

Probiotic and Fermented Food Intake and Its Association with Gastrointestinal Symptoms in
Collegiate Athletes

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Major Project/Report submitted to the faculty of the Virginia Polytechnic Institute and State
University in partial fulfillment of the requirements for the degree of

Online Master of Agricultural and Life Sciences
In
Applied Nutrition and Physical Activity

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May 4, 2021

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I. Abstract

Background: Probiotic supplements have shown the potential to reduce gastrointestinal (GI) distress symptoms; however, there is currently little research on dietary probiotic intake or the impact of probiotic food consumption on GI symptoms, particularly among athletes. **Purpose:** The purpose of this project was to: 1) develop and evaluate a tool for assessing intake of probiotic foods among collegiate athletes, 2) assess intake of probiotic and fermented foods among collegiate athletes at Virginia Tech, and 3) explore associations between intake of these items and GI symptoms in this study population. **Methods:** A literature review, review of 360 dietary recalls previously collected from collegiate athletes (n=120), and evaluation by five experts in sports nutrition and/or probiotic foods informed the development of a brief probiotic food frequency questionnaire (PRO-Q). The PRO-Q was then administered to participants (n=42), all of whom were members of Division I sports teams at Virginia Tech. Validity was assessed by comparing PRO-Q responses with probiotic intake determined using three 24-hour diet recalls (reference method). Participants also completed a questionnaire to evaluate type and severity of GI symptoms. **Results:** Overall intake of probiotic/fermented foods in this population was low (0.77 servings/day), with only 17% of participants reporting daily consumption. Based on Spearman's Rho ($r=0.766$, $p<0.001$), Wilcoxon Signed-Rank test ($p=0.168$, indicating no difference between methods), and Bland-Altman analyses (95% agreement), PRO-Q responses were consistent with dietary recalls for average daily intake of probiotic/fermented foods. A total of 52% of participants reported experiencing regular GI distress symptoms. There was no

association between PRO-Q responses and overall GI symptom score ($r = 0.297$, $p = 0.056$).

Conclusion: These findings suggest that among collegiate athletes, probiotic/fermented food intake was generally low and GI distress symptoms were prevalent. The PRO-Q shows promise as a brief, self-administered method to assess probiotic/fermented food intake among collegiate athletes, although additional research is needed to confirm reproducibility and generalizability in larger samples.

Keywords: Microbiome, GI distress, food frequency questionnaire, dietary assessment tool, validation study

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Keywords/terms:

BMI: Body Mass Index

CFU: Colony Forming Units

FFQ: Food Frequency Questionnaire

NCAA: National Collegiate Athletic Association

NDSR: Nutrition Data System for Research

PRO-Q: Probiotic Questionnaire for Collegiate Athletes

VT: Virginia Tech

II. Introduction & Purpose

According to the Food and Agriculture Organization of the United Nations and the World Health Organization, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002). To be classified as a probiotic, a food or beverage must contain live bacteria. Fermented foods, on the other hand, are defined as “foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action” (Dimidi et al., 2019). Yogurt, sauerkraut, tempeh, kimchi, sourdough, and soy sauce are some of the more commonly consumed probiotic/fermented foods and beverages (Marco et al., 2016).

Intense exercise is known to be associated with gastrointestinal (GI) distress, particularly, bloating, nausea, cramps, and diarrhea (Davidson et al., 2016). Research by de Oliveira et al (2014) showed that 30-50% of athletes experience GI complaints during exercise. A number of factors may predispose athletes to a negatively altered microbiome including inadequate fiber intake, reduced intake of resistant starches, high protein intake, exercise induced stress, increased proinflammatory cytokines, and increased intestinal permeability. Probiotic consumption has been associated with positive effects on multiple health parameters for athletes, including GI conditions, immune function, depression, gut permeability, and inflammation (Wosinska, Cotter, O’Sullivan, & Guinane. 2019). Probiotics may reduce GI complaints by decreasing intestinal permeability, positively altering the microbiome, and reducing symptoms commonly associated with irritable bowel syndrome (IBS) (David et al., 2013; Lamprecht et al., 2012; Sadrin et al., 2020). There is some evidence that, in addition to these health benefits, probiotic intake may also influence athletic performance, with a study by Shing (2013) on endurance runners in the heat

showing a reduction in time to fatigue after four-weeks probiotic supplementation. However, probiotic/fermented food intake among this population has not been well researched.

Presently, no tool for assessing probiotic/fermented food intake among college athletes is available. Food frequency questionnaires (FFQ) are a common methodology for dietary assessment. They allow for assessment of items not consumed on a daily basis as they reflect intake over a specific period of time, making them a better tool than diet recalls when looking at overall usual intake (Institute of Medicine (US) Committee on Dietary Risk Assessment in the WIC Program, 2002). A validated FFQ specific for collegiate athletes is needed to help quantify probiotic/fermented food intake in this population and provide a tool for future assessment of probiotic/fermented food intake among collegiate athletes.

The primary aim of this study was to create and validate a FFQ that can be used to evaluate intake of functional fermented foods that may contain probiotics among collegiate athletes. The secondary aims were: 1) to evaluate intake of probiotic/fermented foods among this population, 2) to describe prevalence and frequency of GI distress symptoms among college athletes, 3) to explore the association between GI distress symptoms and probiotic/fermented intake.

III. Review of Literature

The purpose of this review is to summarize literature related to the effects of probiotics on GI symptoms and physical performance in athletes, and typical intake of probiotics by athlete populations. In some cases, both research on athletes and the general population were reviewed as limited research on the athlete population alone is available.

Probiotics

Probiotics have become increasingly popular due to their proposed health benefits and because of this, are considered functional foods (Yan & Polk, 2006). Probiotics can be consumed in foods or in supplement form. The market for probiotics has been growing at a rate of about 7% per year (Day et al., 2019). There is currently no daily recommended intake for probiotics due to the diversity of the microbiome and insufficient data on how different strains affect different individuals. Day et al. (2019) reported that it is still unclear how different strains and species affect the host making it difficult to define a healthy gut microbiome. Different strains also serve different benefits so targeted intake may depend on the desired outcome. For example, *Clostridium species pluralis* has been shown to specifically impact brain centers suggesting it may be used to address gut-brain axis interactions (Rao et al., 2009).

While many strains of probiotics have been studied, Moller et al. (2016) reports *Lactobacillus* as the most common intervention in studies, with *Bifidobacterium* as the second most common. Traditionally, dairy products have been considered the optimal method for obtaining probiotics from food (Saikia & Dutta, 2019). However, recently non-dairy products have been proven viable sources as well (Saikia & Dutta, 2019). Table 1 lists some specific probiotic species and their respective dietary sources.

Table 1: Probiotic strains and some of their known dietary sources.

Probiotic Species	Dietary Source	References
<i>Lactobacillus</i>	Yogurt, kimchi, sourdough bread, sauerkraut, cheese, olives, sour cream	Marco et al., 2017
<i>Bifidobacterium</i>	Yogurt, kombucha, sauerkraut, kefir, cheese	Marco et al., 2017; Wosinska et al., 2019
<i>Saccharomyces</i>	Wine, beer, yogurt, kombucha, sauerkraut	Marco et al., 2017; Wosinska et al., 2019
<i>Streptococcus</i>	Yogurt	Marco et al., 2017
<i>Bacillus</i>	Soy sauce, natto	Lee et al., 2017
<i>Rhizopus</i>	Tempeh	Marco et al., 2017

In general, 10^6 cells/g is the recommended level of microorganisms at time of consumption; however, several studies have suggested a minimum daily dose of 10^8 - 10^9 cells/g or 100 grams of foods containing 10^6 - 10^7 cells/g to ensure survival of the microorganisms after passing through the GI tract (Karimi et al., 2011). Yogurt, kefir, cheese, sauerkraut, miso, kimchi, and kombucha, contain 10^6 and 10^9 cells/g (Marco et al., 2017). Viability of the probiotics can be impacted by many factors including pH, temperature, artificial flavors or coloring, packaging, storage condition, and presence of other food compounds such as salt or sugar (Terpou et al., 2019). It is important to note that not all fermented foods contain viable microorganisms. For example, the viability of *Bifidobacteria* in Gouda cheese is dependent upon the region of origin, salt content, and any addition of protein hydrolysate, meaning not all Gouda cheeses contain probiotics (Karimi et al., 2011).

Athletes, Exercise, and Gastrointestinal Distress Symptoms

Prevalence

Due to a combination of physiological, nutritional, and mechanical factors, it is estimated that about 30-50% of all athletes experience GI complaints during exercise, with a higher prevalence among endurance athletes (de Oliveira, Burini, & Jeukendrup, 2014). Prevalence of GI symptoms will vary greatly depending on the situation, assessment tool used, and symptoms asked about. Symptoms may include flatulence, belching, nausea, vomiting, diarrhea, abdominal pain, and bloating. A significant increase is often seen among endurance athletes in particular, as one study showed that 84% and 78.3% of male and female runners, respectively, reported at least one GI complaint during training over a 30-day period (Wilson, 2019). A study on 29 trained male triathletes found that 93% endorsed GI distress during competition, 45% of which were considered severe symptoms (Jeukendrup, 2000). Another study by Wilson (2017) reported that more than 75% of runners experienced at least one GI complaint over a 30-day training period. Overall, the majority of the research on GI complaints among athletes focuses on endurance athletes, in particular runners. There is less data available related to frequency of GI distress during team sports such as basketball, football, and soccer (Wilson, 2019).

Causes and Mechanisms

It has been estimated that high intensity exercise can reduce splanchnic blood flow by up to 80% of baseline, which in turn may lead to increased intestinal permeability (ter Steege, & Kolkman, 2012). Often referred to as a 'leaky gut', increased intestinal permeability can lead to nausea, diarrhea, constipation, gas, bloating, and nutrient deficiencies (Lamprecht & Frauwallner, 2013). These physiological effects, combined with during-exercise food and beverage intake, may exacerbate GI symptoms. A study on professional, amateur, and non-athletes reported significantly higher differential sugar absorption in professional athletes than

the other groups (Hałasa et al., 2019). Timing, portion, type and composition of foods and beverages chosen, in addition to overall hydration status, can influence GI symptoms (Jeukendrup, 2017).

Another cause for GI distress among athletes is undesirable food choices. High protein diets, commonly followed by athletes, have been shown to negatively alter the microbiome (Clark & Mach, 2016). Hydration status plays a role in permeability of the gut, with studies showing an increase in gut permeability in individuals who are dehydrated, though it is important to take into account GI comfort in terms of volume of liquid consumed (Lambert et al., 2008). Consumption of foods that delay gastric emptying or lead to a fluid shift in the intestinal lumen such as fiber, fat, protein, and fructose have all been associated with increased GI distress among athletes (de Oliveira et al., 2014). A commonly utilized nutritional consideration for sports drinks is consuming a beverage with multi-transport carbohydrates rather than a single carbohydrate beverage (de Oliveira et al., 2014).

Total energy intake can play a role in GI distress as well, with both excess and inadequate intake having the potential to cause GI distress symptoms (Wilson, 2020). Individuals with anorexia nervosa may suffer from symptoms of bloating, premature or excessive fullness, nausea, and stomach pain (Wilson, 2020). Studies have shown prolonged effects of GI distress following caloric restriction, and GI distress is considered one of the health consequences of relative energy deficiency in sport (Mathisen et al., 2020; Mountjoy et al., 2014).

Anxiety or stress about sport may also cause GI distress (Wilson, 2019). Stress has been linked to delayed gastric emptying and acceleration of large bowel transit (Taché et al., 2001). This occurrence is related to activation of the sympathetic nervous system and various hormone secretion affecting the gut (Taché et al., 2001). Often linked to stress and anxiety, IBS is

commonly underdiagnosed among athletes and may contribute to GI distress (Killian & Lee, 2019). It has been estimated that about 9.8% of endurance athletes meet diagnostic criteria for IBS based on Rome III (Killian & Lee, 2019).

The use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), which can be common among athletes particularly those dealing with injury, can result in GI toxicity (Maseda & Ricciotti, 2020). Toxicity has been associated with nausea, indigestion, constipation, diarrhea, and abdominal pain (Maseda & Ricciotti, 2020). However, Wilson (2019) reported an overall low incidence of nausea among individuals taking NSAIDs compared to other pain killers.

Probiotics

Probiotics and Changes in the Microbiome

There is evidence on the beneficial effects of probiotics on the microbiome as studies have shown an increase in beneficial gut bacteria as well as a decrease in potentially harmful gut bacteria (Yilmaz et al., 2019). Some studies have shown an increase in beneficial gut bacteria in the microbiome following probiotic supplementation. Coman et al. (2017) studied the effect of synbiotic fermented milk, containing *Lactobacillus rhamnosus* and *Lactobacillus paracasei* as well as oat bran fiber, on ten healthy adults who followed an intense gym training program. The trial group was found to have an increase in gut *Lactobacilli* and *Bifidobacteria* following supplementation of the synbiotic milk. Stool consistency, regularity, and ease of defecation were improved as well (Coman et al., 2017). A study on kefir supplementation in patients with inflammatory bowel disease (IBD) detected *Lactobacillus* in feces of all participants of the study, and a reduction in bacteria often associated with proinflammatory response and GI disease was noted as well (Yilmaz et al., 2019).

Probiotics may play a role in alleviating lactose intolerance symptoms. Vitellio et al. (2019) reported that supplementation of a probiotic containing *Bifidobacterium longum* and *Lactobacillus rhamnosus* plus vitamin B6 was effective in increasing gut bacteria beneficial for lactose digestion, specifically *Bifidobacterium*, in lactose intolerant subjects. A reduction in bloating and improvement in constipation was noted in the trial group of this study as well when compared to placebo (Vitellio et al., 2019).

As changes in the gut microbiome have been noted within 24 hours of modifying probiotic content of the diet (David et al., 2013), there may be even more of a benefit to consuming these foods regularly. *Bifidobacterium* strains have been seen to have the most beneficial impact on humans based on a systematic review by Clark and Mach (2016) though other strains may also help reduce GI symptoms due to their ability to increase diversity in the microbiome. Further research is needed to assess how long after consumption these changes last and if a lesser, maintenance dose of supplements or probiotic containing foods may be appropriate once these microbiome changes are initially noted.

Probiotics and Intestinal Permeability

Increased intestinal permeability allows for potential bacterial invasion of the gut (Kelly et al., 2015). Lamprecht et al. (2012) studied triathletes, runners, and cyclists who received a multispecies probiotic for fourteen weeks along with a control group. Zonulin, a protein that is thought to indicate increased intestinal permeability when elevated, decreased by about 20% in the probiotic group compared to the placebo suggesting that probiotic supplementation can reduce intestinal permeability (Lamprecht et al., 2012). Shing et al. (2014) performed a randomized control trial with ten trained male runners. The runners were provided a multispecies probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus*

rhamnosus, *Lactobacillus fermentum*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus* for four weeks. There was a small decrease in urine lactulose: rhamnos concentration following probiotic supplementation which may be related to an improvement in intestinal permeability as well as a small decrease in GI distress symptoms (Shing et al., 2014).

If probiotic consumption can help reduce intestinal permeability, this may improve GI symptoms overall among the athlete population. Further research needs to be done with larger sample sizes and including team sport athletes as this literature only supports improvements in intestinal permeability for endurance athletes.

Probiotics and Irritable Bowel Syndrome

One of the most common GI disorders that physicians come across is IBS, and there is evidence suggesting probiotics may help relieve symptoms of IBS (Ducrotté, Sawant, & Jayanthi, 2012). In 2012, a study was performed on 214 patients with IBS based on Rome III criteria. Frequency and severity of GI symptoms were assessed after supplementation with a capsule of *Lactobacillus plantarum* or a placebo for four weeks. The study found a reduction in abdominal pain and bloating in the probiotic group. Regarding treatment efficacy, 78.1% of the probiotic group found the treatment good or excellent while only 8.1% felt this way in the placebo group (Ducrotté, Sawant, & Jayanthi, 2012). Sadrin et al. (2020) studied males and females diagnosed with IBS, again diagnosed with Rome III criteria. During this double-blind randomized control trial, participants were supplemented with either a probiotic or a placebo for eight weeks while keeping track of abdominal pain and discomfort as well as monitoring stool consistency using the Bristol stool chart. Both the probiotic group and the placebo group reported decreased abdominal pain and discomfort after eight weeks. There was no difference in

stool consistency between the groups. Flatus was reduced significantly in the probiotic group with a slight decrease in bloating and rumbling (Sadrin et al., 2020).

There is evidence indicating a relationship between stress and IBS suggesting that the two should be managed together (Qin et al., 2014). A double-blind placebo study on individuals with stress induced symptoms of anxiety, nervousness, irritability, sleeping problems, and/or GI disturbances reported a reduction in abdominal pain and nausea/vomiting after supplementation with a probiotic containing *Lactobacillus acidophilus* and *Bifidobacterium longum* (Diop, Guillou, & Durand, 2008). Since collegiate athletes are often under significant stress, as shown by a study which estimated that 20-60% of athletes suffer from stress from excessive exercise and inadequate recovery, probiotics could be used to reduce stress related GI symptoms though further research is warranted (Purvis et al., 2010).

Based on these studies, probiotics may be a promising treatment for recusing some symptoms of IBS. Current literature focuses on the general population diagnosed with IBS rather than athletes diagnosed with IBS. This gap in research leaves results open to interpretation in regard to whether or not probiotics are effective in improving symptoms of IBS in athletes.

Effects of Probiotic Supplementation on Gastrointestinal Symptoms with Exercise

Researchers evaluated the effects of probiotic supplementation on 20 male and four female recreational runners. They were studied in a randomized control trial where each participant received either a probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium animalis subspecies Lactis* or a placebo for 28 days before running a marathon race. The study resulted in fewer reported GI distress symptoms and reduced severity with probiotic supplementation in comparison to the placebo group (Pugh et al., 2019). Another

study on *Lactobacillus rhamnosus* supplementation for three months on marathon runners led to a 33% reduction in duration of GI symptoms during training and a 57% reduction during follow up. A probiotic supplement or a placebo was also provided two hours before the start of the race on race day. There was no significant difference in the number of GI symptoms between the two groups (Kekkonen et al., 2007). A randomized single blind placebo-controlled study on 34 elite rugby players found that a multistrain probiotic containing *Lactobacillus gasseri*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* led to a significant reduction in incidence of GI symptoms. However, severity of symptoms did not differ between the probiotic and placebo groups (Haywood et al., 2013).

The positive effects of probiotics have not been observed in all athlete populations. A randomized control trial of 46 female endurance swimmers studied the effects of probiotic yogurt on athletic performance and health. There was no significant difference noted for incidence or duration of digestive symptoms (Salarkia, Ghadamli, Zaeri, & Sabaghian Rad, 2013). Another study on division I collegiate swimmers reported no significant difference in GI inflammatory markers after supplementation with *Bifidobacterium longum* for six weeks. This study concluded that more research needs to be done with different doses of supplementation (Carbuhn et al., 2018).

Based on current literature, the research is mixed regarding whether probiotics affect severity, duration, and/or frequency of GI symptoms. Overall, the majority of studies seem to support positive findings with probiotic supplementation however benefits are often modest. Consumption of foods containing probiotics should, in theory, provide a similar benefit, though there are less studies available on probiotic/fermented food intake versus probiotic supplements. It is possible that the type of exercise may play a role in the effectiveness of probiotics as well

seeing as these two studies done with swimmers did not produce positive results. Further research is needed to determine if there are sport-specific probiotic needs since athletes of different sports likely have a diverse microbiome based on differences in diet and exercise.

Probiotics and Athletic Performance

The use of probiotics as an ergogenic aid is a newer research topic that has revealed mixed results. A recent literature review conducted by the International Society of Sports Nutrition showed seven out of 24 studies reported significant improvement with 17 identifying no effect (Jäger et al., 2019). Multistrain probiotics were more effective ergogenic aids than single strain, though both have demonstrated mixed results (Jäger et al., 2019).

Studies have shown small improvements in run time to fatigue as well as reduced fatigue accumulation in athletes receiving probiotic supplementation (Pugh et al., 2019; Kamano et al., 2018). One study displayed an improved VO₂ max in adolescent female swimmers who received 400 ml of a multistrain probiotic yogurt compared to those who received the placebo over eight weeks of intervention (Salarkia et al., 2012). There is also some evidence that probiotics in combination with exercise may lead to improvements in strength in young sedentary males (Ibrahim et al., 2018). While these studies show the potential for probiotics as an ergogenic aid, improvements were minimal and it is difficult to determine if these results would translate to real-world scenarios. Despite these potential benefits, other studies have shown no statistically significant improvement in performance with probiotic supplementation (O'Brien et al., 2015; Pugh et al., 2019).

The mechanism of probiotics' ergogenic effect in each of these studies is not completely understood, and it is possible that the effect of probiotics on immune function and GI symptoms

may be the underlying reasons for the proposed performance benefits. The majority of these studies have small sample sizes which is a limiting factor. Further research also needs to be performed with more female athletes to see if probiotic supplementation affects males and females differently.

Probiotic Consumption Amongst Athletes

Despite the proposed benefits of probiotics for athletes, probiotic intake among this population has not been thoroughly quantified. In fact, very few studies involving the assessment of probiotics intake among athletes were identified in the literature. Although Sevim et al. (2016), assessed elite volleyball players' probiotic intake via FFQ, dietary assessment results were not published, nor is the FFQ available. However, researchers reported that 60% of the athletes had never heard the term “probiotic” before. Less than 25% had used a probiotic supplement in the past and approximately 45% of these volleyball players reported they would prefer to consume probiotics incorporated into foods over supplements (Sevim et al., 2016). In a second study, only 4% of male and 3% of female 11-13 year-old Canadian athletes-reported regular consumption of probiotic supplements, while 0% of 14-18 year-old athletes reported regular consumption (Parnell et al., 2016). If researchers and dietitians are able to easily quantify intake of probiotics and fermented foods, they will be better able to assess interactions with health and performance as well as target interventions to address intake.

Conclusion

Overall, the literature on probiotics has elucidated potential positive effects on GI distress, the microbiome, intestinal permeability, and IBS symptoms, although further research needs to be done with larger sample sizes, more female representation, and a greater variety of exercise types. Research on the ergogenic effects of probiotics is inconclusive and needs to be

studied further. Longer study durations may be required to truly understand their influence. If the exact mechanism for how probiotics impact health and performance can be determined, then recommendations for their use by athletes can be made. There is little data to quantify consumption of probiotic/fermented foods among collegiate athletes, a gap which makes assessing the influence of probiotic/fermented foods more difficult.

IV. Methods

Experimental Design

This project involves the development and validation of an original FFQ designed to evaluate usual probiotic/fermented food intake in collegiate athletes. Occurrence and frequency of GI distress symptoms and the association with the intake of probiotics/fermented foods were explored. Due to the COVID-19 pandemic, all study-related activities were completed virtually.

Participants

All student-athletes at Virginia Tech were invited to participate in this study. Males and females over 18 years of age on a current roster for any Virginia Tech Division I sport were eligible to participate.

Procedures

Development of a Tool to Assess Probiotic/Fermented Food Intake in College Athletes

In order to develop an original brief FFQ to assess probiotic/fermented food intake among collegiate athletes, the lead researcher (MB) completed the following:

- 1) Review of the scientific and “gray” literature related to probiotics and fermented foods.
- 2) Manual review of dietary recalls that were previously collected by Kostelnik et al. (2021) from 120 male and female athletes from a variety of college athletic teams at Virginia Tech and Radford University (n=360 total days) to determine the most commonly consumed probiotic/fermented foods among this population. Any

probiotic/fermented foods or beverages listed on the recalls that were noted as well as frequency of their consumption. (Appendix A)

- 3) Development of a draft FFQ. The first draft FFQ contained 11 foods and beverages: yogurt, kefir, kombucha, tempeh, sauerkraut, miso, kimchi, apple cider vinegar, sourdough bread, cottage cheese, and raw cheeses. In an effort to reduce systematic error, an “other” category was also included. Respondents would be requested to rate the frequency and amount of consumption, ranging from never to daily, over the previous four weeks.
- 4) Review and evaluation of the FFQ draft by an academic researcher with expertise in probiotics and gut health in athletes as well as four registered dietitians with expertise in sports nutrition, both at Virginia Tech and other universities to ensure face validity. A summary of their feedback is available in Appendix B. Dietitians were asked to answer four questions when providing feedback:
 - a) How many years have you been working in the Sports Nutrition field?
 - b) Does the PRO-Q include the food/beverage items that you think are the most common sources of probiotics consumed by athletes? Should additional items be added?
 - c) Are the instructions written a way that will be understandable to college athletes?
 - d) Any other feedback you wish you provide?
- 5) Modification of the FFQ draft based on feedback. The final FFQ developed, entitled PRO-Q, is included in Appendix C.

While raw cheese was included on the PRO-Q draft, it was not included on the final version due to the difficulty distinguishing between cheeses which do and do not contain live and active cultures.

Validation of the Tool to Assess Probiotic Intake in College Athletes

After the PRO-Q was finalized, a validation study was completed during 2020 Summer I, Summer II, and Fall semesters. Approximately 620 student-athletes were invited to participate via emails from the Virginia Tech (VT) sports nutrition staff, text messages, social media, flyers, and in person. We aimed to recruit athletes who participate on a variety of teams, striving for balanced representation among males/females and sports, where possible. Student-athletes who volunteered to participate filled out an online form then received an email from the lead researcher. The email provided an overview of the study and answered any questions that had been asked. Informed consent was obtained from all student-athletes who agreed to serve as participants.

In an initial session via Zoom with the researcher, demographic and health data was collected from each participant via an online survey (age, class, sport, gender, height, weight, race/ethnicity, hometown, health issues, medications, dietary restrictions, dietary supplements, and probiotic supplement use). (Appendix D). Participants then completed a 24-hour diet recall with the researcher, which required approximately 10-15 minutes to complete. The five-step multiple pass methodology, which has been established as an effective approach for collecting 24-hour diet recalls, was used (Conway, Ingwersen, & Moshfegh, 2004).

Two additional 24-hour diet recalls were completed over the phone on non-consecutive days during the subsequent two-week time frame, ultimately including one weekend day and two weekdays. This was to prevent autocorrelation that may occur if recalls were obtained

consecutively (Kirkpatrick et al., 2019). During the second call, participants were asked to complete the PRO-Q, which required 5-10 minutes of time, in addition to the diet recall. The first recall was completed via Zoom while the second and third were completed over the phone. Initially, recalls were intended to be done in person. However, due to COVID-19, all were completed virtually. The first recall was completed via Zoom to most closely replicate an in-person diet recall, and the remaining recalls were completed over the phone for ease of the participants.

Diet recalls were entered into the 2020 version of Nutrition Data Systems for Research software (NDSR) which is a state-of-the-art database developed by the University of Minnesota used to analyze recalls and provide information regarding a breakdown of nutrient intake. Participants received information regarding analysis of their diet from the NDSR software at the conclusion of the study as incentive for participating. Samples are included in Appendix E.

All dietary recalls were manually reviewed for probiotic/fermented food consumption, based on the items listed on the PRO-Q (Table 2).

Table 2: Foods and beverages included on the PRO-Q and their respective serving size.

Food/Beverage	Serving Size
Yogurt	150 grams, 2/3 cup, ~5.3 ounces
Bread	1 slice
Kefir	8 ounces
Kombucha	240 ml, 8 ounces
Tempeh	3 ounces
Sauerkraut	30 grams, 2 tbsp
Miso	1 tbsp
Kimchi	2 tbsp, 30 grams
Apple Cider Vinegar	1 tbsp
Cottage cheese	1/2 cup
Fermented/pickled vegetables	2 tbsp
Soy Sauce	1 tbsp

Olives	1 tbsp, 15 grams, ~4-5 olives
Pickles	1 ounce, ~4 pieces
Probiotic juice	8 ounces
Probiotic bar	1 bar

Intake of these foods from the diet recalls was documented along with frequency and portion size which was compared to responses on the PRO-Q. The PRO-Q was scored by compiling total estimated intake over one month and then taking the mean average daily intake from this number. Obtaining both the FFQ and diet recalls allowed for review of the potential variation in responses since diet recalls are more vulnerable to random error while FFQs more often result in systematic error (Kirkpatrick et al., 2019). Probiotic/fermented food intake was reported as the daily number of servings consumed after comparing responses from the PRO-Q to what is generally considered one serving size of each food/beverage per the USDA, listed in Table 2 (USDA, Food Data Central). Daily intake of probiotic/fermented foods was then estimated for each participant after determining the mean intake based on the 3-day diet recall (Ganji et al., 2020). This mean daily intake was then compared to responses on the PRO-Q (Hill et al., 2017).

Summary and descriptive statistics were calculated for PRO-Q and diet recall data using IBM Statistical Package for the Social Sciences (SPSS) version 26. Our statistical analyses were informed by those used in previous FFQ validation studies (Fausnacht et al., 2020). Wilcoxon signed-rank test and Bland-Altman plots were utilized to compare intake reported via the PRO-Q with intake reported via food recalls (Fausnacht et al., 2020). Since data were not normally distributed, Spearman's Rho correlation analysis was used to assess the relationship between

PRO-Q and GI symptom data. Differences in PRO-Q results between males and females were evaluated using a Mann-Whitney U test.

Assessment of the Occurrence of Gastrointestinal Symptoms in College Athletes and its Relationship to Probiotic/Fermented Food Intake

To accomplish the secondary aim of this study, a GI symptom questionnaire (Appendix F) was administered to participants during the first Zoom call following the demographic and health questions. The GI symptom questionnaire used has been found to have good reproducibility (Bovenschen et al., 2006). The GI questionnaire asks about the severity of 16 GI symptoms over the last four weeks. Symptoms were rated on a scale ranging from none to unbearable. This questionnaire was modified to only assess GI symptoms that probiotic/fermented food consumption is most likely to influence (Dimidi et al., 2019; Harper et al., 2018).

Summary and descriptive statistics were performed on compiled data from both questionnaires using IBM Statistical Package for the Social Sciences (SPSS) version 26. Spearman's Rho correlation analysis was used to assess the relationship between intake based on the PRO-Q and GI symptoms.

Ethical Considerations

This study was approved by the Institutional Review Board of Virginia Tech (IRB#20-288). All participants verbalized informed consent before participating in the study.

Data Results

A total of 42 participants, 12 males (28.6%) and 30 females (71.4%) from 13 different sports participated in the study. Breakdown of participants by sport is listed in Table 3.

Table 3: Breakdown of participants by sport. Data is reported in n (%).

Sport	# Participants (%)
Baseball	6 (14.3%)
Men's Basketball	1 (2.4%)
Women's Basketball	5 (11.9%)
Cross Country	2 (4.8%)
Football	1 (2.4%)
Women's Soccer	1 (2.4%)
Softball	7 (16.7%)
Spirit Squad	2 (4.8%)
Women's Swim & Dive	4 (9.5%)
Women's Tennis	2 (4.8%)
Women's Track & Field	5 (11.9%)
Volleyball	4 (9.5%)
Wrestling	2 (4.8%)

Participants included five freshmen (11.9%), 12 sophomores (28.6%), nine juniors (21.4%), 13 seniors (31%), and three graduate students (7.1%). The racial breakdown was: 32 White (76.2%), five Black or African American (11.9%), two Asian (4.8%), one Hispanic or Latino (2.4%), and two other (4.8%). Not all participants responded to the ethnicity question, but

of those who did (n=38), 5.3% identified as Hispanic or Latino while 94.7% identified as not Hispanic or Latino.

The PRO-Q took approximately five minutes or less to administer. Table 4 shows each food and beverage item on the PRO-Q and the frequency of consumption in the last four weeks among participants.

Table 4: Food and beverage intake reported via PRO-Q. Data reported as n(%).

Food/Beverage	Less than once per month	1-3 times per month	Once a week	2-4 times per week	5-6 times per week	Once a day	2 or more times per day
Yogurt	11 (26.2%)	7 (16.7%)	4 (9.5%)	13 (31.0%)	1 (2.4%)	5 (11.9%)	1 (2.4%)
Kefir	38 (90.5%)	3 (7.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.4%)
Kombucha	33 (78.6%)	5 (11.9%)	0 (0%)	3 (7.1%)	0 (0%)	1 (2.4%)	0 (0%)
Tempeh	42 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sauerkraut	42 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Miso	37 (88.1%)	5 (11.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Kimchi	39 (92.9%)	3 (7.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Apple Cider Vinegar	31 (73.8%)	6 (14.3%)	3 (7.1%)	2 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Sourdough Bread	21 (50.0%)	16 (38.1%)	2 (4.8%)	3 (7.1%)	0 (0%)	0 (0%)	0 (0%)
Cottage Cheese	34 (81.0%)	7 (16.7%)	0 (0%)	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)
Fermented vegetables	31 (73.8%)	9 (21.4%)	2 (4.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Soy Sauce	13 (31.0%)	16 (38.1%)	8 (19.0%)	4 (9.5%)	0 (0%)	1 (2.4%)	0 (0%)
Olives	31 (73.8%)	7 (16.7%)	3 (7.1%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Refrigerated Pickles	21 (50.0%)	11 (26.2%)	6 (14.3%)	4 (9.5%)	0 (0%)	0 (0%)	0 (0%)
Probiotic Juice	39 (92.9%)	1 (2.4%)	1 (2.4%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Probiotic Bars	39 (92.9%)	2 (4.8%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	No Data						

Only two items, yogurt and kombucha, were reported to be consumed more than once per day. No participants reported consumption of tempeh or sauerkraut and no foods were reported in the “other” category. The most commonly consumed food among participants was yogurt. In total, seven participants (17%) reported consuming probiotic/fermented foods on a daily basis, most commonly yogurt. The mean for the number of different items consumed overall based on the PRO-Q results was 4.07, 25.44% of the 16 items listed. Average daily serving of probiotic/fermented foods for all participants based on diet recalls and the PRO-Q is shown in Figure 1.

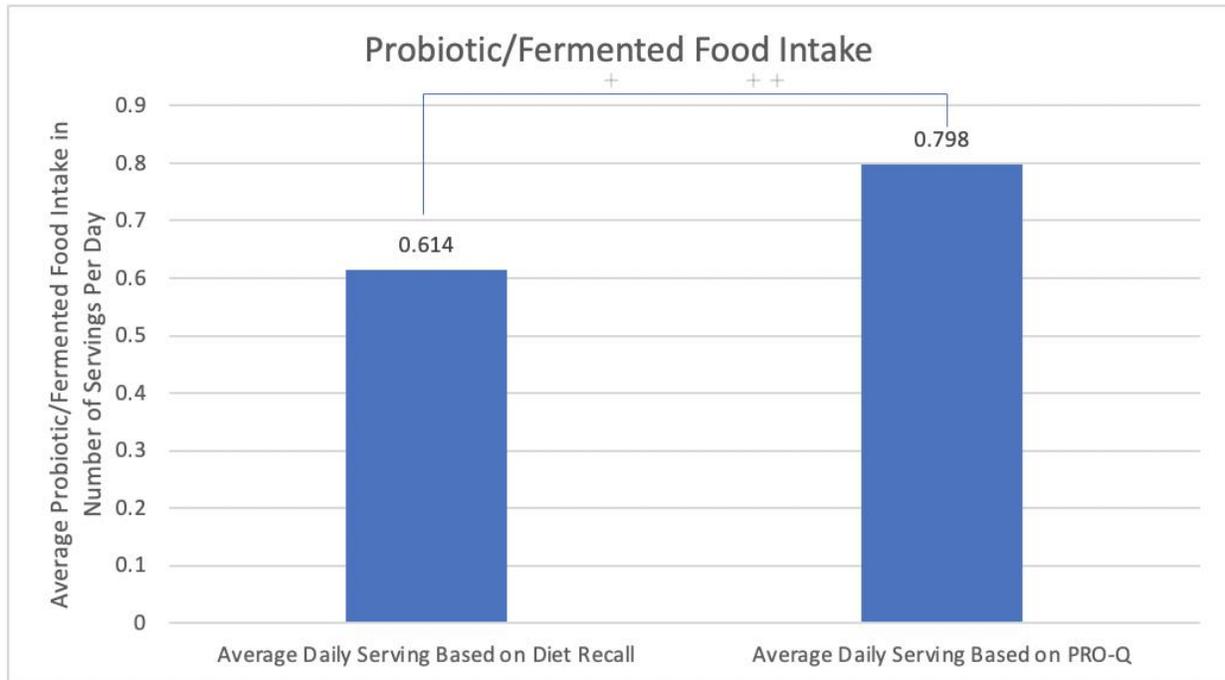


Figure 1: Average daily intake of probiotic/fermented foods. These values were highly correlated based on Spearman’s Rho analysis and Wilcoxon signed-rank test.

+ Spearman’s Rho: $r=0.766$, $p<0.001$; strong correlation

++ Wilcoxon signed-rank test: $p=0.168$; retain the null hypothesis that there was no difference between the average daily intake via diet recalls and average daily intake via the PRO-Q.

The Mann-Whitney U test showed no significant difference in average daily intake via PRO-Q ($p=0.777$) or GI sx ($p=0.523$) based on gender. There was also no correlation between mean daily servings measured by the PRO-Q and body mass index (BMI) ($r = 0.019$, $p = 0.905$) based on a Spearman’s Rho analysis.

Fermented food/probiotic intake as measured by the PRO-Q and diet recalls were highly correlated based on our Spearman’s Rho analysis ($r=0.766$, $p<0.001$). The Wilcoxon signed-rank test comparing average intake via diet recall versus the PRO-Q yielded a significance value of $p=0.168$, which means the difference between the two medians was not statistically significant. A Bland-Altman analysis was utilized to further explore the level of agreement between the PRO-Q and diet recalls. This resulted in a 95% agreement as shown in Figure 2.

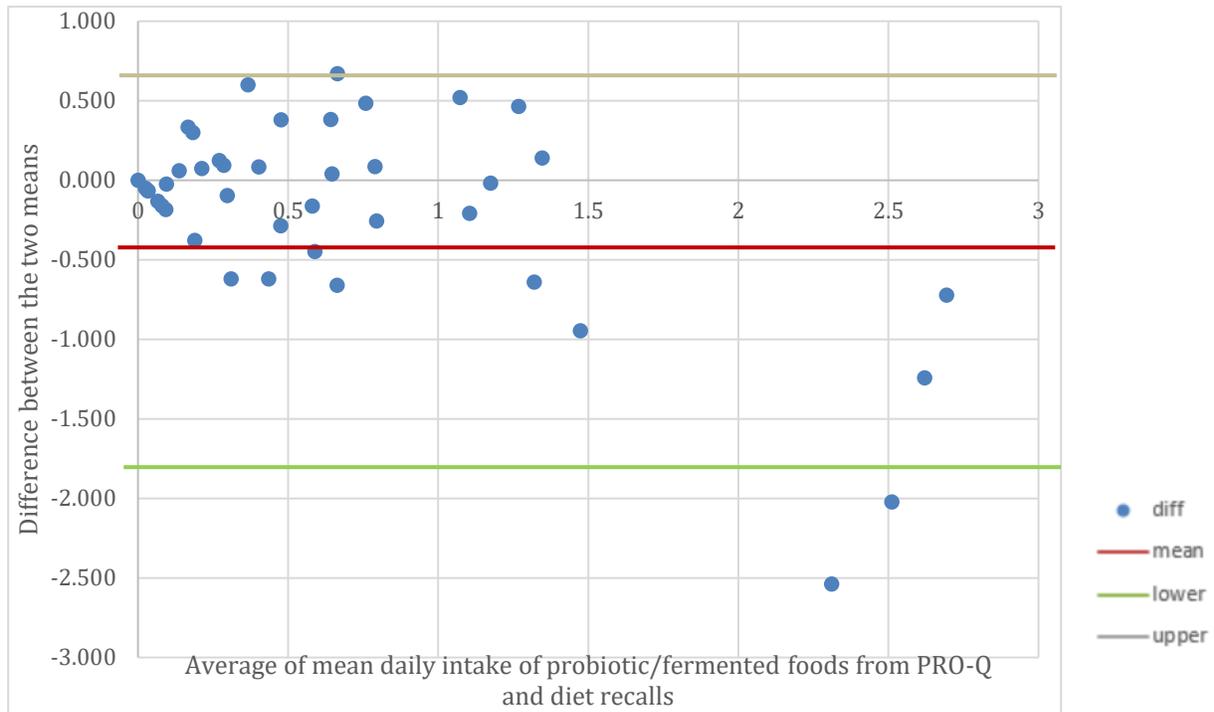


Figure 2: Results from the Bland-Altman analysis on probiotic/fermented food intake based on the PRO-Q and the diet recalls showing 95% agreement.

Table 5: Gastrointestinal symptoms and their reported score of severity/frequency among participants. Data reported as n(%).

Gastrointestinal Symptom	None	Mild	Moderate	Quite A Lot	Severe	Unbearable
Abdominal Pain	27 (64.3%)	9 (21.4%)	4 (9.5%)	2 (4.8%)	0 (0%)	0 (0%)
Epigastric Pain	34 (82.9%)	5 (12.2%)	2 (4.9%)	0 (0%)	0 (0%)	0 (0%)
Bloating	18 (42.9%)	12 (28.6%)	11 (26.2%)	1 (2.4%)	0 (0%)	0 (0%)
Nausea	32 (76.2%)	9 (21.4%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Vomiting	41 (97.6%)	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Loss of Appetite	27 (64.3%)	11 (26.2%)	3 (7.1%)	1 (2.4%)	0 (0%)	0 (0%)
Postprandial Fullness	26 (61.9%)	12 (28.6%)	4 (9.5%)	0 (0%)	0 (0%)	0 (0%)
Belching (burping)	25 (59.5%)	10 (23.8%)	5 (11.9%)	1 (2.4%)	1 (2.4%)	0 (0%)
Flatulence (gas)	18 (42.9%)	15 (35.7%)	8 (19.0%)	0 (0%)	1 (2.4%)	0 (0%)
Frequent Hard Stools	33 (78.6%)	5 (11.9%)	4 (9.5%)	0 (0%)	0 (0%)	0 (0%)
Diarrhea	37 (88.1%)	4 (9.5%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Alternately Solid & Loose stools	28 (66.7%)	7 (16.7%)	5 (11.9%)	2 (4.8%)	0 (0%)	0 (0%)
Constipation	37 (88.1%)	3 (7.1%)	2 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Other	41 (97.6%)	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)

Results from the GI symptom questionnaire (Table 5) revealed the most commonly reported symptoms were bloating and flatulence (gas), where more than 50% of participants reported at least mild symptoms. Belching (burping) was also quite common among participants

with 40.5% reporting at least mild symptoms. The least common symptom was vomiting. No participant marked “unbearable” for any GI symptom on the questionnaire and very few reported “severe” symptoms. The average GI symptom score was one, which equates to “mild” on the symptom scale.

There was no significant correlation between average daily intake of probiotic/fermented foods and overall GI symptom score ($r = 0.297$, $p = 0.056$). Two GI symptoms showed a weak positive correlation to probiotic/fermented food intake based on the PRO-Q: flatulence ($r = 0.379$, $p = 0.013$) and epigastric pain ($r = 0.311$, $p = 0.047$). However, it is worth noting that only seven (16.7%) participants reported any epigastric pain, making this a small sample size. No other GI symptoms were significantly correlated with average daily intake of probiotic/fermented foods based on diet recalls or PRO-Q.

Of the five participants who reported taking a probiotic supplement in the past, none were taking one at the time of the survey, and only one participant had been advised by a dietitian or nutritionist to take a probiotic. Reported reasons for taking a probiotic supplement included post antibiotic treatment, to improve digestion, stomach issues, and stomach flu. Only one participant knew the brand of probiotic supplement consumed, *Lactoflor*. Because no participants were currently taking a probiotic supplement, we did not need to take this into consideration when evaluating the data.

V. Discussion

The primary aim of this study was to create and validate a FFQ to assess intake of probiotic and fermented foods among collegiate athletes. The PRO-Q demonstrated validity as a rapid tool for measuring intake of probiotic and fermented foods among Division I collegiate student-athletes at Virginia Tech based on three statistical analyses (Spearman's Rho correlation analysis, Wilcoxon Signed-Rank test, and Bland Altman plot). Since probiotic intake cannot be evaluated via biomarkers without testing the microbiome which can be costly and invasive, a noninvasive FFQ has value as a rapid evaluation tool for estimated intake of probiotic/fermented foods. Presently, there is no dietary assessment instrument validated to assess probiotic/fermented food intake in collegiate athletes and minimal published literature related to intake in this population exists. Following potential minor modification and reproducibility evaluation, the PRO-Q shows promise for use with collegiate athletes.

Upon administering the PRO-Q, participants frequently associated fermented vegetables with pickles causing some confusion among responses. A suggested edit would be to adjust the order of the foods to have pickles come first. Tempeh and sauerkraut were two foods that none of the participants reported consuming. Despite these foods being commonly consumed among some populations, this study suggests that they are not commonly consumed amongst division I college athletes at VT; therefore, they could potentially be removed from the PRO-Q and entered under the "other" column if needed. Adding options for servings sizes for each item could help simplify the FFQ as well as some participants struggled to estimate average servings.

Gastrointestinal Issues

It was anticipated that GI complaints would be prevalent among 30-50% of athletes based on the literature since this study looked at all sport athletes rather than endurance athletes alone, which would mean about 14-21 of the 42 participants would be expected to have some sort of GI complaints (de Oliveira et al., 2014). However, 24 (57.1%) participants reported “mild”, “moderate”, “quite a lot”, or “severe” for bloating and flatulence, the two most commonly reported complaints among this sample. This means that 57.1% of participants in this study experience some sort of GI symptoms. Wilson (2019) reported flatulence to be perhaps the most common GI symptom among athletes, a finding consistent with the results from this study.

Studies have shown that mild exercise stimulates flatulence which may have contributed to more than 50% of the participants reporting at least mild flatulence symptoms (Dainese et al., 2004). There has been contradictory data in the past regarding the association between intake of probiotics and flatulence among athletes, however this study showed a positive correlation between increased intake of probiotic/fermented foods, suggesting that they may contribute to increased flatulence. Epigastric pain also showed a positive correlation with intake of probiotic/fermented foods, though the small numbers of individuals reporting this symptom may have had an impact on the correlation. It is possible that participants consume higher amounts of these foods in an effort to relieve symptoms of GI distress or perhaps they are not consuming adequate amounts to obtain the potential benefit. Further research on subjects with higher intake of these foods is warranted, as overall in this study intake was low amongst participants.

Per Sandler et al. (2000), bloating is typically more commonly reported among females than males so the distribution of females to males in this study may have been a contributing factor for more than half of all participants reporting bloating. Stress and anxiety levels have been linked to bloating as well (Malagelada et al., 2017). These factors were not evaluated in this

study but could have contributed to bloating among participants. A future study on GI symptoms among collegiate athletes may consider including questions regarding stress and anxiety levels to determine if there is a correlation with bloating.

Relationship between PRO-Q and Gastrointestinal Symptoms

Although a negative correlation between overall intake of probiotic/fermented foods and GI symptoms was hypothesized, there was no significant relationship between these two variables in this study. It is important to note that overall, intake of probiotic/fermented foods was low amongst this population, which may have contributed to the lack of relationship between intake of these foods and GI symptoms. Some additional potential contributing factors include the impact of other dietary factors on GI symptoms, inconsistent consumption of probiotic/fermented foods, exercise induced changes in the microbiome leading to GI distress, or reporting errors either on the PRO-Q or the GI questionnaire. Future research should aim to investigate the relationship between these two variables further.

Strengths & Limitations

Receiving expert feedback on the PRO-Q from various individuals with experience in sports nutrition and gut health helped to create an effective and validated tool for evaluating intake of probiotic/fermented foods among collegiate athletes. Face validity determination by experts in various United States geographic regions was also a strength. Utilizing a manual audit of 360 days of recalls in our target population to develop a list of items to include on the FFQ helped guide us in selection of items for the PRO-Q. Having the same researcher utilize the multiple pass method for all diet recalls for all participants allowed for a consistent, standardized collection of diet recall data. Obtaining participants from 13 different sports allowed for a more

diverse population of athletes rather than focusing on just one sport as many studies on probiotics have in the past.

The study had some limitations as well. Dietary recalls rely on participants' memory and may result in random error due to differences in one's diet from day to day (Carroll et al., 2012). Because no participants responded higher than "less than once a month" for tempeh or sauerkraut, we were unable to validate the questionnaire for these two responses despite being validated for overall intake. It is suspected that while those foods may be more commonly consumed among other populations, they may not be consumed commonly among collegiate athletes. Another limitation is the potential impact that COVID-19 had on the study. Due to stay-at-home orders and campus closures, it was more difficult to recruit participants for the study which affected the sample size. Quarantine and isolation restrictions also may have had an impact on participants' diets due to inability to go to the grocery store or eating out less frequently. Despite the variety in sport for participants, the sample was heavily female (71%) which may have impacted results despite the t-test results displaying no significant difference in PRO-Q score for males and females.

Suggestions for Future Evaluation of the PRO-Q

Considering other dietary factors' potential influence on GI symptoms would be beneficial to incorporate into future studies. Intake of nutrients such as fat, protein, carbohydrates, fiber, caffeine, fructose, sugar alcohols, and artificial sweeteners may influence GI symptoms (Wilson, 2019; Wilson, 2020). For example, a diet higher in carbohydrates would likely translate to a lower fat and protein intake, and fats and protein may cause more lasting feelings of fullness therefore contributing to bloating. Higher fiber intake and higher total carbohydrate intake have been associated with increased flatulence (Christodoulides et al., 2016;

Wilson, 2020). Studies have looked at the potential impact on microbiota dysbiosis related to consumption of artificial sweeteners (Hunter et al., 2019). This impairment could contribute to increased GI symptoms among those who consumed larger amounts of artificial sweeteners.

A second administration of the PRO-Q among this same sample would allow for assessment of reliability, and administering the PRO-Q as well as three 24-hour dietary recalls to other student-athletes at Virginia Tech would then allow us to assess for reproducibility. Both of which are important measures to consider before the PRO-Q can be further utilized. Future research should focus on validating the PRO-Q among other populations; however, it may be prudent to first perform another study to evaluate the needs for inclusions of tempeh and sauerkraut.

Conclusion

In this study, intake of probiotic/fermented foods was relatively low in both frequency and variety, while reported mild-moderate GI symptomatology was rather high (more than half). Thus, Registered Dietitians may be able to use these tools in assessment, counseling, and education of collegiate athletes. Our study findings could also be used to ensure the inclusion of the more popular foods, for example yogurt, in athlete meals and fueling stations. Future studies are needed to evaluate the reproducibility, and generalizability of these results in other athletics populations.

References

Carbuhn, A. F., Reynolds, S. M., Campbell, C. W., Bradford, L. A., Deckert, J. A., Kreutzer, A., & Fry, A. C. (2018). Effects of probiotic (*Bifidobacterium longum* 35624) supplementation on exercise performance, immune Modulation, and cognitive outlook in division I female swimmers. *Sports (Basel, Switzerland)*, 6(4), 1-14. doi: 10.3390/sports6040116

Carroll, R. J., Midthune, D., Subar, A. F., Shumakovich, M., Freedman, L. S., Thompson, F. E., & Kipnis, V. (2012). Taking advantage of the strengths of 2 different dietary assessment instruments to improve intake estimates for nutritional epidemiology. *American journal of epidemiology*, 175(4), 340–347. <https://doi.org/10.1093/aje/kwr317>

Christodoulides, S., Dimidi, E., Fragkos, K. C., Farmer, A. D., Whelan, K., & Scott, S. M. (2016). Systematic review with meta-analysis: effect of fibre supplementation on chronic idiopathic constipation in adults. *Alimentary pharmacology & therapeutics*, 44(2), 103–116. <https://doi.org/10.1111/apt.13662>

Clark, A., & Mach, N. (2016). Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *Journal of the International Society of Sports Nutrition*, 13(1), 1-21. doi: 10.1186/s12970-016-0155-6

Coman, M.M., Verdenelli, M.C., Silvi, S., Cecchini, C., Gabbianelli, R., Amadio, E., Orpianesi, C., Cresci, A. (2017). Knowledge and acceptance of functional foods: A preliminary study on

influence of a synbiotic fermented milk on athlete health. *International Journal of Probiotics and Prebiotics*, 12(1), 33-42.

Dainese, R., Serra, J., Azpiroz, F., & Malagelada, J. R. (2004). Effects of physical activity on intestinal gas transit and evacuation in healthy subjects. *The American journal of medicine*, 116(8), 536–539. <https://doi.org/10.1016/j.amjmed.2003.12.018>

David, L., Maurice, C., Carmody, R., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505, 559-563. <https://doi.org/10.1038/nature12820>

Davidson, G., Marchbank, T., March, D. S., Thatcher, R., & Playford, R. J. (2016). Zinc carnosine works with bovine colostrum in truncating heavy exercise-induced increase in gut permeability in healthy volunteers. *The American Journal of Clinical Nutrition*, 104(2), 526-536. doi: 10.3945/ajcn.116.134403

Day, R. L., Harper, A. J., Woods, R. M., Davies, O. G., & Heaney, L. M. (2019). Probiotics: current landscape and future horizons. *Future science OA*, 5(4), FSO391. <https://doi.org/10.4155/fsoa-2019-0004>

de Oliveira, E. P., Burini, R. C., & Jeukendrup, A. (2014). Gastrointestinal complaints during exercise: prevalence, etiology, and nutritional recommendations. *Sports medicine*,

44(Suppl 1), 79–85. <https://doi.org/10.1007/s40279-014-0153-2>

Dimidi, E., Cox, S. R., Rossi, M., & Whelan, K. (2019). Fermented Foods: Definitions and Characteristics, Impact on the Gut Microbiota and Effects on Gastrointestinal Health and Disease. *Nutrients*, *11*(8), 1806. <https://doi.org/10.3390/nu11081806>

Diop, L., Guillou, S., & Durand, H. (2008). Probiotic food supplement reduces stress induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. *Nutrition Research*, *28*(1), 1–5. doi: 10.1016/j.nutres.2007.10.001

Ducrotté, P. (2012). Clinical trial:Lactobacillus plantarum299v (DSM 9843) improves symptoms of irritable bowel syndrome. *World Journal of Gastroenterology*, *18*(30), 4012-4018. doi: 10.3748/wjg.v18.i30.4012

Fausnacht, A. G., Myers, E. A., Hess, E. L., Davy, B. M., & Hedrick, V. E. (2020). Update of the BEVQ-15, a beverage intake questionnaire for habitual beverage intake for adults: determining comparative validity and reproducibility. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association*, *33*(5), 729–737. <https://doi.org/10.1111/jhn.12749>

FoodData central. (n.d.). Retrieved April 19, 2021, from <https://fdc.nal.usda.gov/>

Ganji, V., Abu-Dbaa, R., Othman H., Zewein, M., Al-Abdi, T., & Shi, Z. (2020). Validation of vitamin D-specific food frequency questionnaire against food records for Qatari women. *Foods*, *9*(2), 1-10. doi: 10.3390/foods9020195

Hałasa, Maciejewska, Ryterska, Baśkiewicz-Hałasa, Safranow, & Stachowska. (2019).

Assessing the association of elevated zonulin concentration in stool with increased intestinal permeability in active professional athletes. *Medicina*, 55(10), 1-10. doi:

10.3390/medicina55100710

Harper, A., Naghibi, M. M., & Garcha, D. (2018). The Role of Bacteria, Probiotics and Diet in Irritable Bowel Syndrome. *Foods (Basel, Switzerland)*, 7(2), 13.

<https://doi.org/10.3390/foods7020013>

Haywood, B.A., Black, K.E., Baker, D.; McGarvey, J., Healey, P., Brown, R.C. (2014).

Probiotic supplementation reduces the duration and incidence of infections but not severity in elite rugby union players. *Journal of Science and Medicine in Sport*, 17(4), 356–360. doi:

10.1016/j.jsams.2013.08.004

Hedrick, V. E., Myers, E. A., Zoellner, J. M., Duffey, K. J., & Davy, B. M. (2018). Validation of a Rapid method to assess habitual beverage intake patterns. *Nutrients*, 10(1), 1-10.

doi: 10.3390/nu10010083

Hill, C. E., MacDougall, C. R., Riebl, S. K., Savla, J., Hedrick, V. E., & Davy, B. M. (2017).

Evaluation of the relative validity and test-retest reliability of a 15-item beverage

intake questionnaire in children and adolescents. *Journal of the Academy of Nutrition*

and Dietetics, 117(11), 1757-1766. <https://doi.org/10.1016/j.jand.2017.05.011>

Hunter, S. R., Reister, E. J., Cheon, E., & Mattes, R. D. (2019). Low Calorie Sweeteners Differ in Their Physiological Effects in Humans. *Nutrients*, *11*(11), 2717.

<https://doi.org/10.3390/nu11112717>

Ibrahim, N. S., Muhamad, A. S., Ooi, F. K., Meor-Osman, J., & Chen, C. K. (2018). The effects of combined probiotic ingestion and circuit training on muscular strength and power and cytokine responses in young males. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, *43*(2), 180–186. <https://doi.org/10.1139/apnm-2017-0464>

Institute of Medicine (US) Committee on Dietary Risk Assessment in the WIC Program. (2002). *Dietary Risk Assessment in the WIC Program*. National Academies Press (US).

Jäger, R., Mohr, A. E., Carpenter, K. C., Kerksick, C. M., Purpura, M., Moussa, A., Townsend, J. R., Lamprecht, M., West, N. P., Black, K., Gleeson, M., Pyne, D. B., Wells, S. D., Arent, S. M., Smith-Ryan, A. E., Kreider, R. B., Campbell, B. I., Bannock, L., Scheiman, J., Wissent, C. J., Pane, M., Kalman, D. S., Pugh, J. N., ter Haar, J. A., & Antonio, J. (2019). International society of sports nutrition position stand: Probiotics. *Journal of the International Society of Sports Nutrition* *16*, 1-44. <https://doi.org/10.1186/s12970-019-0329-0>

Jang, L. G., Choi, G., Kim, S. W., Kim, B. Y., Lee, S., & Park, H. 2019. The combination of sport and sport-specific diet is associated with characteristics of gut microbiota: an observational study. *Journal of the International Society of Sports Nutrition*, *16*(1), 1-10.
doi: 10.1186/s12970-019-0290-y

Jeukendrup, A., Vet-Joop, K., Sturk, A., Stegen, J., Senden, J., Saris, W., & Wagenmakers, A. (2000). Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. *Clinical Science*, 98(1), 47. doi: 10.1042/cs19990258

Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food, London, Ontario, Canada, April 30 and May 1, 2002.

Karimi, R., Mortazavian, A. M., & Gomes Da Cruz, A. (2011). Viability of probiotic microorganisms in cheese during production and storage: a review. *Dairy Science & Technology*, 91, 283-308. doi: 10.1007/s13594-011-0005-x

Kekkonen, R. A., Vasankari, T. J., Vuorimaa, T., Haahtela, T., Julkunen, I., & Korpela, R. (2007). The effect of probiotics on respiratory infections and gastrointestinal symptoms during training in marathon runners. *International Journal of Sport Nutrition and Exercise Metabolism*, 17(4), 352–363. doi: 10.1123/ijsnem.17.4.352

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in cellular neuroscience*, 9, 392.
<https://doi.org/10.3389/fncel.2015.00392>

Killian, L. A., & Lee, S. Y. (2019). Irritable bowel syndrome is underdiagnosed and ineffectively managed among endurance athletes. *Applied Physiology, Nutrition, and Metabolism*, *44*(12), 1329-1338. <https://doi.org/10.1139/apnm-2019-0261>

Kirkpatrick, S. I., Baranowski, T., Subar, A. F., Tooze, J. A., & Frongillo, E. A. (2019). Best practices for conducting and interpreting studies to validate self-reporting dietary assessment methods. *Journal of the Academy of Nutrition and Dietetics*, *119*(11), 1801-1816. <https://doi.org/10.1016/j.jand.2019.06.010>

Komano, Y., Shimada, K., Naito, H., Fukao, K., Ishihara, Y., Fujii, T., Kokubo, T., & Daida, H. (2018). Efficacy of heat-killed *Lactococcus lactis* JCM 5805 on immunity and fatigue during consecutive high intensity exercise in male athletes: a randomized, placebo-controlled, double-blinded trial. *Journal of the International Society of Sports Nutrition*, *15*, 1-9. DOI: 10.1186/s12970-018-0244-9

Kostelnik, S. B., Rockwell, M. S., Davy, K. P., Hedrick, V. E., Thomas, D. T., & Davy, B. M. (2021). Evaluation of Pragmatic Methods to Rapidly Assess Habitual Beverage Intake and Hydration Status in U.S. Collegiate Athletes. *International journal of sport nutrition and exercise metabolism*, *31*(2), 115–124. <https://doi.org/10.1123/ijsnem.2020-0125>

Lambert, G. P., Lang, J., Bull, A., Pfeifer, P. C., Eckerson, J., Moore, G., Lanspa, S., & O'Brien, J. (2008). Fluid restriction during running increases GI permeability. *International journal of sports medicine*, *29*(3), 194–198. <https://doi.org/10.1055/s-2007-965163>

Lamprecht, M., Bogner, S., Schippinger, G., Steinbauer, K., Fankhauser, F., Hallstroem, S., Schuetz B., Greilberger, J. F. (2012). Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *Journal of the International Society of Sports Nutrition*, 9(1), 1-13. doi: 10.1186/1550-2783-9-45

Lamprecht, M. & Frauwallner, A. (2013). Exercise, intestinal barrier dysfunction and probiotic supplementation. *Medicine and Sport Science*, 59, 47-56. doi: 10.1159/000342169

Lee, S., Lee, J., Jin, Y. I., Jeong, J. C., Chang, Y. H., Lee, Y., Jeong, Y., & Kim, M. (2017). Probiotic characteristics of Bacillus strains isolated from Korean traditional soy sauce. *LWT - Food Science and Technology*, 79, 518-524. DOI: 10.1016/j.lwt.2016.08.040

Maher, J. M., Markey, J. C., & Ebert-May, D. (2013). The other half of the story: Effect size analysis in quantitative research. *CBE—Life Sciences Education*, 12(3), 345-351. doi: 10.1187/cbe.13-04-0082

Malagelada, J. R., Accarino, A., & Azpiroz, F. (2017). Bloating and Abdominal Distension: Old Misconceptions and Current Knowledge. *The American journal of gastroenterology*, 112(8), 1221–1231. <https://doi.org/10.1038/ajg.2017.129>

Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., Gänzle, M., Kort, R., Pasin, G., Pihlanto, A., Smid, E., & Hutkins, R. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology*, *44*, 94-102. doi: 10.1016/j.copbio.2016.11.010

Mathisen, T. F., Heia, J., Raustøl, M., Sandeggen, M., Fjellestad, I., & Sundgot-Borgen, J. (2020). Physical health and symptoms of relative energy deficiency in female fitness athletes. *Scandinavian journal of medicine & science in sports*, *30*(1), 135–147. <https://doi.org/10.1111/sms.13568>

Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R., & Ljungqvist, A. (2014). The IOC consensus statement: beyond the Female Athlete Triad--Relative Energy Deficiency in Sport (RED-S). *British journal of sports medicine*, *48*(7), 491–497. <https://doi.org/10.1136/bjsports-2014-093502>

O'Brien, K. V., Stewart, L. K., Forney, L. A., Aryana, K. J., Prinyawiwatkul, W., & Boeneke, C. A. (2015). The effects of postexercise consumption of a kefir beverage on performance and recovery during intensive endurance training. *Journal of Dairy Science*, *98*(11), 7446-7449. <https://doi.org/10.3168/jds.2015-9392>

Parnell, J., Wiens, K., & Erdman, K. (2016). Dietary intakes and supplements in pre-adolescent and adolescent canadian athletes. *Nutrients*, *8*(9), 526. doi: 10.3390/nu8090526

Pugh, J. N., Sparks, A. S., Doran, D. A., Fleming, S. C., Langan-Evans, C., Kirk, B., Close, G. L. (2019). Four weeks of probiotic supplementation reduces GI symptoms during a marathon race.

European Journal of Applied Physiology, 119(7), 1491–1501. doi: 10.1007/s00421-019-04136-3

Purvis, D., Gonsalves, S., & Deuster, P. A. (2010). Physiological and psychological fatigue in extreme conditions: overtraining and elite athletes. *PM & R : the journal of injury, function, and rehabilitation*, 2(5), 442–450. <https://doi.org/10.1016/j.pmrj.2010.03.025>

Qin, Hong-Yan & Cheng, Chung-Wah & Tang, Xu-Dong & Bian, Zhao-Xiang. (2014). Impact of psychological stress on irritable bowel syndrome. *World Journal of Gastroenterology*, 20, 14126-14131. DOI: 10.3748/wjg.v20.i39.14126

Rao, A. V., Bsted, A. C., Beaulne, T. M., Katzman, M. A., Iorio, C., Berardi, J. M., & Logan, A. C. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut pathogens*, 1(1), 6.

<https://doi.org/10.1186/1757-4749-1-6>

Reid, G., Gadir, AA., Dhir, R. (2019). Probiotics: reiterating what they are and what they are not. *Frontiers in Microbiology*, 10, 1-6. doi: 10.3389/fmicb.2019.00424

Ritchie, M. L. & Romanuk, T. N. (2012). A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLOS One*, 7(4), 1-11. doi:10.1371/ journal.pone.0034938

Sadrin, S., Sennoune, S., Gout, B., Marque, S., Moreau, J., Zinoune, K., Grillasca JP., Pons O., Maixent, J.M. (2020). A 2-strain mixture of *Lactobacillus acidophilus* in the treatment of irritable bowel syndrome: A placebo-controlled randomized clinical trial. *Digestive and Liver Disease*. doi: 10.1016/j.dld.2019.12.009

Sandler, R. S., Stewart, W. F., Liberman, J. N., Ricci, J. A., & Zorich, N. L. (2000). Abdominal pain, bloating, and diarrhea in the United States: prevalence and impact. *Digestive diseases and sciences*, 45(6), 1166–1171. <https://doi.org/10.1023/a:1005554103531>

Saikia, A., & Dutta, F. (2019). Probiotic Foods. In S.C. Deka, D. Seth, & N.R.S Hulle (Eds.), *Food Bioactives: Functionality and Applications in Human Health* (pp. 87-104). Apple Academic Press.

Salarkia, N., Ghadamli, L., Zaeri, F., & Sabaghian Rad, L. (2013). Effects of probiotic yogurt on performance, respiratory and digestive systems of young adult female endurance swimmers: a randomized controlled trial. *Medical Journal of the Islamic Republic of Iran*, 27(3), 141-146.

Schober, P. & Vetter, T. (2019). Two-sample unpaired t tests in medical research. *Anesthesia & Analgesia*, 129(4), 911. doi: 10.1213/ANE.0000000000004373

Serra-Majem, L., Frost Andersen, L., Henríque-Sánchez, P., Doreste-Alonso, J., Sánchez-Villegas, A., Ortiz-Andrelluchi, A., Negri, E., & La Vecchia, C. (2009). Evaluating the quality of dietary intake validation studies. *British Journal of Nutrition*, 102(1), S3-S9. doi:10.1017/S0007114509993114

Sevim, Y., Onur, H. N., Bayram, H. M., & Ergun, M. (2016). P-94 Knowledge and consumption frequency level of probiotics in elite volleyball players – a pilot study. *British Journal of Sports Medicine*, 50(Suppl 1). Doi: 10.1136/bjsports-2016-097120.147

Shing, C. M., Peake, J. M., Lim, C. L., Briskey, D., Walsh, N. P., Fortes, M. B., Ahuja KD., Vitetta, L. (2013). Effects of probiotics supplementation on gastrointestinal permeability, inflammation and exercise performance in the heat. *European Journal of Applied Physiology*, 114(1), 93–103. doi: 10.1007/s00421-013-2748-y

Taché, Y., Martinez, V., Million, M., & Wang, L. (2001). Stress and the gastrointestinal tract III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors. *American journal of physiology. Gastrointestinal and liver physiology*, 280(2), G173–G177. <https://doi.org/10.1152/ajpgi.2001.280.2.G173>

ter Steege, R. W., & Kolkman, J. J. (2012). Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Alimentary pharmacology & therapeutics*, 35(5), 516–528. <https://doi.org/10.1111/j.1365-2036.2011.04980.x>

Terpou, A., Papadaki A., Lappa I. K., Kachrimanidou V., Bosnea, L. A., Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, 11(7), 1-32. doi: 10.3390/nu11071591

Vitellio, P., Celano, G., Bonfrate, L., Gobetti, M., Portincasa, P., & Angelis, M. D. (2019). Effects of *Bifidobacterium longum* and *Lactobacillus rhamnosus* on gut microbiota in patients with lactose intolerance and persisting functional gastrointestinal symptoms: A randomised, double-blind, cross-over study. *Nutrients*, *11*(4), 1-15. doi: 10.3390/nu11040886

Wilson P. B. (2017). Frequency of Chronic Gastrointestinal Distress in Runners: Validity and Reliability of a Retrospective Questionnaire. *International journal of sport nutrition and exercise metabolism*, *27*(4), 370–376. <https://doi.org/10.1123/ijsnem.2016-0305>

Wilson, P.B., (2019). ‘I think I’m gonna hurl’: A narrative review of the causes of nausea and vomiting in sport. *Sports*, *7*, 1-13. doi: 10.3390/sports7070162

Wilson, P.B. (2020). *The Athlete’s Gut*. VeloPress.

Wosinska, L., Cotter, P. D., O’Sullivan, O., & Guinane, C. (2019). The potential impact of probiotics on the gut microbiome of athletes. *Nutrients*, *11*(10), 1-23. doi: 10.3390/nu11102270

Yan, F. & Polk, D. B. (2006). Probiotics as functional foods. *Current Opinion in Clinical Nutrition and Metabolic Care*, *9*(6), 717-721. doi: 10.1097/01.mco.0000247477.02650.51

Yilmaz, İ., Dolar, M. E., & Özpınar, H. (2019). Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: A randomized controlled trial. *The Turkish Journal of Gastroenterology: the Official Journal of Turkish Society of*

Appendices

Appendix A: Data from Diet Recall Review

Participant #	Did he/she consume probiotic rich foods? (Y/N)	Food(s) consumed & frequency	Notes
1	Y	Cheddar: 44	
2	Y	Yogurt x 1 / Cheese x 3 (type unknown)	
3	Y	Yogurt x 1	
4	Unknown	Cheese x 2 (type unknown)	Cheese types not specified, likely not probiotic rich foods
5	Y	Kombucha x 2, yogurt x 3, sour cream x 1	
6	Y	Kombucha x 2, parmesan cheese x 1	
7	Unknown	Cheddar x 1, unknown cheese x 4, parmesan x 1	
8	Y	Sourdough bread x 3 (slices)	
9	Unknown	Cheddar cheese x 3	
10	Unknown	Parmesan cheese x 2	
11	Y	Yogurt x 2, Parmesan cheese x 1, unknown cheese x 1	
12	Y	Yogurt x 1	
13	Y	Yogurt x 1, unknown cheese x 1	
14	Y	Yogurt x 1, cheddar x 2, parmesan x 1	
15	Unknown	Cheddar cheese x 1	
16	Unknown	Cheddar x 1, parmesan x 1	
17	Y	Parm: 17	
18	Unknown	Unknown cheese x 2	
19	Unknown	Cheddar x 2, unknown cheese x 1	
20	Unknown	Cheddar x 1	
21	Unknown	Parmesan x 1	
22	Unknown	Unknown cheese x 2	
23	N		
24	Unknown	Parmesan x 1	
25	N		
26	N		
27	Y	Yogurt x 2, parmesan x 1	
28	Unknown	Cheddar x 2, sour cream x 1	
29	N		
30	Unknown	Cheddar x 3	
31	Y	Yogurt x 1, olives x 1, unknown cheese x 1	
32	Unknown	Cheddar x 1	
33	Unknown	Unknown cheese x 2, Cheddar x 1	
34	Y	Cheddar x 1, olives x 1	
35	Y	Sour cream x 2	
36	Y	Dill pickle x 1, cheddar x 1, unknown cheese x 1	
37	Y	Feta x 1, parmesan x 1	
38	Y	Sour cream x 2	
39	Y	Olives x 1, feta x 1	
40	Y	Sour cream x 1	
41	Y	Yogurt x 2,	
42	Y	Yogurt x 2, Cheddar x 2	
43	Unknown	Cheddar x 2	
44	Y	Cheddar x 3, yogurt x 1, sour cream x 1,	
45	Unknown	Cheddar x 1	
46	Y	Cheddar x 2, sour cream	
47	Y	Cottage cheese x 3, yogurt x 2	
48	Y	Olive x 2	
49	Y	Olives x 2, cheddar x 1	
50	N		

51	Y	Cheddar x1, sour cream x2	
52	Y	Cheddar x1, sour cream x1	
53	Y	Olives x2, yogurt x2, unknown cheese,	
54	Y	Yogurt x2, unknown cheese x2, sour cream x1	
55	Unknown	Unknown cheese x1, cheddar x2	
56	Unknown	Cheddar x1, unknown cheese x2	
57	Y	Olives x2, cheddar x1, sour cream x3, yogurt x1,	
58	Unknown	Cheddar x1, parmesan x1,	
59	Y	Yogurt x1, sour cream x1, cheddar x1	
60	Unknown	Cheddar x3	
61	N		
62	Y	Miso soup x1	
63	Y	Yogurt x2	
64	Y	Yogurt x1, cheddar x1, olive x1	
65	Y	Cheddar x3, sour cream x1, yogurt x1	
66	Y	Olives x1, pickle x1	
67	Y	Sour cream x1,	
68	N		
69	Y	Olives x1, yogurt x1, unknown cheese x3	unknown cheese is likely not probiotic rich
70	Unknown	Parmesan x1, unknown cheese x2	
71	Y	Yogurt x1	
72	Y	Yogurt x1	
73	Y	Yogurt x1, kombucha x1, sour cream x1,	
74	Y	Cheddar x4, yogurt x1	
75	Y	Yogurt x2, Cheddar x1, , parmesan x1	
76	Unknown	Cheddar x2	
77	Y	Sour cream x1	
78	Unknown	Cheddar x1	
79	Y	Sour cream x4	
80	Y	Yogurt x2	
81	Y	Yogurt x3, sourdough bread x1, sour cream x1, parmesan x1	
82	Y	Yogurt x2, goat cheese x1,	
83	Unknown	Parmesan x1	
84	Y	Yogurt x1	
85	N		
86	Y	Yogurt x1	
87	Y	Yogurt x1, Brie x1	
88	N		
89	Unknown	Cheddar x1, unknown cheese x1	
90	Y	Yogurt x2, cottage cheese x1,	
91	Y	Olives x2, unknown cheese x2, Cheddar x1, yogurt x1	
92	Unknown	Cheddar x2,	
93	Y	Yogurt x2	
94	Y	Cheddar x1, sourdough x1	
95	Y	Olives x1, kimchi x1, parmesan x1,	
96	N		
97	N		
98	N		
99	Unknown	Cheddar x1	
100	Unknown	Cheddar x1	
101	Y	Sourdough bread x1, cheddar x2, probiotic juice	
102	Y	Yogurt x1, cheddar x1	
103	N		

104	N		
105	Y	Kombucha x1, unknown cheese x1, feta x1	
106	Unknown	Unknown cheese x2	
107	N		
108	N		
109	Unknown	Cheddar x1	
110	N		
111	Unknown	Cheddar x1	
112	Unknown	Unknown cheese x1	
113	Y	Sour cream x1	
114	Y	Sour cream x1	
115	Y	Yogurt x1	
116	Unknown	Unknown cheese x1	
117	N		
118	N		
119	N		
120	Unknown	Cheddar x1, unknown cheese x1	

Appendix B:

Dietitians were asked to answer four questions when providing feedback:

a) How many years have you been working in the Sports Nutrition field?

b) Does the PRO-Q include the food/beverage items that you think are the most common sources of probiotics consumed by athletes? Should additional items be added?

c) Are the instructions written a way that will be understandable to college athletes?

d) Any other feedback you wish you provide?

Responses for a)	Responses for b)	Responses for c)	Responses for d)
<ul style="list-style-type: none"> ● 20 years ● 30 years ● 31 years ● 10 years 	<ul style="list-style-type: none"> ● Probiotic shots ● Natto ● Kvass ● Refrigerated pickles ● Probiotic bars ● Probiotic juice ● Other vinegars ● Supplements ● Olives ● Fermented vegetables ● Soy sauce 	<ul style="list-style-type: none"> ● Long intro, consider breaking up ● Consider defining probiotic 	<ul style="list-style-type: none"> ● Change color for ease of readability ● Consider adding 3 or more times per day as an option ● Specify serving size ● Consider brand of products ● Adjust overlapping time frames ● Changing “unpasteurized sauerkraut” to “refrigerated sauerkraut” ● Specifying types of cheese versus listing “raw cheese” ● Clarification that not all fermented foods contain probiotics

Appendix C:

The following food frequency questionnaire (FFQ) was developed by researchers at Virginia Tech in the Department of Athletics and the Department of Human Nutrition, Foods, and Exercise as part of a research study to evaluate intake of foods containing probiotics among collegiate athletes and associated gastrointestinal symptoms. Participants are asked to complete the FFQ as well as the gastrointestinal symptom questionnaire which has been adapted from another study. Participation is voluntary and there is no compensation or direct benefit for participating. Responses will be kept anonymous, and you may withdraw from the study at any time. By completing this questionnaire, you are giving consent to participate in this study. The questionnaire should take less than 10 minutes to complete.

Please contact Mackenzie Bowman, RD, CNSC at mackenzieb@vt.edu with any questions regarding the study. If you have questions about the protection of human research participants, contact the Virginia Tech Institutional Review Board at irb@vt.edu or (540) 231-3732.

Please mark the frequency of consumption of each of the following foods over the **last 4 weeks**. Select the serving size that most closely matches your intake.

	Never or less than once per month	1-3 x per month	Once a week	2-4 x per week	5-6 x per week	1 x per day	2 or more times per day	<u>Serving Size Each Time</u>
Yogurt								
Kefir								
Kombucha								
Tempeh								
Refrigerated Sauerkraut								
Miso								
Kimchi								
Apple Cider Vinegar								

Sourdough bread								
Cottage Cheese								
Fermented vegetables								
Soy sauce								
Olives								
Refrigerated pickles								
Probiotic Juice								
Probiotic Bar								
Other foods not listed: _____								

Appendix D:

Dietary Assessment of Foods Containing Probiotics in Collegiate Athletes

Please fill out the following to the best of your ability

- 1) ID Number: _____
- 2) Age: _____
- 3) Class (i.e. Freshman): _____
- 4) Sport: _____
- 5) Gender: _____
- 6) Height: _____
- 7) Weight: _____
- 8) Hometown: _____
- 9) Race/ Ethnicity: _____
- 10) Prescription Medication(s): _____
- 11) Supplements (i.e. Vitamin, Herbal, Creatine, etc): _____
- 12) Dietary Restrictions (i.e. Gluten free, vegetarian): _____
 - a) Reason for following (Prescribed or choice): _____
- 13) Health Issues (i.e. IBS, Diabetes): _____
- 14) Have you ever taken a probiotic supplement (capsule or powder)? Yes ____ No ____

*If yes, please answer questions 15-17. **If no**, please move on to the next section.*
- 15) What brand/strain did you take? _____
- 16) Was this supplement prescribed by a physician? _____
 - a) If so, was it prescribed for a specific condition? _____
- 17) Are you currently taking this supplement? Yes ____ No ____

Appendix E: Sample NDSR Printout



NDSR 2020 Daily Values Report for Adults and Children 4 Years of Age and Older

Project Abbreviation: PROQ

Participant ID: 1

Date of Intake: 8/2/2020

Nutrient	Amount Reported	Daily Value ⁴	Percent Daily Value
Calories	2728 kcal		
Total Fat	128.6 g	78 g	165 %
Saturated Fat	42.5 g	20 g	213 %
Trans Fat	2.7 g		
Cholesterol	367 mg	300 mg	122 %
Sodium	4941 mg	2300 mg	215 %
Total Carbohydrate	242.3 g	275 g	88 %
Dietary Fiber	17.1 g	28 g	61 %
Total Sugars	105.3 g		
Added Sugars	43.7 g	50 g	87 %
Protein	156.3 g		
Vitamin D	6.3 mcg	20 mcg	32 %
Calcium	1268 mg	1300 mg	98 %
Iron	13.7 mg	18 mg	76 %
Potassium	3282 mg	4700 mg	70 %
Vitamin A (RAE) ¹	726 mcg	900 mcg	81 %
Vitamin C	146.1 mg	90 mg	162 %
Vitamin E	7.8 mg	15 mg	52 %
Vitamin K	118.2 mcg	120 mcg	99 %
Thiamin	3.7 mg	1.2 mg	308 %
Riboflavin	3.0 mg	1.3 mg	233 %
Niacin (NE) ²	66.3 mg	16 mg	414 %
Vitamin B6	2.4 mg	1.7 mg	140 %
Folate (DFE) ³	709 mcg	400 mcg	177 %
Vitamin B12	6.6 mcg	2.4 mcg	275 %
Pantothenic Acid	6.4 mg	5 mg	128 %
Phosphorus	2128 mg	1250 mg	170 %
Magnesium	324 mg	420 mg	77 %
Zinc	17.7 mg	11 mg	161 %
Selenium	222.9 mcg	55 mcg	405 %
Copper	1.1 mg	0.9 mg	121 %
Manganese	2.7 mg	2.3 mg	119 %
Choline	436.0 mg	550 mg	79 %

Note: DSAM nutrients are not included in these totals. Nutrient totals may not equal the sum of their parts. (Refer to the NDSR User Manual.)

¹ RAE = Retinol Activity Equivalents

² NE = Niacin Equivalents

³ DFE = Dietary Folate Equivalents

⁴ Nutrient calculations are performed for NDSR nutrients for which a Daily Value has been established. Daily Values are based on a caloric intake of 2000 kcal per day. Source: Code of Federal Regulations, Title 21- Food and Drugs, Volume 2, Sec 101.9, Nutrition labeling of food, 2018.

Appendix F:

Abdominal or Epigastric Symptoms

Adapted from the GI symptom questionnaire used by Bovenschen et al., 2006

Did you experience during the **last 4 weeks**:

	None	Mild	Moderate	Quite a lot	Severe	Very Severe	Unbearable
Abdominal Pain							
Epigastric Pain (Upper abdomen, right below ribs)							
Bloating							
Nausea							
Vomiting							
Loss of appetite							
Postprandial Fullness							
Belching							
Flatulence							
Frequent hard stools							
Diarrhea							
Alternately solid or loose stools							
Constipation							
Other: _____							