn, Rucklidge, Kuijer, & Frampton, 2017). However, these findings are inconsistent with past research demonstrating a reduction in anxious behaviour in mice given a probiotic supplement (Matthews & Jenks, 2013; Savignac, Tramulla, Kiely, Dinan, & Cryan, 2015). Although the bacterial species utilized by Matthews and Jenks differed from that used in the present study, the effects in Savignac et al. were observed using one of the two species (Bifidobacterium longum) investigated in the present study. It is not possible to make a
direct comparison of relative dosage between human and mice trials but it should be noted that the mice in Savignac et al. were given $1 \times 10^9$ CFU daily of B. longum while participants in the present study were given a combined daily dose of B. longum and Lactobacillus helveticus totaling $3 \times 10^9$. Relative to total body mass, the dose given to the mice was many times higher than participants in the present study, suggesting that higher dosages of probiotics may be required to obtain anti-anxiolytic effects.

These results were also inconsistent with the anti-anxiolytic effects observed by Messaoudi et al. (2011) in a sample of healthy adults with no reported problems related to anxiety and/or ADHD, that utilized a probiotic formulation and dosage identical to the present study. Differences between the two studies include participant age as well as type and severity of reported mental health problems. Taken together, the differences in the study participants are highly relevant, as self-report measures of anxiety in children with comorbid ADHD have been shown to lack reliability (Tsang et al., 2015). Indeed, self-report measures of anxiety failed to discriminate between children with an established diagnosis of anxiety disorder and those without a diagnosis in children who were also diagnosed with ADHD (Tsang et al., 2015). Research also points to the difficulty recognizing anxious symptoms in children with ADHD by outside observers. In a study of data from the Norwegian Patient Register, Hansen, Oerbeck, Skirbekk, and Kristensen (2016) determined that referral letters for half of children meeting the criteria for an anxiety disorder made no mention of anxiety as a symptom. Measures of anxiety in the present study may not have reliably captured the actual level of anxiety in the sample due to the more outwardly apparent symptoms of ADHD.
No conclusions can be made regarding the clinical utility of probiotics in the treatment of anxiety in children based on these results. An unexpectedly large proportion of participants who volunteered for the study experienced clinical or near-clinical levels of symptoms related to ADHD based on parental report on the CBCL. Based on the same measure, only 12 participants were classified in the clinical or near-clinical range for Anxiety problems without comorbid ADHD problems. Future studies should seek to determine the effect of probiotic supplementation on anxiety in children with a structured clinical interview to obtain a diagnostically pure sample of anxious participants.

_Probiotics and ADHD_

The hypothesized effects of probiotic supplementation on symptoms of ADHD were not supported when using the total sample. Indeed, post-treatment scores on the ADHD symptom measure were virtually the same for both groups, and exploratory analyses of the hyperactivity and inattention sub-scores on the DBD were also non-significant. However, when participants who were being treated with stimulant medications were removed from the sample, there was a clear trend toward a reduction in overall ADHD symptoms and hyperactivity sub-scores in those who were given a probiotic, as well as improvement in symptoms related to inattention. It must be noted that although p-values approaching or below the customary cut-off for significance of .05 were observed for overall symptoms and hyperactivity, these results must be considered exploratory as no group-wise error rate correction was applied. That said, the removal of stimulant-medicated participants resulted in a total sample of only 36 participants, making the near-significant trend even more notable.
Probiotics have been shown to prevent the development of ADHD when given in infancy, prior to the development of the disorder (Pärty et al., 2015). However, no previous published research has demonstrated the effectiveness of probiotics in reducing symptoms related to extant ADHD. Past studies have documented improvements in children given a combination of various nutritional supplements (Harding et al., 2003) but not probiotics in isolation and not in a placebo-controlled study design. Though exploratory in nature, the finding that behavioural symptoms of ADHD, particularly hyperactivity, were reduced through probiotic supplementation should be explored further. If the trends observed in the present study are replicated in a larger sample, the use of probiotics in the treatment of ADHD could be recommended as a viable first option prior to the use of stimulant medications. Though efficacious, stimulants such as methylphenidate and amphetamine cause problematic side-effects in many children that can impact treatment compliance (Sahin et al., 2014). In comparison, probiotic supplements are well-tolerated and have a very low side-effect profile (van den Nieuwboer et al., 2015).

**Probiotics and Memory**

The hypothesized effects of probiotic supplementation on memory were partially supported. Analyses of the full sample of participants revealed differential effects of probiotics on verbal and visual memory. Post-treatment visual memory scores were significantly higher (i.e., better) for the probiotic-treated group compared to the placebo-treated group but a non-significant effect of probiotics on verbal memory scores was also noted. However, when participants being treated with stimulant medications were removed from the sample, post-treatment memory scores among participants in the
probiotics group improved on both visual and verbal memory tests. These results suggest that probiotic supplementation is beneficial for memory function in children affected by ADHD.

The sample size of the current study did not allow for direct comparisons between those with Anxiety, those with ADHD, and those with a combined presentation of Anxiety and ADHD. Further research is required to clarify how probiotics might differentially improve memory across clinical groups. Furthermore, the investigation into the effects of probiotics could be expanded to include other areas of cognition, such as working memory, processing speed, and learning. Should probiotic supplementation be shown to facilitate wide-ranging cognitive enhancement, they could be employed to improve learning outcomes, particularly in those experiencing clinical problems.

In addition to clarifying the clinical uses of probiotics, underlying mechanisms of action should be explored. The improvement seen in visual memory is consistent with past research showing that people with digestive dysfunction also tend to have deficits in visuospatial memory (Kennedy et al., 2014). This finding points to a possible mechanism of action related to digestive function. Further studies examining the relationship between improvements in cognitive function and biological markers (such as bacterial diversity in the microbiome) following probiotic treatment would help to further clarify this.

Dysfunction of the HPA axis has been suggested as a contributor to the cognitive deficits experienced by people with ADHD (Ma et al., 2011). Investigating the association between cognitive improvements and a measure of HPA axis function such as cortisol levels would help to determine if the improvements in memory are due to regulation of
HPA function. This question is being investigated in the current sample of children as part of the larger research project.

*Stimulants, Diet, and Probiotics*

The surprising differences observed in the results between the full sample and the treatment-naïve subsample suggest several areas for further investigation. The sample size of the current study does not allow for a direct comparison of the effects of probiotics on medicated versus non-medicated participants. Future studies could look at these as separate groups to determine the extent to which stimulant medications reduce the effectiveness of probiotic treatment. The results of the present study strongly suggest that future probiotics research should avoid using stimulant-treated participants unless this comparison is one of the stated aims of the research. With regards to clinical uses, these results also suggest that probiotic supplementation (at least at the dosage used in this study) might be of limited usefulness in those taking stimulants.

A review of the respective effects of stimulant medications and probiotics on the microbiome and brain may provide plausible explanations for the suppression of any beneficial effects of probiotics among individuals taking stimulants. At the symptom level, stimulant medications are known to alter digestive function. Loss of appetite and weight loss are frequently reported by those taking methylphenidate, and abdominal pain is also a consistent, though less frequent, side-effect (Sahin et al., 2014). It is reasonable to consider whether the same mechanisms of action underlying these side effects may influence the activity of probiotics in the gut, which are known to improve symptoms of gastrointestinal dysfunction (Didari et al., 2015).
Sahin et al. (2014) investigated alterations in key hormones related to digestive function in people treated with methylphenidate and found that there were significant increases in ghrelin in blood samples of those taking the medication compared to controls. They suggested that this increase may have been a compensatory response to the appetite suppressing effects of the stimulant. Ghrelin is a peptide hormone created in the gut which stimulates vagal afferent nerves and functions as a neuropeptide in the central nervous system (Diano et al., 2006). In addition to regulating hunger and satiety, increased levels of ghrelin in the hippocampus are related to synaptic development and improvements in spatial memory and learning in mice (Diano et al., 2006). Laboratory results indicate that Lactobacillus and Bifidobacterium strains modulate ghrelin receptors found in the hypothalamus (Fuentes et al., 2016). The hypothalamus has been shown to have significantly higher uptake of peripheral ghrelin than the hippocampus in mice (Diano et al., 2006). Applying these findings to the current results, we could speculate that by inhibiting the uptake of ghrelin in the hypothalamus, probiotics may enhance memory function by promoting increased availability of ghrelin to the hippocampus. The increase in ghrelin observed in those treated with stimulants may “drown out” this effect due to an overabundance of peripheral ghrelin.

Stimulant medications and probiotics may also have differential effects on brain-derived neurotrophic factor (BDNF). BDNF is a protein found in the brain and periphery and its presence in the hippocampus has been shown to play an important role in learning and memory (Yamada & Nabeshima, 2003). In situ research has shown that Bifidobacterium longum can correct BDNF deficiencies in the hippocampus of mice (Bercik et al., 2010). It has also been demonstrated that a diet high in prebiotics increases
bifidobacteria in the gut of mice and BDNF in the hippocampus (Savignac et al., 2013). Conversely, methylphenidate use has been shown to reduce BDNF in humans (Sahin et al., 2014). The potential increase in BDNF, and associated enhancement in memory function, may be cancelled out by the reduction in BDNF caused by stimulant medication.

The interaction between nutrient intake, probiotics, and mental health symptoms should also be explored. The present study did not include a measure of participants’ diet throughout the intervention phase but there is growing evidence that nutrient intake, either in the form of food or supplementation, can substantially reduce symptoms of even very serious mental illnesses (Kaplan, Rucklidge, Romijn, & McLeod, 2015). It has been suggested that the introduction of a probiotic supplement may potentiate the absorption of nutrients, thereby augmenting their mental health benefits (Kaplan et al., 2015). Additionally, many foods contain forms of fiber (e.g., inulin, oligofructose) that resist being broken down in the upper gastrointestinal tract and are fermented by microflora in the large intestine (Slavin, 2013). This fermentation process leads to the growth of potentially beneficial bacteria that exert positive health effects in the colon. The inclusion of diet tracking in future trials would allow researchers to answer questions about how the food we eat impacts or interacts with the effect of probiotics.

The inclusion of diet tracking in future research would also allow for an investigation of the even more complex interactions that might exist between stimulant medications, probiotics, nutrition, and mental health. It is conceivable that the appetite-suppressing effects of medication leads to a reduction in the intake of prebiotic food, which in turn impacts the effectiveness of probiotic supplementation. There may also be
significant differences in the dietary habits of those who choose stimulant medications to treat their children’s ADHD symptoms versus those who are resistant to do so. For example, parents who opt for stimulant medications may tend to include fewer prebiotic foods in their child’s diet while those who do not use these medications tend to serve their children more prebiotic food. If prebiotic intake does augment the effect of probiotic supplementation, then these differences in dietary habits may explain some of the differences between medicated and unmedicated participants in the current study.

If probiotic supplementation is to be recommended as an option to improve cognitive function in children with ADHD, interactions with the most common medications prescribed for the disorder must be explored. It is possible that treatment effects are dose-dependent and higher doses of probiotics can be effective even when stimulant medications are also being used. It is also possible that no dosage of probiotics can overcome the potential suppressive effects of stimulants. The relationship between symptom and side-effect severity must be considered when making clinical decisions regarding the use probiotics as an alternative to medications. Further research may demonstrate a role for probiotics in less severe cases, especially when stimulants are not well-tolerated due to gastrointestinal distress.
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doi:10.1177/1087054712446830


Appendix A

Acadia University’s
Probiotics and Mental Health Research Lab

Does your child exhibit symptoms of

ADHD or ANXIETY?

or

Are you an adult who suffers from

ANXIETY or LOW MOOD?

Visit www.probioticstudy.com
to participate in our research
Appendix B

What are PROBIOTICS?

We can consume probiotics in certain foods as well as in supplements. Health Canada considers probiotics to be a Natural Health Product.

The gut microbiome, which lines the digestive tract, plays a role in normal development, digestion, nutrition, and immune system regulation, as well as influencing neurological functions. Factors such as cereal enterics, antibiotics, illness, stress, and diet can disrupt the balance of an individual's microbiome and contribute to health problems. Probiotics may help restore the balance.

Does your child have any of the following symptoms?

- Problems paying attention, concentrating, and finishing things?
- Difficulty sitting still, making their turn, and playing quietly?
- Problems with anxiety, worrying, and fearfulness?
- Overly shy compared to other children their age?

We are looking for children aged 6 to 14 to participate in a natural treatment research study. Children will be compensated for their participation.

Visit our website
www.probioticstudy.com

for more information about our studies and eligibility criteria. If you or your child would like to participate, please complete the online questionnaire on the website and a member of our research team will contact you.

Horton Hall, Room 242
Acadia University
18 University Avenue
Wolfville, NS B4P 1X5

Purpose of the study:
To determine whether probiotics, a natural treatment, will help reduce or eliminate symptoms of ADHD and/or anxiety, and associated gastrointestinal symptoms in children aged 6 to 11 years.

Questions and Answers

What compensation will my child receive for participating in the study?
Your child will be entered in a weekly draw for prizes such as movie passes, toys, gift certificates, etc. In addition, if your child completes the study, he or she will receive $20 cash at the last meeting.

Participants will also receive a two-month supply of probiotic/placebo provided as well as payment to help cover the costs of meals or food items to go with the probiotic.

Can I withdraw my child from the study if we change our minds about participating?
You may withdraw your child from the study at any time for any reason. Participation is completely voluntary.

Will the information my child and I provide be kept confidential?
Once the researcher has contacted you, all identifying information will be replaced with a code number. From that point on, all your questions and other data will be identified by code number only.

Who should I contact if I have questions about the study?
For more information, visit our website: www.probioticstudy.com.

Look for us on Facebook!
Appendix C

Recruitment Poster

CALL FOR PARTICIPANTS!

For a Study on the Effects of a Probiotic Supplement on the Symptoms of Anxiety and Attention Deficit-Hyperactive Disorder (ADHD)

Do you have a child between the ages of 6 and 14 living in the Wolfville or Halifax area who has been diagnosed with ADHD or an anxiety disorder, or experiences difficulty at home and school because of any of the following groups of symptoms?

- Problems paying attention, concentrating, and finishing things
- Difficulty sitting still, waiting their turn, and playing quietly
- Problems with anxiety, worrying, and fearfulness?

We are looking for children, aged 6 to 14 years, who live in the Wolfville or Halifax areas to participate in a natural treatment study. This study will examine the effects of a probiotic supplement on symptoms of attention deficit-hyperactive disorder (ADHD) and/or anxiety.

Your child must meet certain eligibility requirements in order to participate. For more information, please visit www.probioticstudy.com.
Appendix D

Study Information Sheet

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON
SYMPTOMS OF ADHD AND ANXIETY

Department of Psychology, Acadia University

Researchers
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

Please read the following information carefully and contact the researchers at probiotickids@gmail.com if you have any questions.

Definitions of terms used in this consent form:
Randomized: Selections are made in a manner similar to drawing numbers from a hat.

Double-Blind: Neither the child nor the researchers know who has been given which product (probiotic or placebo) until the end of the study. Should it become necessary, that information can be obtained.

Cross-over: Each child will be given each study product (probiotic or placebo) in turn. There will be a washout period between study products to ensure that the first product has cleared the body before giving the next product.

Parallel: Children are divided into different groups of the trial that take place at the same time. For example, one half of the children in the study take the probiotic powder while the other half of the children take a placebo.

Placebo: An inactive substance with no health effects.

1. Background
You and your child have been invited to participate in a study examining the effects of probiotics, consumed with dairy products, on the symptoms of attention deficit-hyperactivity disorder (ADHD), anxiety disorders, and related physiological and psychological factors.

Each individual’s gut contains many different strains of bacteria, which together form the gut microbiome (also known as the microflora). The microbiome consists of both good and bad bacteria, and it influences many processes in a person’s body, such as nutrient absorption, body weight, normal development, and brain functioning. Probiotics are the
good bacteria that exist within the gut microbiome and they are also present in some foods. Factors such as antibiotics, diet, cesarean birth, prolonged stress, and illness can affect the composition of one’s microbiome and cause imbalances. Imbalances among gut bacteria are associated with digestive disorders such as irritable bowel syndrome and Crohn’s disease; but gut bacteria also influence brain functioning. Microbiome disturbances have been linked to autism, depression, anxiety, and may also influence ADHD symptoms. One way that gut bacteria may exert their effects is through their influence on the regulation of a stress hormone called cortisol. Cortisol is often elevated in individuals with anxiety but low in those with ADHD. Consuming probiotics is thought to help restore proper balance within the gut, which in turn may have a positive effect on brain functioning and cortisol levels, thereby improving symptoms of anxiety and ADHD.

2. Purpose
The current study will examine the effect of a probiotic supplement consumed with milk or ice cream on the symptoms of ADHD and anxiety in children. The main goal is to determine if probiotics might be useful as a treatment for anxiety and ADHD symptoms in children. A second goal is to examine the effects of probiotics on saliva cortisol levels. Finally, we are also interested in the effects of the probiotics on children’s digestive health. This research is being conducted by Dr. Susan Potter, a clinical psychologist and associate professor in the Department of Psychology at Acadia University and Dr. Mark Johnston, a psychiatrist at True North Clinical Research in Kentville and Halifax. This research will also contribute to student theses at Acadia University.

3. Eligibility
In order to participate in this project, your child must be between the ages of six and fourteen years and be exhibiting inattentive, hyperactive, and/or anxiety symptoms that are causing some degree of difficulty for him or her at school and at home. You must live within reasonable driving distance of Wolfville or Halifax; you and your child must be able to meet with a researcher at Acadia University, your child’s school, or a clinic in Halifax four times during the study period (at the beginning and end of each phase - approximately every four weeks). You will need access to the internet and an email account that you check regularly. Your child must already drink milk with no adverse health effects (children who are dairy intolerant cannot participate). Children diagnosed with cancer, AIDS/HIV, Crohn’s Disease, active ulcerative colitis, or any other serious illness cannot participate (less serious digestive disturbances such as irritable bowel syndrome are permitted).

4. Overview of the Study
If you are interested in having your child participate in this research study, and you have not already visited the study website, http://probioticstudy.com, please visit the study website and complete the online questionnaire asking about your child’s ADHD and anxiety symptoms, health, medical history, and diet. If your child is eligible to participate, you will be contacted by one of the researchers to schedule an appointment to meet, either at Acadia University, your child’s school, or a clinic in Halifax (Roth & Associates). At this
meeting, the study procedures will be explained to you and you will be given the materials you need for the study. You will be asked to complete some questionnaires asking about your child’s behavior and ADHD and/or anxiety symptoms. You will complete some of these questionnaires again at the end of each phase of the study so we can determine if there has been any change in your child’s symptoms. With your permission, your child’s teacher will also be asked to complete some questionnaires regarding your child’s behavior and ADHD and/or anxiety symptoms at school.

The study will be carried out over a thirteen-week period. The first week is an information-gathering week during which your child will complete a daily food diary with your assistance (that is, they will be asked to write down what they ate each day) and answer a few questions about his/her bowel health, anxiety, and ADHD symptoms. Following this week, your child will consume either a probiotic powder or placebo powder (an inactive substance with no health effects) once each day for four weeks, taken with either milk or ice cream. This will be followed by a four-week “wash-out” period (no probiotic or placebo), then they will switch and take the other, probiotic or placebo, (whichever they did not receive during the first four weeks) for four weeks. Throughout the study period, you and your child will be asked to answer a few questions about your child’s mood and bowel health at the end of each week. Your child will be asked to provide a saliva sample four times over the course of the study, which will be analyzed for cortisol, an important stress hormone that may be influenced by anxiety, ADHD, and probiotics.

You will be provided with enough probiotics/placebo to give your child every day in each of the four-week phases. You will also be provided with an $8 Sobey’s gift certificate when you meet with the researcher at the beginning of each four-week phase to help cover the cost of the milk or ice cream.

5. What is a placebo?
A placebo is an inert substance (sometimes called a “sugar pill”) that would not be expected to have any health effects. A “placebo effect” is an improvement in health, or sometimes the experience of side-effects, that cannot be attributed to a treatment (i.e., it occurs in response to a placebo). Placebo effects often occur when a person believes they are taking an active treatment and, as a result, expects or anticipates that it will have an effect. Therefore, it is important to have a “placebo control” in a study like this because if your child’s symptoms get better simply because they are taking something they hope will work (i.e., the probiotics), we won’t know if the improvement was due to the probiotics or to your child’s expectations.

6. What will my child and I be asked to do?
Prior to your first meeting with the researchers, one of the researchers will contact you and explain the initial one-week information-collection period. The researcher will explain to you how to keep a food diary to keep track of what your child eats each day, and a few questions that we would like you to answer each evening about your child’s anxiety and
ADHD symptoms and gut/bowel health during this information collection week. These can be completed online, but a paper option is available and can be mailed (or picked up) for those who would prefer. In addition, an appointment will be scheduled for you to meet with one of the researchers at the end of the information collection week. You will not have to complete the food diaries again, but you will continue to answer the questions about ADHD/anxiety symptoms and gut/bowel health at the end of each week of the study period. This part is described in more detail below.

A. One-week Information Collection Period

- Each day for the first week you will help your child complete an online food diary (or paper version if preferred). You (or he/she) will be asked to list what he or she ate each day. The amounts don't need to be exact but it is important to list all foods and drinks consumed. This should take 5 to 10 minutes to complete.
- Each evening during this first week, you will be asked to answer a few questions about your child’s overall anxiety/ADHD symptoms that day and he or she will answer a few questions about his/her bowel health (e.g., number and type of bowel movements). We are interested in gastrointestinal symptoms because of their strong association with anxiety, ADHD, and microbiome disturbances. Answering these questions should only take a few minutes.

B. Initial Appointment

You will be asked to come to our office to meet with one of the researchers. This appointment will be approximately one hour long. During this session, the following things will happen:

- The study requirements will be explained to you and your child in detail and, if you and your child agree to participate, you will be asked to sign an informed consent form.
- You will talk with the researcher about your child’s anxiety and/or ADHD, other mental health issues and any physical health problems he or she may have, and complete some questionnaires asking about these types of issues.
- You will be given two saliva collection kits to take home. These consist of a small test-tube and straw that your child will put in his or her mouth and drool or spit into. Instructions on when and how to take the saliva samples are provided below. We will analyze the saliva samples for cortisol, a stress hormone, to see if probiotics have any effect on cortisol levels.
- You will receive your child’s probiotics or placebo packets and an $8 Sobey’s gift card to help cover the costs of the milk or ice cream, for the first four-week phase of the study.

Saliva Sample Collection

- You will help your child collect his or her first saliva sample one morning shortly after meeting with the researchers, BEFORE beginning to take the probiotic or placebo powder. Instructions for saliva sample collection are as follows:
  - First sample (Labeled “#1”): You should collect your child’s first baseline saliva sample as soon as possible after your first meeting with the researchers, before
beginning to take the probiotics or placebo. Do this first thing in the morning immediately after your child wakes up (have the kit ready the night before, in the child’s bedroom). They should not eat or drink anything until after you have collected the saliva sample. Write the date and time on the label. Follow the instructions with the kit provided. If his or her mouth is too dry, get him/her to try to imagine biting into a lemon or eating a favourite food. After collecting the sample, immediately place it in your freezer (deep freezer if you have one). Bring the saliva sample to your first meeting with the researcher.

- **Second sample (Labeled “#2):** Your child’s second saliva sample should be collected near the end of the first probiotic or placebo phase (do it during the last week, prior to your appointment with the researcher). As before, collect the sample first thing in the morning. Write the date on the label. Place the sample in your freezer.

- **Third sample (Labeled “#3”):** Collect this as described above, at the end of your child’s four-week wash-out period (i.e., the four weeks during which they do not take the powder). Write the date and time on the label.

- **Fourth sample (Labeled “#4”):** Collect this as described above, at the end of your child’s last four weeks in the study in which they take probiotics or placebo powder (during the last week, before your final meeting with the researcher). Write the date and time on the label.

- Bring the saliva samples to your next meeting with the researcher. It is okay if the samples cannot be refrigerated for an hour or two, but try to keep them frozen for as long as possible.

C. **First four week probiotic or placebo phase**

- During this phase, your child will consume one packet of probiotics or placebo (whichever you were given) each day for four weeks.
- To take the probiotic or placebo powder, your child should open the packet, tilt his/her head back, and pour the powder onto his/her tongue. He/she should then drink some milk (a half cup or more). Another option is to take the powder by mixing it with a small amount of ice cream.
- Try to give your child the probiotic/placebo at approximately the same time each day.
- Neither you nor the researcher will know whether your child is taking probiotics or placebo -- the packages all look alike but have different lot numbers (they will be identified at the end of the study).
- The probiotic packages will contain a flavoured probiotic powder (Probio'Stick) that contains a minimum of 3 billion good bacteria (cfu), produced by Lallemand Health Solutions. These probiotics are microencapsulated to protect them from stomach acids so that they can travel to the intestines and colon where they are believed to exert their beneficial effects.
- The placebo packages contain a flavoured powder that is inert (i.e., it will not have any effects on any of your child’s symptoms) that looks and tastes just like the Probio'Stick.
- You and your child will answer the questions about the severity of your child’s anxiety/ADHD symptoms and bowel health at the end of each week.
- You will receive emails from the researchers every few days during the first week to check in and see how your child is doing and answer any questions you may have. You will receive an email at the end of each week with a link to the weekly questionnaire.
Part way through this four-week period, you will be contacted by the researchers to set up your second appointment, which will take place near the end of the fourth week.

Towards the end of the fourth week of taking the probiotics or placebo, you will collect another morning saliva sample from your child, as before. Use the test-tube labeled “#2” and write the date and time on the label. After collecting the sample, place it in your freezer. Bring the sample with you to your next meeting with the researcher.

D. Second Appointment with the Researcher (End of first probiotic or placebo phase/Beginning of four-week wash-out period)

Your second appointment with the researcher will take place when your child is within a few days of finishing the first supply of probiotics or placebo (remember to collect your second saliva sample on a morning shortly before this meeting). This appointment marks the beginning of the four-week wash-out period. Please remember to bring your frozen saliva samples with you. This meeting should last about a half hour, during which:

- You will complete some of the questionnaires again that you completed at the beginning asking about your child’s anxiety/ADHD symptoms and gastrointestinal health during the past week.
- You will be given another saliva collection kit to collect a sample during the final week of this four-week wash-out period (use test-tube labelled “#3”).

E. Four week wash-out phase

- Researchers believe that when we consume probiotics, they do not colonize (set up house) in our gut, but move on out unless we keep taking them. The four-week wash-out period is necessary to make sure that probiotics taken in the first phase (for those who received probiotics first) have left your child’s body before moving on to the second phase.
- During the four-week washout phase, you will continue to answer the questions about your child’s anxiety/ADHD and bowel health at the end of each week.

F. Third Appointment with the Researcher (end of four-week wash-out period/start of second probiotic or placebo phase)

This phase is just like the first four-week phase, except that if your child took probiotics during the first phase, they will now take placebo, and if they took placebo during the first phase, they will now take probiotics.

- As before, your child will consume one packet each day, with milk or ice cream. Try to have them take it at approximately the same time each day as in the previous phase.
- You will be given an $8 Sobey’s gift card to help cover the cost of the milk or ice cream.
- You and your child will continue to answer the questions about your child’s anxiety/ADHD symptoms and bowel health at the end of each week.
- You will receive emails from the researchers every few days during the first week to check in and see how your child is doing and to answer any questions you may have.
- Part way through this four-week period, you will be contacted by the researchers to set up your final appointment, which will take place close to the end of the last week.
As before, towards the end of the fourth week of this phase, you will collect another morning saliva sample from your child. Use the test-tube labeled “#4” and write the date and time on the label. After collecting the sample, place it in your freezer. Bring the sample with you to your final meeting with the researcher.

G. Final Meeting with Researcher

Your final meeting with the researcher will take place near the end of the fourth week of the second probiotic/placebo phase. At this meeting:

- You will complete the questionnaires again that you filled out at the last meeting, focusing on your child’s symptoms and behaviour during the previous week.
- Any questions you have will be answered.
- Your child will receive $50 for his/her participation if they complete the entire thirteen weeks of the study. If you decide to not continue after the first phase your child will receive $20.

H. Six-Month Follow-Up

- In order to examine whether there are any long-term effects of taking probiotics during the study period, you will be contacted by a member of the research team approximately six months after your child completes the study. You will be interviewed and asked questions about your child’s health, probiotic consumption, diet, ADHD symptoms, and anxiety since completing the study and you will be asked to complete questionnaires again, similar to those completed at your last meeting with the researcher.

7. What are the ingredients of the probiotic and placebo powder and will my child feel any beneficial or adverse effects from taking them?

- The probiotic powder is made up of the following ingredients: two strains of probiotics, Lactobacillus helveticus and Bifidobacterium longum subsp. longum (3*10^9 CFU per sachet), artificial fruit flavour, xylitol (sweetening agent), matodextrin (filler), and malic acid (acidifying agent). The placebo powder has the same ingredients minus the probiotics.
- When your child is taking the probiotic powder, if it has a beneficial effect, you may notice an improvement in your child’s mood, anxiety levels, concentration, and behavior, and any digestive issues he or she may have had may get better. Some people report experiencing mild bloating and/or gas when they first begin taking probiotics, but these complaints are usually mild and subside within the first week or so.
- On the other hand, because the placebo powder is an inert substance with no health effects, it will not cause improvements in your child’s mood, anxiety levels, concentration, behavior, or digestion. However, some children taking the placebo may experience a “placebo effect” and feel an improvement in their symptoms (or sometimes negative side-effects) that they will attribute to the powder, assuming (incorrectly) that it must be the probiotic.
- We ask that you tell your child’s health care provider that your child is taking part in this study.
8. What are the risks and advantages of participating?

- There are no known risks associated with participating in this study. The probiotics have been studied before, are available over-the-counter in pharmacies and are generally considered safe. Some people may experience mild gastrointestinal side effects such as gas and bloating. It is possible that there are other risks of which we are not aware at this time. If we become aware of other risks, we will notify all research participants immediately. In the event that your child becomes ill or experiences any adverse reactions that you believe are caused by the probiotic or placebo powder, please notify the researchers immediately and stop giving your child the powder. Arrangements will be made for your child to be seen by Dr. Mark Johnston at the True North Clinical Research clinic in either Halifax or Kentville. If your child is seriously ill, take him or her to your family doctor or emergency department, and let them know that your child is participating in a probiotic study, then please notify the researchers right away.

- There will be no direct benefits to your child from participating in this study (other than a small financial compensation) because we do not know for certain that probiotics will help anxiety and ADHD, although the results of other past research are promising. However, your child will be contributing to the advancement of science and furthering our knowledge about the potential therapeutic effects of probiotics on ADHD/anxiety.
9. **Can my child participate if they are taking other medications?**
   Yes, but please discuss this with your physician before enrolling your child in this study and please notify the researchers of any medications your child is taking. For the purposes of the research product, it is okay if your child is taking medications for ADHD/anxiety as long as they have been taking a stable dose for at least two months and the dose is unlikely to change during the study period. However, your child cannot participate if they are taking antibiotics, undergoing chemotherapy, or they are taking medications for HIV. There may be other medications that are also contraindicated so please discuss this with the researchers.

10. **Will my child be paid for participating in this study?**
   - As a token of our appreciation, each month that your child is in the study, your child will be entered into a draw for the chance to win a prize (e.g., movie passes, toys, gift certificates, etc valued at approximately $20).
   - If your child completes the study, he or she will receive $50 cash at the last meeting. If you decide to not continue after the first phase your child will receive $20.

11. **Will the milk or ice cream required in the study be provided?**
   You will be provided with an $8 Sobey’s gift card at the beginning of each phase to help cover the cost of milk or ice cream for that phase.

12. **Can I withdraw my child from the study if we change our minds about participating?**
   You may withdraw your child or your child may withdraw himself/herself from the study at any time for any reason. If your child withdraws, he or she will no longer be entered into the prize draws and will not receive the $50 compensation at the end. If you decide to not continue after the first phase your child will still receive $20. If you wish to withdraw your child’s data, you must notify the researchers within one week of your child completing the study or withdrawing from it. If your child withdraws from the study, we would appreciate it if you would let us know why; this is important for Health Canada monitoring purposes and it may help us to improve the design of future studies.

13. **Can the researcher withdraw my child from the study?**
   You and your child may be withdrawn from the study by the researchers for failing to complete the required tasks; e.g., not completing the questionnaires, not taking the probiotics or placebo, missing appointments with the researcher, etc. If you are withdrawn by the researcher for not completing the required tasks, your child will not be entered into the prize draws and will not receive the $50 compensation at the end.

14. **What should I do if my child refuses to take the probiotic or placebo powder or doesn’t want to complete his or her questionnaires?**
   Participation in this research is completely voluntary. We fully respect your child’s right to withdraw from the study. If, however, your child is having a bad day and you believe he or she may feel differently later on, ask him or her again a bit later or the next day. It is okay if your child misses a day as long as it happens only occasionally. Do not coerce or force your child to take the powder if he or she does not want to.
15. **Will the information my child and I provide be kept confidential?**

- Once you have completed the online eligibility questionnaire and the researcher has contacted you, all identifying information (your child’s name, your name, and your contact information) will be removed from the server and you will be assigned a code number. From that point on, all your questionnaires and other data will be identified by code number only, except for teacher rating forms which will have your child’s first name until received by the researcher, at which point names will be removed and replaced with code numbers. Apart from this, neither your name nor your child’s name will appear on anything except the master code list and this will be stored separately in an encrypted, password-protected file on a password-protected computer. All online questionnaires will be housed on Canadian servers and your child’s data will be identified by code number only.

- Contact information collected during this study will be used only for the purpose of this study and will not be shared with anyone.

- Your child’s teacher will be asked to answer a few short questions asking about your child’s anxiety and ADHD symptoms at school. However, we will not share information we gather from you about your child with his or her teachers (unless you want us to and provide written permission), nor will we share with you information that your child’s teacher provides about your child (unless the teacher wants us to and provides written permission); that is, information your child’s teacher provides will be treated as confidential.

- Saliva samples will be identified by code number only and will be analyzed by a technician in the Biology Department at Acadia University.

- Although certain persons known as study monitors, auditors, and other regulatory authorities may be granted access to our research records for verification of clinical trial procedures and/or data, as required by Health Canada’s Good Clinical Practice Guidelines, the information they view will be identified by code number only; your child’s identity will not be revealed.

- If you are completing online questionnaires about your child from your workplace, be aware that your employer is legally entitled to monitor electronic communications in the workplace, and could view your responses.

- All information you and your child provide will be kept confidential, except where confidentiality must be waived by law (e.g., reports of child abuse, subpoenas, etc).

- When the results of this project are presented at professional conferences, discussed in student theses, and published in scholarly journals, only group results will be presented and individual participants will not be identified.

- All data files will be encrypted and storage disks will be password protected. Except when being transported, code-numbered paper data, saliva samples, and electronic data will be stored securely in a locked office at Acadia University.

- Your child’s code number will be entered in the monthly prize draw and you will be contacted by phone or email if your child’s number is drawn.

16. **Who is funding this study and are there any conflicts of interest?**

Funding for this study comes from four different sources:

- Mitacs – Accelerate grant (a government program to support graduate student training and research)
• Acadia University Faculty Association research grant (Article 25.55)
• Lallemand Health Solutions, Montreal, QC – Lallemand Health Solutions is providing the probiotics and placebo as well as financial support. Although they could benefit from the results should the study reveal any therapeutic effects of their encapsulated probiotics, the company is at arm’s length and is not involved in the data collection or analysis. This research may provide further scientific evidence of the therapeutic potential of Lallemand’s Probio’Stick; however, they are equally open to the possibility that the results might not be in their favour.
• Milk 2020 – this is a non-profit organization based in New Brunswick that is funded by dairy farmers and the government, aimed at developing and enhancing knowledge and innovation in order to support the growth of the dairy industry. Milk 2020 has provided financial support.

17. Are there other effective treatments available for treating ADHD and Anxiety Disorders in children?

Health Canada’s Good Clinical Practice Guidelines require us to discuss the potential benefits and drawbacks of other treatments that are available for your child’s condition.

ADHD: There are a variety of medications approved for the treatment of ADHD in children, and many children with ADHD are helped by these medications. The most commonly prescribed drugs for the treatment of ADHD are stimulants such as Ritalin™, Concerta™, and Adderall™. These medications help many students by improving their attention and reducing their hyperactivity. Some children taking these medication experience unpleasant or undesirable side-effects such as insomnia, loss of appetite, and headaches, and they don’t work for everyone; approximately one third of children with ADHD do not respond well to treatment with stimulants. There is a non-stimulant medication approved for children with ADHD called Strattera™ that is effective for some children. Cognitive-behavioural therapy can help children cope with their ADHD and teach them strategies to help focus and sustain their attention and reduce their impulsivity, but children with ADHD typically continue to struggle. Consistency in effective parenting, routines, and expectations, along with good nutrition, exercise, and sleep may also be helpful.

Anxiety: There are currently no medications approved by Health Canada for the treatment of anxiety disorders in children under the age of 18. Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly used to treat anxiety disorders in adults, but there are very few studies with children. The studies that do exist have yielded mixed results with most showing that SSRIs are not effective in children. Moreover, adverse emotional and behavioural reactions to SSRIs and other newer antidepressants can occur in children, including agitation, irritability and suicidal ideation. Another class of anti-anxiety medications known as benzodiazapenes (tranquilizers such as Valium™ and Ativan™) are often prescribed for adults, but these should not be given to children because they are highly addictive and can trigger anger and irritability in children. When a qualified and
experienced child psychologist is available, psychotherapy, particularly behaviour therapy (and cognitive-behaviour therapy for older children) can often help children with anxiety problems. Psychotherapy is most successful when combined with positive lifestyle changes (good nutrition, sleep, and exercise) and effective, consistent parenting.
18. **By participating in this study, will my child or I receive free counseling or psychotherapy for my child’s anxiety and/or ADHD?**

Unfortunately, we are not able to provide our study participants with counseling or psychotherapy for a number of reasons. However, we would be happy to refer you or your child to a mental health professional if you felt you needed to see someone.

We recognize that it is possible that by answering questionnaires focusing on your child’s anxiety, ADHD, and/or physical ailments, you may feel upset as you reflect on these issues. If this should happen and you or your child find yourselves in need of counseling, please contact one of the following:

- If you have a therapist, please contact him or her
- The appropriate Mental Health Clinic at
  - Mental Health, King’s County, Child & Youth Program (902) 679-2873
  - Valley Regional Hospital, Kentville (902) 679-2870
  - Annapolis Valley Health, Health Education (877) 365-1735
  - Hants Community Hospital, Windsor (902) 792-2042
  - Queen Elizabeth II Hospital, Halifax (902) 473-2043
  - Halifax Community Mental Health (902) 422-1611
- If you would like a specific referral, please contact Dr. Susan Potter or Dr. Mark Johnston (contact information is at the top of this information sheet).
- If you or your child are in need of urgent psychological help, please visit your nearest hospital emergency department.

19. **Who should I contact if I have questions about the study or concerns about the research?**

- For enquiries about the research project, or specific questions about your child’s participation, please email probiotickids@gmail.com
- The project supervisor, Dr. Susan Potter, can be reached at susan.potter@acadiau.ca or (902) 585-1220.
- For ethical concerns, please contact the Chair of the Acadia University Research Ethics Board, Dr. Stephen Maitzen, at smaitzen@acadiau.ca or (902) 585-1498.

This clinical trial has been approved by the Acadia University Research Ethics Board and Health Canada’s Natural Health Product Directorate.
Appendix E

Eligibility Criteria

Please read all of the following statements carefully. If your child meets ALL of the following requirements and you wish for him or her to participate in the study, please click NEXT to continue. If your child does not meet one or more of the following criteria, we are sorry, but your child is not eligible to participate in this study. Thank you for your time.

- My child is between the ages of 6 and 14 years
- My child is not dairy intolerant (he/she can consume milk without any adverse effects)
- My child is not allergic to soy
- My child is not currently taking any antibiotics or probiotics
- My child has not been diagnosed with any of the following:
  - Cancer
  - HIV/AIDS
  - Crohn’s disease
  - Ulcerative colitis
  - Other serious illness
- I have daily access to the internet and a valid email address that I check regularly

It is important that your child avoids foods containing probiotics throughout the thirteen-week study period. Foods that should be avoided for the duration of the study include:

- Probiotic yogurt (or foods containing it, such as frozen yogurt or yogurt drinks/smoothies) – you will be given a list of yogurt brands that are okay to eat
- Kefir
- Kimchi (Kim chee)
- Unpasteurized/uncooked tempeh
- Unpasteurized/uncooked miso
- Sauerkraut (fermented, unpasteurized)
- Sour cream
- Cottage cheese
- Most uncooked cheese (cheddar, feta, gouda, etc.)
  - Cheese produced by Arla and Saputo may be eaten during the study
  - Processed cheese slices are also okay to eat
  - Do not eat cheese made by Cow’s Dairy (PEI) because it contains probiotics
- Tamari and soy sauce – unless it is pasteurized
- Any other foods that contain probiotics
Appendix F

Online Consent Form for Eligibility Questionnaire, Food Diary, and Daily Symptoms Questionnaire

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON SYMPTOMS OF ADHD AND ANXIETY

Researchers:
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

You and your child have been invited to participate in a study examining the effects of probiotics on the symptoms of attention deficit-hyperactivity disorder (ADHD), anxiety, and associated physiological and psychological factors, as described in the project information sheet. Please review the information sheet before consenting to participate.

This consent form is for the initial online questionnaire (described in section 4 on the information sheet) and one-week information collection period (food diary and daily questions about symptoms described in section 6 on the information sheet) only. These questionnaires and food diary will help the researchers determine if your child is a good candidate for the study and provide them with useful information if your child meets the eligibility criteria and you and your child decide to participate.

By checking the box below you are indicating that you are the legal parent or guardian of your child and that you have signing authority for your child, that you have read the information sheet, that you understand the nature of the study and what is required of you and your child, and you are providing your free and informed consent to participate in the initial screening questionnaire for this study. By consenting to participate, you are not waiving any of your legal rights by consenting to participate in this study. Your participation in this research project is greatly appreciated.

Contact information collected during this study will be used only for the purpose of this study.

☐ By clicking “SUBMIT”, I acknowledge that I have read and understand the above information and hereby consent to participate in the initial screening portion of this study. I am free to discontinue my participation at any time.

SUBMIT
Note: if you encounter any difficulties while completing this questionnaire, please click the back button on your browser and try clicking “next” again. If you continue to encounter difficulties please email the researcher.
Appendix G

**Online Questionnaire (Demographic and Screening Questions)**

Please provide all of the following information:

Your Name: ________________________________________________

Relationship to child: _______________________________________

Address: __________________________________________________________________________

E-mail Address: _________________________________________________

Phone: Home: __________________________________ / Cell: ______________________________

Child’s Name: _________________________________________________

Child’s Age: _________

Child’s Gender:  
☐ Male  ☐ Female

**NEXT**

Is your child currently taking any medication for inattentive and/or hyperactive/impulsivity symptoms?

☐ Yes  ☐ No

If you answered yes to the above question, please specify which medication(s) your child is taking, the dose, and how long they have been taking it:

Is your child currently taking any medication for anxiety symptoms?

☐ Yes  ☐ No

If you answered yes to the above question, please specify which medication(s) your child is taking and how long they have been taking it:
If your child is currently taking any other medications (for symptoms other than those associated with attention deficit-hyperactivity disorder or anxiety disorders) please list them here:

______________________________________________________________________________

Does your child have any diagnosed illnesses?

☐ Yes

☐ No

If yes, please specify the illness/illnesses:

______________________________________________________________________________

Has your child been tested for any thyroid diseases?

☐ Yes

☐ No

If yes, please specify any diagnosis:

______________________________________________________________________________

Has your child ever had his or her cortisol levels tested?

☐ Yes

☐ No

If yes, please specify the results:

______________________________________________________________________________

Has your child experienced any yeast- or fungal-infections in the past two years? (including skin rashes/fungal infections such as athlete’s foot or Candidiasis vaginal yeast infection, thrush of the mouth/throat, etc.)

☐ Yes

☐ No

If yes, please specify:

______________________________________________________________________________

Does your child have any diagnosed psychiatric, developmental, or neurological conditions?
Does your child currently attend sessions with a mental health professional?

☐ Yes

☐ No

If yes, please specify:

_______________________________________________________________

Does your child currently attend sessions with a mental health professional?

Please answer the following questions with respect to your child's behavior.

Indicate how much the following statements apply to your child during the past 6 months, using the following scale:

1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Inattention Symptoms:</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has difficulty giving close attention to details in schoolwork or other activities</td>
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<tr>
<td>Has difficulty sustaining attention in chores or play</td>
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<tr>
<td>Does not appear to listen when spoken to</td>
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<tr>
<td>Cannot follow through on given instructions (but instructions are understood)</td>
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<tr>
<td>Cannot organize his or her own tasks or activities</td>
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<tr>
<td>Avoids or dislikes activities that require sustained attention</td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td>Often loses track of his or her possessions (pencils, schoolwork, toys, etc.)</td>
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<tr>
<td>Easily distracted by other things in his or her environment</td>
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<tr>
<td>Is forgetful in his or her everyday activities</td>
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</tbody>
</table>
Indicate how much the following statements apply to your child during the past 6 months, using the following scale:
1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Hyperactive/Impulsive Symptoms</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fidgets or squirms in his or her seat; has difficulty sitting still</td>
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<tr>
<td>Has difficulty staying in his or her seat in situations where it is expected</td>
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<tr>
<td>Is overly active, running and climbing when not appropriate for the situation</td>
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<tr>
<td>Difficulty engaging in leisure activities quietly</td>
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<tr>
<td>Talks excessively</td>
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<tr>
<td>Could be considered “on the go” or “driven by a motor”</td>
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<tr>
<td>Has difficulty waiting for his or her turn when in a group</td>
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<tr>
<td>Interrupts other children’s games or intrudes into other’s activities inappropriately</td>
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<tr>
<td>Frequently answers questions before the speaker has finished saying the question</td>
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</tbody>
</table>
Indicate how much the following statements apply to your child \textbf{during the past 6 months}, using the following scale:
1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Anxiety Symptoms</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is more shy and anxious than other children his or her age</td>
<td></td>
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<tr>
<td>Is more worried than other children his or her age</td>
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<tr>
<td>Is afraid of many more things than other children his or her age.</td>
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<tr>
<td>Worries that something terrible is going to happen to him/herself or his/her family</td>
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<tr>
<td>Is afraid to sleep away from home (e.g., sleep-overs at his or her friends’ houses)</td>
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</tr>
</tbody>
</table>

Thank you for your time and interest in this research project. A researcher will be in contact with you within approximately one week to discuss your potential participation in this study, and to set up an initial meeting for you and your child.

If any of these questions have left you concerned about your child’s behavior and you would like to speak a professional, please contact one of the following:

- Your child’s therapist, if applicable;
- Halifax Community Mental Health, Tel: (902) 422-1611
- Annapolis Valley Health, Health Education, Tel: 1-877-365-1735
• Mental Health, King’s County, Child & Youth Program, Tel: (902) 679-2873
Once you click the SUBMIT button at the bottom of the page, you will be redirected to the Probiotic Study homepage.
Appendix H

Consent Form – Intervention Phase

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON
SYMPTOMS OF ADHD AND ANXIETY IN CHILDREN

Researchers
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

You and your child have been invited to participate in a study examining the effects of probiotics on the symptoms of attention deficit-hyperactivity disorder (ADHD), anxiety, and associated physiological and psychological factors, as described in the project information sheet. Please review the information sheet before consenting to participate. Section 6 of the information sheet describes what you and your child will be asked to do at each step in the study. Contact information collected during this study will be used only for the purpose of this study.

By signing below you are indicating that:
- you are the legal parent or guardian of the child named on the form and that you have signing authority for the child
- you have read the information sheet and understand the nature of the study and what is required of you and your child
- you have explained the study to your child and he or she has agreed to participate
- you are providing your free and informed consent to participate in this study with your child
- you are not waiving any of your legal rights by consenting to participate in this study

Please check the box below if you consent for your child’s teacher to know of their participation in the study and comment on any change in your child’s ADHD and/or anxiety symptoms. Your child’s name will appear on the teacher questionnaire until it has been received by the researcher, at which point it will be changed to your child's code number.

☐ I consent for my child’s teacher to know of his or her participation in this study.

☐ I consent for my child’s teacher to answer questions about my child’s ADHD and/or anxiety symptoms and acknowledge that the teacher’s answers will not be shared with me.

We may carry out similar studies in the future. Please check the box below if you are willing to be contacted in the future about participating in other similar studies. Your time, participation, and contribution to this research project and science in general are greatly appreciated. Any contact information disclosed below will be used only for the purpose of informing you of our
future studies. Checking this box does not mean you have to participate in these studies, you will simply be notified.

☐ I am willing to be contacted about participating in other similar studies in the future.

Name: _________________  Email address: _______________  Phone number: ______________

Please indicate why you and your child are choosing to participate in this study:

______________________________________________________________________________

Name of Child: _____________________________________

Name of Parent: _________________________________

Signature of Parent: _______________________________  Date: __________________

Signature of Researcher: ___________________________  Date: __________________