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Exploring The Short-Term Effects Of Probiotic And Prebiotic Supplementation On The Microbiota And Physiology Of Male C57bl/6 Mice

Miguel Angel Rivas
Wayne State University,

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**EXPLORING THE SHORT-TERM EFFECTS OF PROBIOTIC AND
PREBIOTIC SUPPLEMENTATION ON THE MICROBIOTA
AND PHYSIOLOGY OF MALE C57BL/6 MICE**

by

MIGUEL A. RIVAS

THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2017

MAJOR: NUTRITION AND FOOD SCIENCE

Approved By:

Advisor

Date

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2017

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DEDICATION

I dedicate this thesis to my mother, Gladys González Ortíz, who showed me the importance of education, perseverance, and humility from a young age. My mom survived a stroke while I was beginning my thesis work. I am very fortunate that she gets to witness its completion.

I also dedicate this work to my father, Arcilio Rivas Rodríguez, and brother, Angel Rivas González. Their support through my life challenges is what keeps me strong and focused.

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I want to acknowledge my outstanding advisor **Dr. Yifan Zhang**. She gave me wings to explore places I thought were unreachable and never hesitated to pick me up the times I fell while getting there.

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CHAPTER 1: INTRODUCTION

Microbiota

The human body is home to around 3.9×10^{13} bacterial cells [1]. They are present in several tissues like: gastrointestinal, urogenital, oral, nasal, and skin [2]. These microorganisms are collectively known as the microbiota. Much research has been directed at understanding the role that these communities play in our health. In 2008 advances in molecular biology and genome sequencing facilitated the implementation of the Human Microbiome Project (HMP) [2, 3] by the National Institutes of Health (NIH). This was established not long after the Human Genome Project, but was not as widely known at the time. However, the HMP has proven to have as powerful impact on our understanding of many diseases. A growing number of research studies have focused on the microbiota of the gut, in particular, since it has been correlated with chronic diseases such as type 2 diabetes, Chron's disease, and obesity [4]. It is also a popular field of study since the microbiota can be manipulated through different models using diet modification [5], fecal transplant [6], and even antibodies [7]. The gut microbiota is not unique to humans. Bacterial communities are also found in other mammalian species like mice [8]. This makes them easier to study since mice can act as a surrogate for studying in vivo interactions between host and microbiota.

Bacteroidetes, Firmicutes, and Proteobacteria are the most abundant phyla in our gut [9], yet no two individuals share the same microbiota [2]. The exact composition changes through our lifetime in both diversity and structure. It can also be affected by antibiotic therapy, overseas traveling, and temporary illness [10]. This constant change in the microbiota can many times go unnoticed and can have an influence on our health.

We know that gut bacteria can play a beneficial role by increasing levels of short-chain fatty acids (SCFA) and preventing food allergies [11]. They are also capable of protecting against pathogens [12] and toxins [13] as well as maintaining the integrity of the intestinal epithelial barrier [14]. However, they can also be detrimental to our health. The gut microbiota can influence gastrointestinal disorders, colon cancer, hypertension, and even asthma [9]. Some associated conditions like obesity or colitis have also been associated with the western lifestyle [15]. This makes us question whether the rise in certain diseases is due to an impaired or dysbiotic flora. If true, we have to wonder if there are ways to stabilize the microbiota and what that would look like for each individual.

LPS + LBP

Lipopolysaccharides (LPS) provide structure and protection to the outer membrane of almost all Gram-negative bacteria, especially those that are pathogenic such as *Escherichia coli*, *Salmonella enterica*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, and *Helicobacter pylori* [16]. They consist of a hydrophobic domain known as lipid A endotoxin, a non-repeating oligosaccharide, and a distal polysaccharide, or O-antigen [17]. LPS that enters the mammalian bloodstream can cause many negative health effects. Cells in the intestinal lining known as enterocytes can internalize Gram-negative bacteria through phagocytosis mediated by toll-like receptor 4 (TLR4) [18]. Lipoproteins like chylomicrons become associated with a lipopolysaccharide binding protein (LBP) and are responsible for inactivating the LPS from the dead bacteria and transporting it to target tissues [19].

LBP is a 58 kDa glycoprotein [20] synthesized in the liver [21] that forms a complex with LPS which can bind to CD14 receptors in macrophages or neutrophils and trigger

an inflammatory response involving interleukin-1 (IL-1) and IL-6 cytokines [22]. Elevated serum LBP levels have been associated with heavy drinking [23], metabolic syndrome, and obesity [24]. Levels tend to be higher in males and increase with age [25]. We know that lifestyle factors can contribute to higher LBP levels, but LBP resulting from endotoxemia can also play a role in obesity and insulin resistance [26]. This means there is more to obesity than an excess of food intake and that the microbiota can also play an important role.

Bifidobacteria

The Bifidobacterium genus consists of Gram-positive, non-motile, non-spore-forming, rod-shaped, and often branched anaerobic bacteria. They belong to the Actinobacteria phylum. These bacteria are associated with animal habitats and have been isolated from feces, sewage, human vagina, dental caries, and honey bee intestine [27]. They cannot grow below 20°C or above 46.5°C and prefer a pH close to 6.5. In humans, bifidobacteria are most abundant at birth, likely due to the fermentation of maternal milk [28]. While populations normally decrease with age, they can still be boosted through consumption of certain foods [5, 29].

Commercially, we can find these bacteria added to food products like yogurt which always propose some kind of health benefit. There does seem to be some protective effect against enterocolitis [13, 30]. Studies have also shown a negative correlation between bifidobacteria abundance and abdominal pain in healthy adults [10]. Models have shown that they can help reduce inflammation [31]. Still, there is another study suggesting that some bifidobacteria species may be associated with obesity [32].

Lactobacillus

Lactobacillus bacteria are Gram-positive, non-spore-forming, fermentative, microaerophilic, chemo-organotrophic, and can occur as rods or coccobacilli [27]. They belong to the Firmicutes phylum and are known for their ability to convert sugars into lactic acid, which can function as a preservative in foods. Lactobacilli grow best at a temperature of 30°C to 40°C. They are commonly incorporated as viable microorganisms in many foods and supplements. The list of lactobacilli used commercially is extensive and many have shown health benefits in mammals [33]. Despite this, there have been other strains that have been known to cause infections. The most common species that are associated with diseases are *L. casei*, *L. rhamnosus*, and *L. plantarum* [34].

VSL#3

Probiotics consist of live microorganisms, like those usually found in yogurt, that may have beneficial health effects. Most claim to provide some form of digestive or gastrointestinal relief. Few probiotics have had the same success as VSL#3. This particular probiotic is a well studied mixture containing high concentrations of freeze-dried bacteria (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*) [35]. The label states it contains over 225 billion bacteria that are 10 times as potent than the average probiotic [36]. This product is sold in capsule as well as powder form. Many studies have demonstrated positive effects in the dietary management of irritable bowel syndrome (IBS) [37, 38], ulcerative colitis [39-41], and an ileal pouch [42].

Potato Resistant Starch

Potato starch is a commercially available polysaccharide extracted from the potato root/vegetable. A serving of Bob's Red Mill Unmodified Potato Starch, 1 tablespoon, has 40 calories and 10 g of total carbohydrates. It is mainly used as a thickener in soups with similar cooking functionality to corn starch. Recently, it has grown in popularity among consumers afflicted with gastrointestinal issues like IBS. This product is classified as a resistant starch (RS), "defined as that fraction of dietary starch, which escapes digestion in the small intestine" [43]. It is also considered a prebiotic because its indigestible compounds can act as a substrate for the growth of healthy bacteria in the gut.

Amylopectin and amylose polymers are the main components of potato starch. These starches get fermented by the gut microbiota which produce short chain fatty acids (SCFA) that are primarily composed of acetic, propionic, and butyric acids [44]. This can lower the pH in the lumen and protect against the formation of cancerous tumors [45]. Resistant starches seem to provide a higher proportion of butyric acid than other indigestible carbohydrates [44]. This is important because butyric acid can act as a powerful anti-inflammatory agent for colonic cells by inhibiting IL-12 and up regulating IL-10 [46]. It also keeps toxins out of the bloodstream by reducing intestinal permeability [47].

Considering Supplements

Although supplements are regulated by the Food and Drug Administration, they do not undergo the same approval process as medications. Supplements are assumed to be safe until proven otherwise. The lack of clinical trials makes it difficult to assess the effectiveness of one supplement against another. Consumers rely on labels and per-

sonal experience. This is sometimes not enough to make informed decisions. For example, VSL#3 has been found to restructure the gut microbiota into a community that protects against diseases like colitis [48]. Potato starch also changes the microbiota into one that produces high levels of butyrate [49] which strengthen the intestinal wall [47] and reduce inflammation [46]. Consumers report similar health benefits using either VSL#3 or potato starch. These products are especially popular among groups suffering with IBS. However, VSL#3, can be many times more expensive than potato starch. This makes it unattainable to many. Therefore, there is a need to understand the differences between supplements to justify a higher price point. Our study is the first to compare the short-term effects of VSL#3 against potato starch in a healthy mouse model.

CHAPTER 2: OBJECTIVES

- 1) Design a replicable, non-invasive, mouse model for testing supplements.
- 2) Test two commercially-available supplements associated with a beneficial microbiota and gastrointestinal relief: VSL#3 and potato starch.
- 3) Study the associated physiological changes in response to 15 days of dietary supplementation.
- 4) Compare the effectiveness of each diet in modifying the host microbiota.

CHAPTER 3: METHODS

Animals

Twenty-four male C57BL/6 mice, 21 days old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and used in this study following experimental protocol (16-03-061) approved by The Institutional Animal Care and Use Committee (IACUC) at Wayne State University (Detroit, MI). Sani-chips bedding was used instead of corn cob in order to minimize consumption by the animals. Mice were randomly assigned to one of 6 small cages (n = 4, per cage) with free access to food and water. After 2 days of acclimation, the mice were placed in one of three treatment groups and were labelled as shown in Figure 2. Briefly, three out of four mice in every cage were labelled using permanent markers (Sharpie, Oak Brook, IL) at the base of their tails in colors: red, green, and blue. The first two cages (n = 8) were fed a control chow diet: PicoLab® Laboratory Rodent Diet 5L0D (LabDiet, St. Louis, MO). The remaining 4 cages were divided into two test groups: potato starch and VSL#3. The 10% potato starch supplemented diet (Bob's Red Mill, Milwaukie, OR) was formulated, pelleted (Figure 1), and irradiated by TestDiet (St. Louis, MO) and kept at 4 °C. Water for all groups was changed daily. The VSL#3 group was given the control chow diet with modification of water, formulated daily as published previously [50]. Briefly, one pack of unflavored VSL#3 probiotic mixture was dissolved in 1 L of water. Five-hundred milliliters was given to each of the two VSL#3 group cages. Water intake was monitored daily for all groups. Food intake was monitored periodically as described in Table 1. Body weight was measured on day 0, 8, and 14. Following 15 days of dietary intervention, mice were euthanized using CO₂ followed by tissue harvest. Blood was collected through cardiac puncture, processed to obtain

serum with SST Microtainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), and stored at -80°C (Figure 1). Animals were 5 weeks of age at the time of sacrifice.

Measurement of Fecal Bacteria

Fresh fecal samples were collected from the animals at specific times (Table 1). Each mouse was placed into individual empty cages for a short period until defecation and collection. Stools were stored at -80°C . Microbial DNA was extracted from the feces using the FastDNA spin kit for soil (MP Biomedicals, LLC, Solon, OH). DNA concentrations were quantified using a NanoDrop 2000 (Thermo Fisher Scientific Inc., Asherville, NC) and diluted to $4\text{ ng}/\mu\text{L}$ before being stored at -20°C until analysis. Microbial profiles were obtained using 96-well plates in a CFX96 Touch System (Bio-Rad Inc., Mississauga, ON). Samples were run in triplicates with a final volume of $10\mu\text{L}$ containing $5\mu\text{L}$ of SsoAdvanced Universal SYBR Green Supermix, $0.5\mu\text{M}$ primers, and 4ng of template DNA. Standard curves were generated using reference strains *Escherichia coli* (Total bacteria), *Bifidobacterium infantis* (Bifidobacteria), and *Lactobacillus rhamnosus* (Lactobacillus) diluted 10-fold. The curves were normalized to copy number using previously published 16S rRNA gene copy number values [51]. Group-specific primers are shown in Table 2 along with reference strains for generating standard curves.

Amplicon Validation

Amplicon sizes of group specific primers for total bacteria, bifidobacteria, and lactobacillus were identified through ethidium bromide stained-agarose gel electrophoresis after end-point polymerase chain reaction (PCR). Size was determined using a 100 base pair ladder. F1 and F2 were female mouse samples on a control diet from another study and used for comparison. C1, C2, and C5 were control mice. PS1, PS2, and PS5 were

samples from mice given a 10% potato starch diet. V1, V2, and V5 were mice drinking VSL#3 probiotic water. EC-ref, B-ref, and L-ref are DNA samples from our reference strains: *Escherichia coli*, *Bifidobacterium infantis*, and *Lactobacillus rhamnosus*. VSL#3 was DNA extracted from the live bacteria found in the VSL#3 probiotic. NTC was the no template control that only contained master mix, H₂O, and primers.

LBP ELISA

Serum LBP levels were measured in duplicates with the Mouse Lipopolysaccharide-binding protein ELISA Kit (Aviva Systems Biology Corporation, San Diego, CA) read at 450 nm.

Glucose Assay

Serum glucose levels were measured in triplicates using a Glucose Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI) read at 500nm.

Statistical Analysis

Statistical analyses were performed in SPSS version 24.0 (Armonk, NY) at a significance level of 0.05. Differences among the three groups were assessed using the Kruskal-Wallis test and subsequent nonparametric Mann-Whitney U test.

CHAPTER 4: RESULTS

Non-Invasive Labelling

The red label was easier to visualize and outlasted the blue as well as the green. The green labelled mice required more reapplication of marker dye than the other two. Mice did not show any differences in weight, food intake, or water intake because of the labelling.

Intake and Physiology

The mice showed normal food and water consumption after exposure to the diets (Figure 3). There was an increase in water intake for the VSL#3 group. Food intake remained the same across all groups. Body weight at day 14 was statistically different between the potato starch group and the control only (Table 3). Tissue measurements in the same table indicate an increase of large intestine weight in mice given the 10% potato starch supplementation. This is statistically different against the control and the VSL#3 group. Blood glucose levels were almost identical in all the diet groups.

Fecal Microbial Profile

Quantitative PCR data showed similar numbers of total bacteria among the three groups (Figure 4). Bifidobacteria levels were higher after potato starch supplementation (9.25 ± 0.26) compared to control group as well as VSL#3 group (6.77 ± 0.60 , 7.33 ± 0.21 respectively). VSL#3 supplementation was associated with more lactobacillus (8.24 ± 0.21) compared to control and potato starch groups (7.71 ± 0.52 , 7.73 ± 0.37 respectively). Values are given in log 16S rRNA gene copies/ng of total DNA (mean \pm SD). Differences among the three groups were assessed using the Kruskal-Wallis test and subsequent nonparametric Mann-Whitney U test at a significance level of 0.05. Primers

were validated through endpoint PCR and showed normal amplification (Figure 5). The amplicon sizes matched those found in literature (Table 2).

LBP

Supplementation with VSL#3 was associated with lower LBP levels (Figure 6) with a mean of 0.97 ± 0.38 $\mu\text{g/mL}$. LBP levels in potato starch (1.82 ± 0.60 $\mu\text{g/mL}$) have a higher mean than controls (1.47 ± 0.45 $\mu\text{g/mL}$) but did not reach significance ($p > 0.05$) using the Kruskal-Wallis test and subsequent nonparametric Mann-Whitney U test. Samples were measured in duplicates (total $n = 48$). Values given as mean \pm SD.

CHAPTER 5: DISCUSSION

Many studies have shown the health benefits of incorporating probiotics and prebiotics in our diet. Few studies have looked at whether one is more beneficial than the other. Both supplements have grown in demand and are commonly used to relieve gastrointestinal and digestive issues. Probiotics might appear more beneficial since they contain actual living bacteria. These have been shown to help with diarrhea [52] and decrease the numbers of pathogenic bacteria in our gut [53]. They are also common in foods like yogurt. The disadvantage is that viability and effectiveness of a probiotic can depend on product brand, shelf life, bacterial count, temperature, pH, tissue adhesion, and host immune response [54]. Many prebiotics, on the other hand, have longer shelf lives and make it all the way to the colon without being absorbed. Therefore, instead of introducing new bacteria, they can help increase the abundance of beneficial ones that are already part of our GI tract. These would then produce SCFAs and help prevent colon carcinogenesis [55]. Since prebiotics are dietary fiber they can be found in many affordable foods like fruits and vegetables. This makes them more accessible to the average consumer.

In this study we compared the VSL#3 probiotic against the potato starch prebiotic in vivo. We introduced mice to 15 days of potato starch or VSL#3 supplementation and were surprised by the quick changes in their physiology and microbiome.

Both mice groups as well as controls consumed similar amounts of food. This was an important observation since one group had customized food pellets that incorporated 10% potato starch. There was a concern that they would not like the food. Even though this group showed normal food intake they were associated with increased body

weight on day 14. This was an unexpected outcome since all groups had gained the same amount of weight from day 0. The weight difference may have been due to slower digestion since their large intestines were also heavier. We also know that potato starch acts like fiber and increases colonic transit time. This slower digestion has not been associated with any detrimental outcomes. In fact, potato starch has been found to produce high levels of butyrate which has many health benefits [56]. One concern for the mouse weight gain is that the potato starch may be selecting a gut microbiota that increases energy harvest [57]. However, this would likely involve higher levels of blood glucose compared to the other groups. In our study we found that all groups had similar blood glucose levels. The potato starch group did have a slight increase in LBP levels which have been associated with obesity. Still, this increase was not statistically significant.

Our other test group had been given the VSL#3 probiotic supplement through a water mixture that was made fresh daily. Interestingly, this group was found to have consumed more liquid than the others. This may have been due to the small traces of cornstarch in VSL#3 that possibly improved the taste of the water. VSL#3 is a well established probiotic and there is extensive research demonstrating its benefits in ulcerative colitis [39-41] and IBS [37, 38]. The VSL#3 product formula includes a mixture of both lactobacillus and bifidobacteria species. However, when we looked at the mice fecal microbial profiles we found that supplementation with VSL#3 only increased lactobacillus abundance. This differs slightly from another study that showed an increase in both lactobacillus and bifidobacteria [58]. It is possible that 15 days of supplementation is not enough time for bifidobacteria to become established. There may also be more lac-

tobacillus in the original product, since the actual numbers for each probiotic species in VSL#3 is not disclosed. Potato starch supplementation did a better job at increasing bifidobacteria abundance. This is supported by other studies [59, 60].

Lactobacillus and bifidobacteria are known to displace pathogens [61]. Therefore, both bacterial groups can be beneficial. Studies have demonstrated that the combination of both bacterial groups may provide more benefits by suppressing the pro inflammatory cytokines IL-6 as well as IL-7 and promoting the expression of tight junction proteins claudin-1 and occluding, thus reinforcing the intestinal barrier [62]. It is possible that a combination of potato starch and VSL#3 into a synbiotic might be the best way to increase both lactobacillus and bifidobacteria. This has not yet been tested.

The VSL#3 probiotic was also associated with lower serum LBP levels, which is consistent with the literature [63]. It was impressive to see how LBP levels dropped so quickly after only 15 days of supplementation. Low LBP levels are associated with lower risk of obesity [24] and inflammation [64].

Our results have validated some of the benefits of potato starch and VSL#3 that are found in literature. While both products had short term effects on the microbiota, it is difficult to say which is more favorable. VSL#3 seemed to trigger an increase in lactobacillus, while potato starch demonstrated an increase in lactobacillus. In the future, we would like to see the effects of supplementation combining both VSL#3 and potato starch as a synbiotic. This could provide better health effects since the inclusion of bifidobacteria and lactobacillus along with prebiotics have been found to enhance the survival and activity of a host in both in-vitro and in-vivo experiments [65]. Limitations of

this study did not allow us to include female mice or a larger sample size for parametric statistical analysis. This should also be incorporated in future research.

CHAPTER 6: CONCLUSION

The VSL#3 probiotic and potato starch prebiotic are in high demand because of their potential health benefits. In this study we used a non-invasive mouse model to observe how the microbiota and murine physiology changed after just 15 days of supplementation. We found that the fecal bacteria can change significantly in only 15 days. There was an increase in bifidobacteria after potato starch supplementation. This group also showed higher body weight, although this may have been due to slower bowel movements. Mice given the VSL#3 probiotic were associated with greater lactobacillus abundance. They also had lower levels of serum LBP which might suggest that they are at lower risk for inflammation and obesity.

TABLE 1. ANIMAL MODEL TIMELINE

	DESCRIPTION	PROCEDURE	DATE
DAY - 2	Acclimation Period		Tue, Aug 30, 2016
DAY - 1	Acclimation Period		Wed, Aug 31, 2016
DAY 0	Experimental / Control Diet	<i>fecal sample, start diet</i>	Thu, Sep 1, 2016
DAY 1	Experimental / Control Diet		Fri, Sep 2, 2016
DAY 2	Experimental / Control Diet	<i>food intake</i>	Sat, Sep 3, 2016
DAY 3	Experimental / Control Diet		Sun, Sep 4, 2016
DAY 4	Experimental / Control Diet	<i>fecal sample, food intake</i>	Mon, Sep 5, 2016
DAY 5	Experimental / Control Diet		Tue, Sep 6, 2016
DAY 6	Experimental / Control Diet	<i>food intake</i>	Wed, Sep 7, 2016
DAY 7	Experimental / Control Diet		Thu, Sep 8, 2016
DAY 8	Experimental / Control Diet	<i>fecal sample, food intake</i>	Fri, Sep 9, 2016
DAY 9	Experimental / Control Diet		Sat, Sep 10, 2016
DAY 10	Experimental / Control Diet	<i>food intake</i>	Sun, Sep 11, 2016
DAY 11	Experimental / Control Diet		Mon, Sep 12, 2016
DAY 12	Experimental / Control Diet	<i>food intake</i>	Tue, Sep 13, 2016
DAY 13	Experimental / Control Diet		Wed, Sep 14, 2016
DAY 14	Experimental / Control Diet	<i>fecal sample, food intake</i>	Thu, Sep 15, 2016
DAY 15	Morning Fast (6 hours)	<i>euthanasia, tissue harvest</i>	Fri, Sep 16, 2016

TABLE 2. PRIMERS FOR Q-PCR

MICROBIAL GROUP	PRIMER SEQUENCE, 5'-3' (FORWARD, F AND REVERSE, R)	GENOMIC DNA STANDARD	AMPLICON (BP)	REFERENCE
Total Bacteria	F: ACTCCTACGGGAGGCAG R: CCGTMTTACCGCGGCTGCTGGCA	<i>Escherichia coli</i>	200	Amann, Krumholz, & Stahl, 1990; W.T. Liu, Mirzabekov, & Stahl, 2001
Bifidobacteria	F: CGCGTCYGGTGTGAAAG R: CCCCACATCCAGCATCCA	<i>Bifidobacterium infantis</i>	242	Delroisse et al., 2008
Lactobacillus	F: GCAGCAGTAGGGAATCTTCCA R: GCATTYCACCGCTACACATG	<i>Lactobacillus rhamnosus</i>	348	Castillo, et al., 2006

TABLE 3. ANIMAL CHARACTERISTICS IN RESPONSE TO DIETARY INTAKE

DESCRIPTION	control (N=8) Mean (SD)	potato starch (N=8) Mean (SD)	VSL#3 (N=8) Mean (SD)
daily food intake per mouse (g)	3.63 (0.26)	3.70 (0.36)	3.74 (0.29)
daily water intake per mouse (mL)	5.54 (0.77)	5.55 (1.21)	6.65 (1.39) ^a
body weight at day 14 (g)	20.61 (0.94) ^a	21.84 (0.71) ^b	20.89 (1.05) ^{a,b}
total bodyweight gain from day 0 (g)	7.71 (0.94)	8.65 (0.71)	8.27 (1.33)
spleen weight (g)	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)
spleen length (cm)	1.30 (0.2)	1.40 (0.1)	1.40 (0.1)
large intestine weight (g)	0.95 (0.08)	1.23 (0.10) ^a	0.95 (0.07)
large intestine length (cm)	10.10 (0.6)	10.70 (0.9)	10.50 (1.0)
blood glucose (mg/dL)	105.57 (34.52)	113.81 (21.51)	116.05 (22.07)

*All tissues were collected on day 15. Values in the same row not sharing the same superscript are **significantly different at $p < .05$** in the two-sided test of equality for column means. Tests assume equal variances and are adjusted for all pairwise comparisons within a row using the Bonferroni correction.

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**10% (w/w) potato starch
+ irradiation**

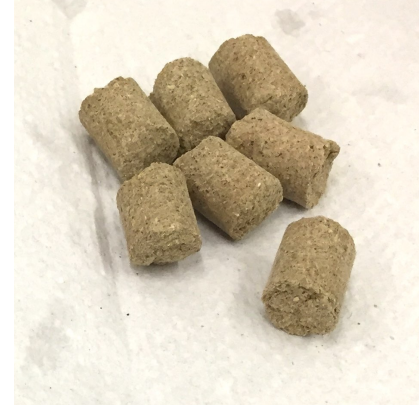


FIGURE 1. CONTROL VS. FORMULATED DIETS

The 10% potato starch food pellets (TestDiet) were similar in color to the control (LabDiet). The cross-sectional shape of the custom pellet was more circular rather than oval. The custom pellets were also smaller. Both were irradiated.



FIGURE 2. NON-INVASIVE ANIMAL LABELLING

Three out of four mice in every cage were labelled using permanent markers (Sharpie, Oak Brook, IL) at the base of their tails in colors: red, green, and blue.

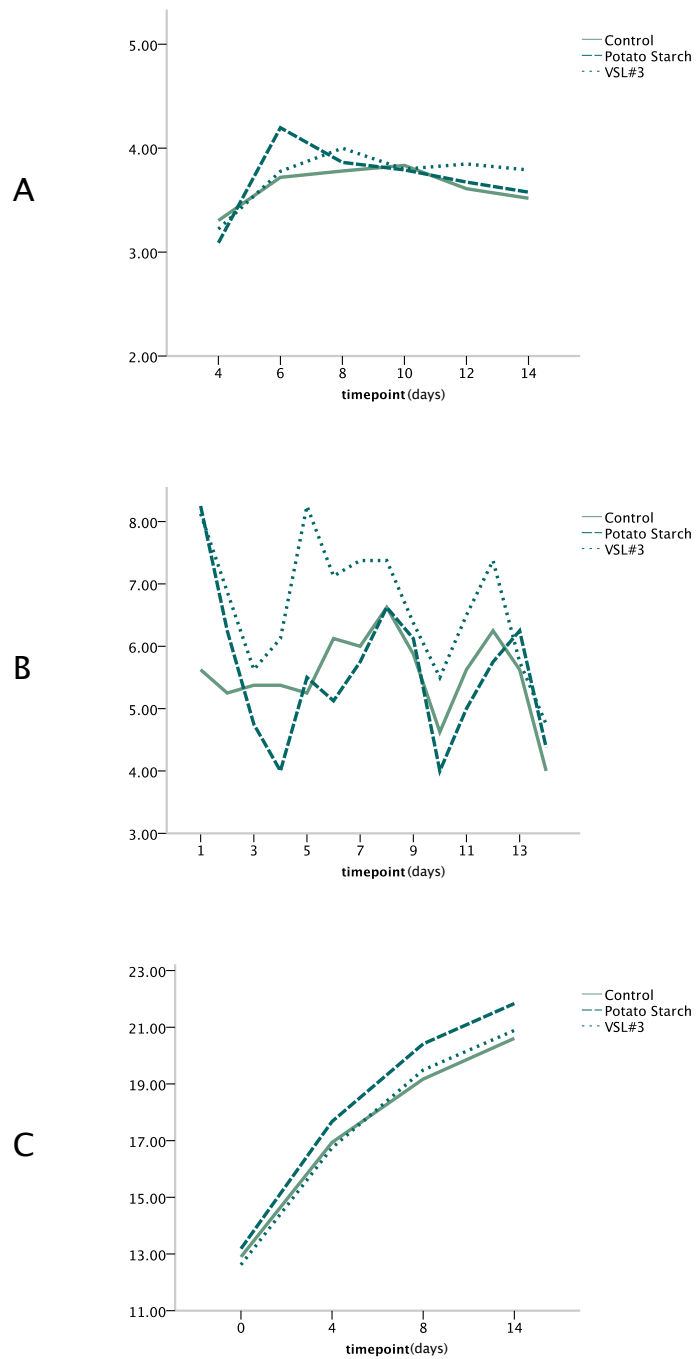


FIGURE 3. INTAKE AND BODY WEIGHT OVER TIME

A. Mean daily food intake per mouse (g). Intake was measured periodically for each cage and was divided by the total mice per cage (n=4).

B. Mean daily water intake per mouse (mL). Intake was measured per cage and divided by the total mice per cage (n=4).

C. Mean mouse body weight (g).

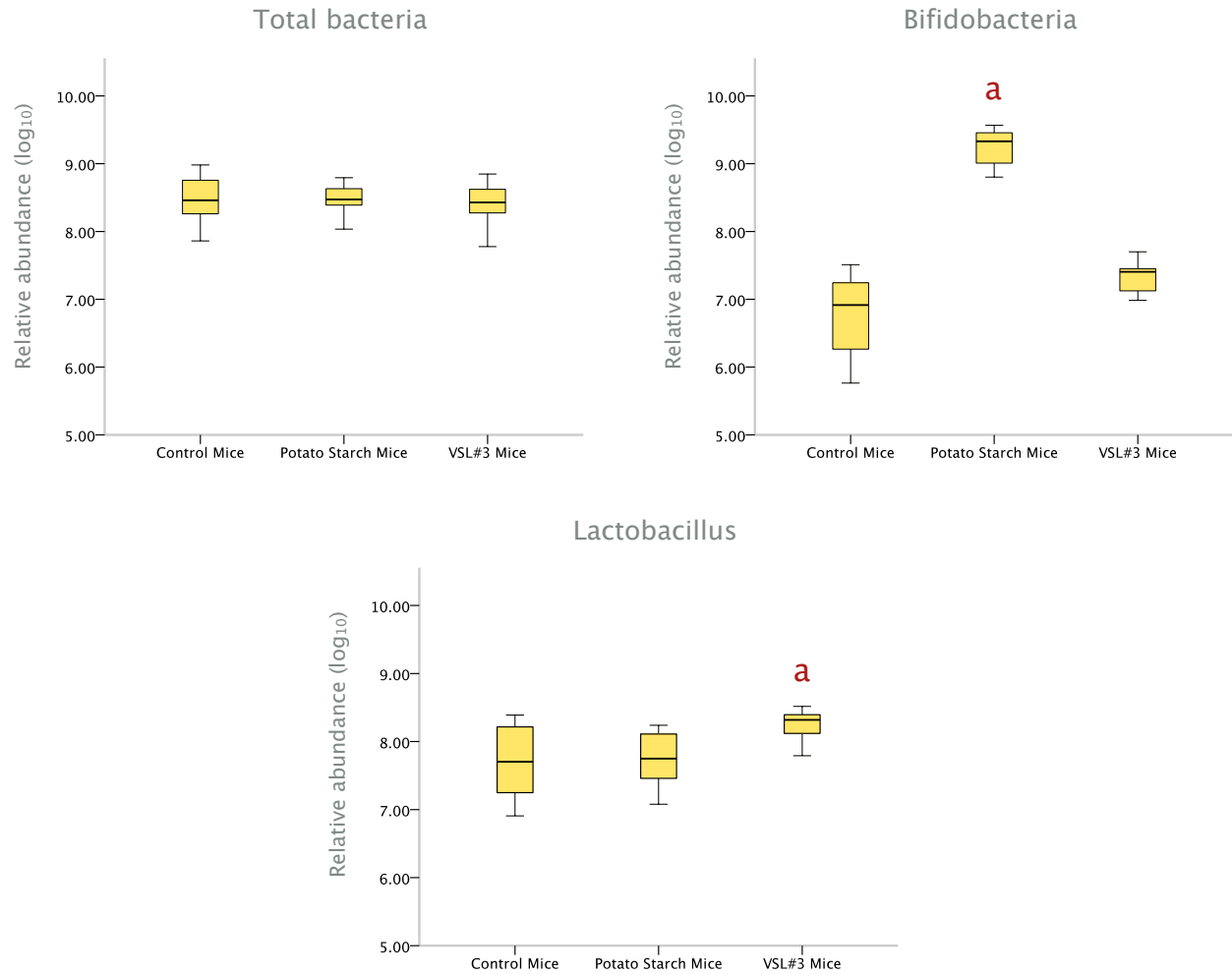


FIGURE 4. QUANTIFICATION OF FECAL BACTERIA

Abundance of microbial groups in fresh fecal samples measured by quantitative polymerase chain reaction (qPCR). Values are given in log 16S rRNA gene copies/ng of total DNA. Values not sharing the same letter are significantly different.

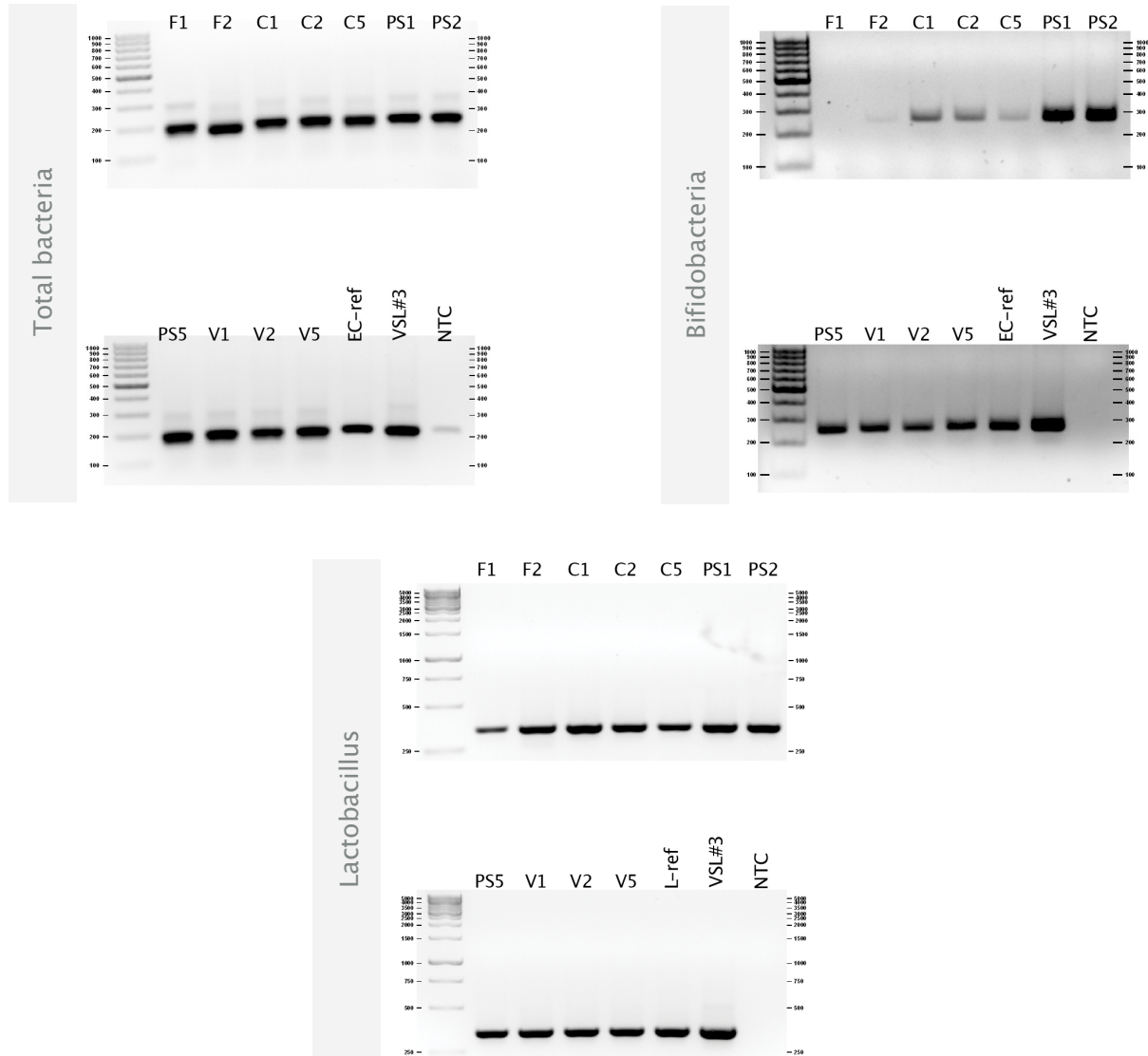


FIGURE 5. PRIMER VALIDATION AND AMPLICON SIZE

F1 and F2 were female mouse samples on a control diet from another study and used for comparison. C1, C2, and C5 were control mice. PS1, PS2, and PS5 were samples from mice given a 10% potato starch diet. V1, V2, and V5 were mice drinking VSL#3 probiotic water. EC-ref, B-ref, and L-ref were DNA samples from our reference strains: *Escherichia coli*, *Bifidobacterium infantis*, and *Lactobacillus rhamnosus*. VSL#3 was DNA extracted from the live bacteria found in the VSL#3 probiotic. NTC was the no template control that only contained master mix, H₂O, and primers.

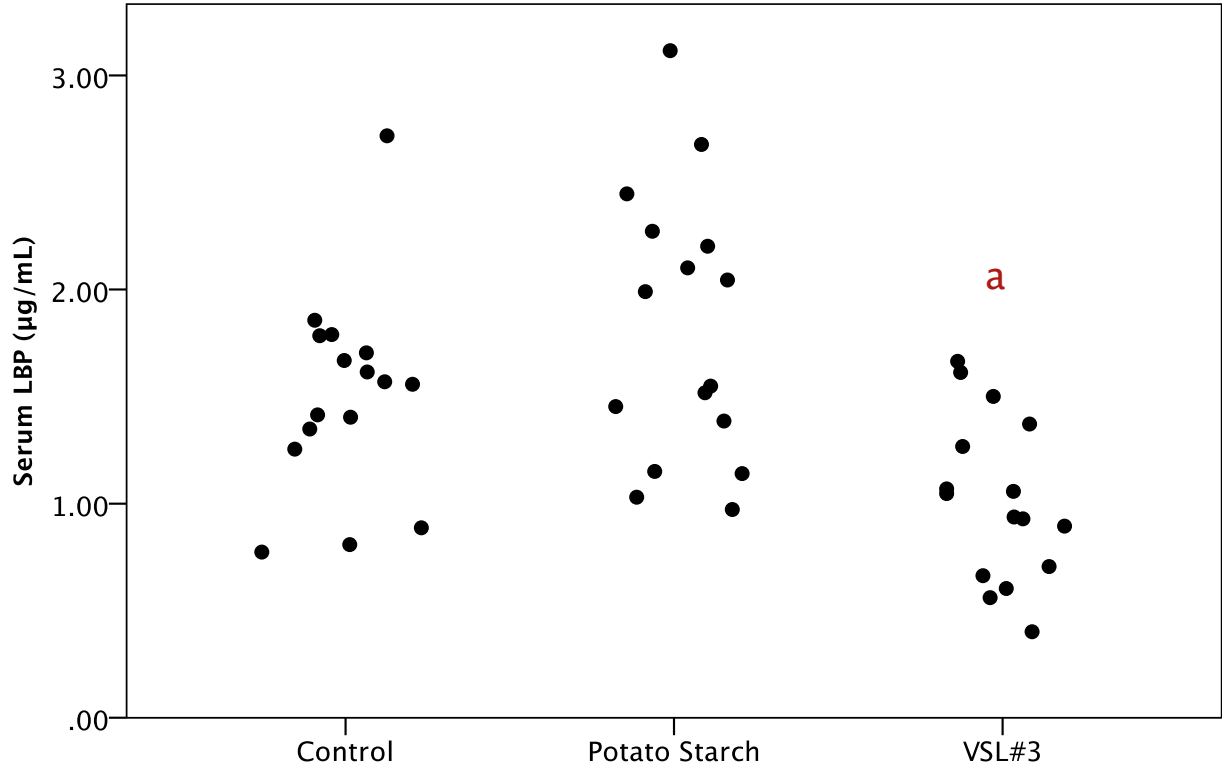


FIGURE 6. LEVELS OF SERUM LBP AFTER DIETARY SUPPLEMENTATION

Groups not sharing the same letter are significantly different.

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ABSTRACT**EXPLORING THE SHORT-TERM EFFECTS OF PROBIOTIC AND
PREBIOTIC SUPPLEMENTATION ON THE MICROBIOTA
AND PHYSIOLOGY OF MALE C57BL/6 MICE**

by

MIGUEL A. RIVAS**December 2017****Advisor:** Dr. Yifan Zhang**Major:** Nutrition and Food Science**Degree:** Master of Science

The gut microbiome may play a role in the development of chronic diseases like obesity, diabetes, and heart disease. Diets including prebiotics or probiotics can alter the abundance of gut bacterial groups and have subsequent health effects. In this study we wanted to establish a method for comparing the benefits of commercial supplements that alter the microbiota by monitoring fecal microbial profiles in male C57BL/6 mice (n = 24) exposed to 15 days of dietary supplementation. A probiotic diet (VSL#3) and a prebiotic diet (potato starch) were compared to a standard diet (n = 8 for each group). Microbial profiles were obtained through qPCR using group-specific 16S RNA primers.

The potato starch group showed higher body weight than the control ($p < 0.05$), but was similar to the VSL#3 group. The large intestine weight of the potato starch group was higher than the control and the potato starch group ($p < 0.05$). Food intake remained the same across the groups. Daily water consumption was higher in the VSL#3 group (6.65 ± 1.38 mL) as opposed to the potato starch and the control group

(5.55 ± 1.21 mL, 5.54 ± 0.77 mL respectively). Blood glucose levels were similar between all groups. Quantitative PCR data showed higher abundance of bifidobacteria at a significant level compared to control and VSL#3 groups. VSL#3 supplementation was associated with more lactobacillus ($p < 0.05$) and lower serum LBP levels ($p < 0.05$) compared to control and potato starch groups.

The microbiota changes observed with VSL#3 and potato starch supplementation were mostly consistent with the literature. However, VSL#3 probiotic did not demonstrate the same increase in bifidobacteria as other studies. This may suggest a need for prolonged consumption or combination with a prebiotic like potato starch. Weight increase after potato starch supplementation might have been due to slower digestion. There was a reduction in LBP levels after VSL#3 consumption, which may help prevent inflammation and obesity.

AUTOBIOGRAPHICAL STATEMENT

Miguel Angel Rivas González was born on July 26, 1990 in San Juan, Puerto Rico. He is the son of Gladys González and Arcilio Rivas. His parents and older sibling, Angel Rivas, encouraged him to work hard on academics from a young age. In high school, Miguel excelled in advanced biology, calculus, and physics. He also took part in several NSF funded research projects at Universidad Metropolitana. By sophomore year of high school he was learning microscopy and gel electrophoresis for the study of dinoflagellates in the bioluminescent bays of Puerto Rico. Outside of school and research he participated in dance recitals at Agnes Colón Dance Studio and dedicated over 100 hours of community work to his local chapter of United Way: Club Me Importas Tú.

In 2008, Miguel was awarded the Kinesis Scholarship for his outstanding academic achievement and community service. This gave him the financial support to work on his B.S. degree in Microbiology at University of Michigan, Ann Arbor. He joined the Undergraduate Research Opportunity Program (UROP) which introduced him to the work of Dr. David Markovitz at the Department of Internal Medicine and Infectious Diseases. Miguel remained as a member of the Markovitz lab for several years after graduation, where he studied the role of human DEK protein in juvenile idiopathic arthritis.

In Fall 2015, Miguel entered the M.S. program in Nutrition & Food Science at Wayne State University. Here he incorporated his laboratory skills into Dr. Yifan Zhang's food microbiology research. He wanted to understand the impacts of dietary supplementation on murine gut microbiota. His work in graduate school was recognized by a Diversity Scholarship from the Institute of Food Technologists and a Graduate Professional Scholarship from Wayne State University. Miguel also presented his work at the International Association of Food Protection Conference.

Miguel is now working on his professional career and aspires to become a role model for the continued development of his community.