# EFFECTS OF PSYLLIUM IN MILK REPLACERS FOR NEONATAL DAIRY CALVES

BY

STEPHANIE J. CANNON

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2009

Urbana, Illinois

Adviser:

Dr. James Drackley

**Abstract**

Psyllium has been shown to exert beneficial effects on intestinal health and function in several non-ruminant species. The effects of psyllium inclusion in milk replacers for neonatal dairy calves have been minimally researched. The purpose of my research was to determine the impact of psyllium inclusion in milk replacer on feed intake, water intake, growth, illness, digesta viscosity, rate of passage, nutrient digestibilities, blood components, VFA concentrations and microbial populations throughout the gastrointestinal tract, size and scale of gastrointestinal tissues, ion transport measures, and histomorphological development in the gastrointestinal tract in neonatal dairy calves.

Male Holstein calves, less than 36 h of age, were purchased for this trial. Calves were blocked by pairs and randomly assigned within pair to one of two dietary treatments. Treatments consisted of milk replacers (reconstituted to 12.5% DM and fed at a rate of 12% of BW) formulated to contain 22% protein and 20% fat either with (PSY) or without (CON) a 1.1% inclusion of psyllium. Pairs of calves were harvested at wk 1, 2, 3, and 4 for analysis.

Feed intake (DM, protein, and metabolizable energy), water intake, growth, and illness did not differ between treatments. Digesta viscosity was increased in the abomasal and colon digesta for the PSY treatment. In addition, DM content of digesta from the proximal colon, distal colon, and feces was decreased for the PSY treatment. Apparent digestibility of DM was greater for the CON treatment. The predominant effect of psyllium inclusion in the milk replacer on VFA concentrations was in the lower gastrointestinal tract as indicated by the higher total VFA concentrations in the jejunum,

proximal colon, and distal colon. Greater lower gut fermentation is logical because psyllium is a fiber that can be fermented by intestinal bacteria. Effects of inclusion of psyllium on bacterial counts were minimal.

Size and scale of the gastrointestinal tract were impacted by psyllium predominantly in the lower gastrointestinal tract. Calves fed PSY had heavier duodenums, more duodenal tissue per kilogram BW, increased duodenal wet weight, increased jejunal mass per unit of BW, denser jejunal tissue, and greater jejunal tissue density per unit of BW. Length of the jejunum per unit of calf BW was increased for the CON calves. In the ileum, the PSY calves had denser tissue. Colon wet weight, as well as dense colon tissue per unit of calf BW was increased for the PSY calves. The increase in size and scale of the gastrointestinal tract could be of benefit to the neonatal dairy calf from a standpoint of potentially greater absorptive capacities of the tissue and greater tissue resistance to disease intrusion into the bloodstream. The inclusion of a fermentable fiber could also potentially "set-up" the gastrointestinal tract to allow more rapid growth once calf starter ingestion begins.

In isolated segments of gastrointestinal tissues, inclusion of psyllium in the diet significantly affected the change in short circuit current induced by addition of 10 mM glucose. Glucose transport was greater in rumen, ileum, and colon from CON calves compared with calves fed PSY . These data could potentially indicate that psyllium inclusion slowed the rate of glucose absorption in the rumen, ileum, and colon.

Psyllium inclusion in milk replacer resulted in physiological changes consistent with improved performance and health. The effects of psyllium inclusion on growth and health should be determined in large numbers of calves under field conditions.

**Dedication**

In loving memory of my Grandpa, Charles Everett Cannon-

You taught me that nothing is impossible. After all, Vermont could be as flat as Illinois with the proper application of bulldozers and dynamite.

**Acknowledgements**

Ah, the conclusions of my M.S. work. This is a time that I have been looking forward to for a long time, and I know many of those who've supported me in my endeavors appreciate seeing this thesis project come to a close. I truly could never have made it this far without the support that I've received along the way.

First and foremost, thank you to Mom & Dad, for raising me in a barn. If it wasn't for my Tonka truck (that I spent hours feeding cows with) & Ted-Ted, my first Jersey calf (who inspired my 4-H career), who knows where I would have been today. Those two gifts inspired a life-time love, passion, and enthusiasm for the dairy industry.

The opportunity to research neonatal dairy calves working with Dr. Drackley at the U of I is truly an honor. I appreciate Dr. Drackley's wisdom, kindness, and patience. Lots of patience. Thank you.

Due to the complexity of my trial, I was also afforded the opportunity to work with Dr. Fahey and his laboratory, and Dr. Tappenden and her laboratory, and Dr. Wallace. Furthermore, Dr. Murphy was also willing to step in and explain things that were beyond my comprehension. Dr. Hutjens always had a way of making things real and applicable. This team of professors was truly a gift and blessing, and I am very grateful for this.

I also would like to extend thanks to all of my fellow grad students and co-workers who helped me succeed in this endeavor: Heather, Nicole, Jennifer, Joe, Noah, Dave, Lynda, Cheryl, Amy, Brandon, Lizzy, Amanda, Lauren and Deena. Your support and patience is much appreciated!

A very special thank you goes out to Amy, for not only being the best roommate, but also being a close friend and always being there for me. You always stepped in when you didn't have to, you did it because you wanted to (even assisting with a few 1 am diaper changes!!). You are truly an inspiration.

And also, to the QPRZ. You mean the world to me, and your never ending support (and influx of lawn gnomes) is truly appreciated. To Erin and Tex, words can not describe how much you mean to me. You've always supported me in all of my endeavors, have been a constant inspiration, and have never, ever let me give up; one target at a time. I am also truly grateful to my three thesis writing "assistants", whose constant companionship has been comforting and amusing; despite pen thefts, assaults and guardianship of the printer, and tricks with the powerstrip…And, of course, the five foot radius rule.

I am also truly grateful to Dr. Kesler, for not only giving me the opportunity to go to Brazil once, but to go twice. The impact that those trips made on my life is indescribable, and I am very fortunate and blessed to have been afforded those opportunities.

I thank Land O'Lakes for the sponsorship of this trial. I thank the Department of Animal Sciences for providing the facilities, equipment, and resources to complete my graduate research and Master's degree. And, I truly appreciate my 33 little dudes, who gave this trial their all.

## Table of Contents

# Chapter 1

# Literature Review

## Rumen Development in the Neonatal Dairy Calf

The dairy calf is born a unique and complex animal. At birth, the rumen, reticulum, and omasum of the ruminant stomach are present; however, these three compartments are underdeveloped. This fact, in conjunction with the esophageal groove and developmental state of abomasal and intestinal enzymes, compels the neonatal dairy calf to function as a monogastric animal (Heinrichs, 2005). The abomasum is considered the "true stomach" of the calf; this is attributed to the ability of the abomasum to carry out gastric digestion of proteins similar to the stomach of non-ruminants (Davis and Drackley, 1998).

Ingestion of dry feeds and the microbial end-products (short-chain fatty acids or volatile fatty acids; VFA) produced as a result of fermentation of the dietary carbohydrate and protein fractions have been shown to stimulate rumen epithelial development (Davis and Drackley, 1998). Rumen development can be attributed to the presence and absorption of VFA. Each VFA is unique in its metabolism, and the effects of each VFA on rumen development are also unique. Butyrate is the VFA most responsible for stimulation of rumen epithelial development, with propionate being the second most stimulatory (Heinrichs, 2005).

In newborn calves (0-3 days), the papillae of the rumen are partially developed. However, if the calf receives a diet of only milk or milk replacer, the papillae will show regressive changes, both in length and shape (Tamate *et al.*, 1962). This regression of

growth is theorized to result from the lack of VFA reaching the rumen from the milk diet, because of esophageal groove closure. Rumen development present in newborn calves can be attributed to VFA in the maternal blood, which then supplied the fetal rumen tissue with VFA for papillae growth and development (Tamate *et al.*, 1962).

The esophageal or reticular groove is a unique muscular fold whose closure allows liquid feeds to bypass the reticulo-rumen and be shunted into the abomasum of the calf (Warner *et al.,* 1956). When solid feeds are consumed, they are directed into the reticulo-rumen (Church, 1988). Therefore, milk or milk replacer consumed by the calf has limited ability to stimulate rumen development (Warner *et al.,* 1956). When calves consume only milk or milk replacer, minimal rumen epithelial metabolic activity and VFA absorption occur, and this is not affected by increasing age. On the contrary, the size of the rumen of calves fed milk or milk replacer has been shown to increase proportionally with body size (Vazquez-Anon *et al.,* 1993).

The impact of VFA to stimulate development of the structure and absorptive ability of the ruminal mucosa is through stimulation of metabolism in the ruminal mucosa (Sutton *et al.*, 1963). In neonatal sheep, infusion of physiological concentrations of VFA may stimulate rumen morphological and metabolic development (Lane and Jesse, 1997).

**Dietary Fiber**

Polysaccharides, one of the three classified groups of carbohydrates, are polymers of simple sugars (monosaccharides) and derivatives thereof (Garrett and Grisham, 1999). The physical properties of polysaccharides are dominated by the conformation of the individual chains and the way in which they interact with one another (Morris, 2001).

Dietary fiber is a complex mixture of polysaccharides with different functions and activities during passage through the gastrointestinal tract. Many of the functions and activities of fiber are dependent upon its physicochemical properties. These properties include solubility, viscosity, water-holding capacity, and adsorption of small molecules and ions (Oakenfull, 2001).

The current definition of dietary fiber (plant material that resists digestion by the enzymes of the human alimentary tract; Blackwood *et al.*, 2000) includes a number of non-starch polysaccharides (NSP) composed of gums and mucilages from a variety of seeds and fruits, as well as the cellulose and hemicellulosic polysaccharides and lignin (Blackwood *et al.,* 2000).

The predominant role of dietary fiber in the stomach and small intestine is to limit the rate of release of nutrients. This may occur by physical trapping of sugars, lipids, and proteins within solid particles or hydrated networks. Another mechanism is by increasing the viscosity of the digesta, therefore reducing the rate of nutrient transport to the epithelium. Both of these mechanisms focus on physical properties of fiber, as opposed to its chemical composition (Morris, 2001). By definition, fiber is not broken down by enzymes of the gastrointestinal tract. However, virtually all fiber fractions are fermented to some extent by the microorganisms in the cecum and colon. Fermentation depends upon accessibility of the polysaccharide molecules to the microorganisms, which in turn depends upon chemical structure and physical properties, particularly solubility (Oakenfull, 2001). The physiological properties of dietary carbohydrates are dependent on site, rate, and extent of absorption or fermentation in the intestine. In humans, consumption of dietary fiber has been found to increase stool weight, alter gut transit

time, alter activity of colonic microflora, modify absorption of fats, sugars, minerals, and bile acids, influence appetite, and absorb toxins. The extent to which specific dietary fibers exert these physiological effects is dependent on a complex mixture of structural, chemical, and physical properties (Blackwood *et al.* 2000).

In the field of ruminant nutrition, the most common measures of fiber are crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF). None of these three measures are chemically uniform. Crude fiber is considered obsolete and typically is not measured except for feed regulatory purposes. Neutral detergent fiber is predominantly a measure of the structural or cell wall components of plant cells, including cellulose, hemicellulose, and lignin. Acid detergent fiber is a measure of cellulose and lignin. Non-structural carbohydrates (NSC) are not recovered in the NDF fraction and comprise sugars, starches, organic acids, and other carbohydrates. NSC can be classified as water soluble, although some larger polysaccharides are insoluble (National Research Council, 2001).

**Psyllium**

Psyllium (*Plantago ovata* Forsskaol*)* is a herbaceous low-growing annual plant native to India and Iran, and is also referred to as Ispaghul (Blumenthal *et al.,* 2000). The seed husks of this plant, *Plantago ovata*, are commonly referred to as ispaghula husks (Leng-Peschlow, 1991) or psyllium (Washington *et al.,* 1998).

Psyllium seed husk is composed of mucilaginous polysaccharides, with a highly branched acidic arabinoxylan containing a xylan backbone (Washington *et al.,* 1998; Blumenthal *et al.,* 2000). Arabinoxylans are natural polysaccharides with highly

branched structures (Edwards *et al.*, 2003). Marlett and Fischer (2003) reported that psyllium seed husk is composed of 902 mg/g carbohydrate, 35 mg/g crude protein, and 34 mg/g ash.

Psyllium husk is a gel-forming, water-soluble fiber (McCall *et al.*, 1992a; Sierra *et al.*, 2002). Psyllium possesses limited ion-exchanging capacity (McCall *et al.*, 1992a). Psyllium interacts with water to form three fractions: a soluble fraction, a gel fraction, and the matrix fraction (Al-Assaf *et al.*, 2003). Marlett and Fischer (2002) have identified these same three fractions, and have identified them as Fraction A, Fraction B, and Fraction C. Fraction B is a physiologically active gel-forming component unique to psyllium seed husk, with a highly branched arabinoxylan polysaccharide (Fischer *et al.*, 2004), a xylose backbone, and arabinose and xylose side chains (Marlett and Fischer, 2003).

Arabinoxylans are an important fraction of non-starch polysaccharides (NSP) in dietary fiber that have been linked to increased fecal bulking as a result of the high water holding capacity and low degradability (Edwards *et al.*, 2003). Psyllium husks contain a high concentration of mucilage polysaccharides that gel over a wide range of concentrations (Washington *et al.*, 1998). The psyllium husk interacts with water to yield a visco-elastic system that is preserved throughout its transit in the colon. The solid matrix of the psyllium husk interacts with water to yield a gel fraction and a completely soluble fraction capable of rapid fermentation to short-chain fatty acids (i.e., VFA). These physical and chemical states can initiate a series of physical and physiological processes during transit through the colon (Al-Assaf *et al.*, 2003).

Current applications of psyllium in the United States include uses in human over-the-counter dietary supplements for increased fiber, cholesterol reduction, and laxative activity. The Food & Drug Administration in 1998 amended its soluble fiber health claim regulation to include blonde psyllium husk as a source (Blumenthal *et al.*, 2000).

**Viscosity**

In order to understand the viscous nature of psyllium and the resultant effects it is imperative first to define viscosity. Viscosity is the ratio of shear stress to shear rate, where shear stress is the resistance to rotation and shear rate is the amount of strain applied per second. Since stress is defined as units force per unit area, it has units of pressure, or pascals (Pa). Because strain is a dimension-less ratio, shear rate has units of reciprocal time ($s^{-1}$). The resultant measurement of viscosity is Pa·s. One Pa·s is equivalent to 1 centipoise (cP). More simply, viscosity is the tendency of a liquid to reduce flow (Morris, 2001).

Viscosity is caused by physical interactions between polysaccharide molecules in a solution, and most water-soluble fibers form viscous solutions (Oakenfull, 2001). Arabinoxylans (such as psyllium) exhibit a high viscosity in aqueous solution due to their extended conformity (Blackwood *et al.*, 2000).

The viscosity of solutions of disordered coils is strongly dependent upon concentration. In dilute solutions, the individual coils are free to move independently, and generate viscosity by tumbling and interfering with the flow of the solvent. Dilute solutions tend to exhibit Newtonian behavior, where the resistance to flow is almost directly proportional to the shear rate, and viscosity will remain virtually constant. As the

polymer concentration is raised, a point is reached at which the polymers begin to touch, and as concentration rises, the polymers overlap and interpenetrate one another. The transition from a solution of individual coils to an entangled network of overlapping chains is accompanied by a sharp increase in the concentration dependence of viscosity, and by the onset of non-Newtonian behavior. At higher shear rates, extent of random coil overlap decreases, and viscosity falls. This is known as shear thinning (Morris, 2001), which psyllium exhibits (Dikeman *et al.*, 2006).

Gastric emptying is known to be powerfully influenced by meal viscosity, with more viscous meals allowing less deep, and therefore less effective, antral contractions associated with delayed gastric emptying (Washington *et al.,* 1998). As fiber meal viscosity increases, meal emptying time is prolonged in dogs (Russell and Bass, 1985). Including psyllium at 1% of the diet resulted in digesta that exhibited a near Newtonian flow with a low viscosity compared to a meal containing 3% psyllium meal (Russell and Bass, 1985).

The effect of dietary fiber is related to viscosity and methods of administration. The mechanism of action of gel-forming fiber is related to its ability to increase viscosity of gastrointestinal contents and thus interfere with motility and nutrient absorption (Sierra *et al.*, 2002). By lowering the viscosity of digesta, the rate of flow should be increased (McRorie *et al.*, 1998). If soluble polysaccharides are consumed as part of a dry or low-moisture diet, their effect on digesta viscosity will be limited by their rate of dissolution and release, which in turn will depend upon the overall structure of the food and its susceptibility to mechanical and enzymatic degradation (Morris, 2001).

In a study utilizing pigs, a relationship between viscosity of digesta and water content of digesta was observed (McRorie *et al.*, 1998). It was concluded, therefore, that digesta viscosity is a function of water content when the diet is held constant. Furthermore, it was observed that when dry matter increases past 25%, a small increase in dry matter will result in a large increase in digesta viscosity (McRorie *et al.*, 1998).

**Effects of Psyllium on Digestive Function and Metabolism**

*Viscosity.* Polysaccharides are hydrophilic, with an ability to hold water and undergo gelation. The ability to hold water is due to the entrapment of water within the three-dimensional network of molecules. Insoluble fibers hold water more like a sponge (Oakenfull, 2001).

The high water holding capacity of soluble fibers has an effect on their viscosity and their interactions with other molecules in the gut. The high visco-elastic system provides roughage action, lubricates, and modifies absorption of certain nutrients. Psyllium is a particularly effective stool-bulking agent and is widely used in treatment of constipation and diarrhea. The water holding capacity improves consistency of liquid stools, and the laxative effect is a result of its ability to form a gel and hold many times its own weight in water. The water holding capacity affects stool weight and intestinal transit time (Blackwood *et al.*, 2000). Stool softening can be dramatically affected by relatively small increases in water content (McRorie *et al.*, 1998).

*Rate of Passage.* Psyllium hydrates in water to yield a viscous gel that could slow the gastric emptying of meal carbohydrates (Russell and Bass, 1985). Using dogs,

researchers found that as test meal fiber content increased, gastric emptying was slowed. Psyllium, at 1% inclusion, produced the same stomach flow rate in duodenally cannulated dogs as did the saline control meal. At 3% inclusion, however, the psyllium meals emptied slower, as if a constant amount of meal emptied per unit of time (Russell and Bass, 1985). Upper gut contractile activity regulates food transit through the small intestine, as well as the degree to which food is mixed with digestive enzymes and the exposure of digestive nutrients to the absorbing surface (Cherbut *et al.*, 1994).

In humans, psyllium has been shown to ameliorate diarrhea (Washington *et al.*, 1998; Blumenthal *et al.* 2000), while during cases of constipation, psyllium has been shown to decrease passage time through increased stool volume (Blumenthal *et al.*, 2000; Al-Assaf *et al.*, 2003). This effect in humans helps to restore and maintain regularity, and psyllium has been approved for use as a secondary medication for treatment of diarrhea (Blumenthal *et al.*, 2000).

Inclusion of psyllium in the diet increases daily fecal mass. Tomlin and Read (1998) observed increased fecal mass in humans consuming 14 g of psyllium per day and noted an inverse relationship between stool mass and transit time. Therefore, they determined that transit time and fecal mass are not necessarily dependent variables, and that stool frequency is more associated with transit time, not fecal mass (Tomlin and Read, 1998). However, in rats, Leng-Peschlow (1991) found that colonic motility and propulsion of contents was influenced by load of material. Furthermore, they observed that retention time determined the degree of digestion of fiber components (Leng-Peschlow, 1991). Fecal bulking results in decreased colonic transit time, thereby reducing exposure of colonic mucosa to intestinal contents (Edwards *et al.*, 2003).

In African green monkeys fed psyllium husk at 9.7% inclusion rate, a large day to day variation in fresh fecal output was noted (Costa *et al.,* 1989b). Psyllium significantly decreased fecal content of dry matter compared to monkeys on cellulose diets (Costa *et al.,* 1989b). In rats, fecal output increased with psyllium at 5% inclusion, which was attributed to increased fecal water and dry weight (Edwards *et al.,* 1992). Leng-Peschlow (1991) also observed increased fecal water and dry weights in rats fed psyllium. Asvarujanon et al. (2004) observed that fecal dry weights in rats were higher when fed psyllium; dry weights decreased as viscosity of psyllium preparations fed decreased. Additional hydration of stool by psyllium is an uncommon mechanism of laxation for a dietary fiber source, and is a feature used to treat loose stools (Marlett *et al.,* 2000). When psyllium is included, it has been noted that feces tend to exhibit a gel-like quality in monkeys (McCall *et al.,* 1992a), humans (Marlett *et al.,* 2000), and calves (Cebra *et al.,* 1998).

In humans, feces are approximately 75% water and 25% dry matter, which is composed of undigested dietary residues plus bacteria and bacterial cell debris. The ability of fiber to increase fecal bulk depends on a complex relationship between the chemical and physical properties of the fiber and the bacterial population in the colon (Oakenfull, 2001). Psyllium does not increase fecal bacterial mass in humans, but may increase stool frequencies (Marlett *et al.,* 2000). Non-fermented fiber can absorb water, which increases fecal bulk and results in dilution of intestinal contents (Edwards *et al.,* 2003). Tomlin and Read (1998) concluded that the major mechanism by which soluble polysaccharides increased fecal mass is through increased water holding capacity, not by stimulation of bacterial growth. An area of concern in some human studies is that

subjects have perceived increases in flatulence when taking psyllium, although others have not detected increased flatulence (Marlett *et al.,* 2000).

In the study by Costa *et al.* (1989b), fecal viscosity was greater for psyllium-fed monkeys than for cellulose-fed monkeys. The increase of viscosity in the psyllium-fed monkeys suggests that not all the psyllium was fermented, and that there may have been an increase in microbial mass. The water-holding capacity of psyllium husk is high (3.2 ml/g). Due to the highly viscous nature of psyllium seen even in a 1% solution, a small unfermented portion could have caused increased fecal viscosity and weight (Costa *et al.,* 1989b).

McRorie *et al.* (1998) noted that when pigs were administered psyllium, the water content of the colon was significantly increased in 9 out of 13 bowel segments compared to control pigs fed no psyllium in the diet. The 9 significant locations were located distal to the 4 non-significant yet numerically higher locations; therefore, the higher water holding capacity in pigs fed psyllium is most noted in the distal colon. Consequently, it is concluded that psyllium resists the dehydrational effects of the distal colon, resulting in softer digesta and feces (McRorie *et al.,* 1998).

*Blood Cholesterol Reduction*. Psyllium is an effective blood cholesterol-lowering agent in human studies (Blackwood, 2000; Blumenthal *et al.,* 2000; Sierra *et al.,* 2002), as well as in rats (Asvarujanon *et al.,* 2004). The proposed mechanism for cholesterol reduction is the adsorption of bile acids to dietary fiber through a large number of weak binding sites on the polysaccharide structure. This adsorption of bile acids leads to increased fecal excretion of bile acids. In turn, there is an increased metabolism of

cholesterol to bile acids in the liver, therefore removing more serum cholesterol and thus decreasing serum cholesterol concentrations (Blackwood *et al.,* 2000).

To test this theory, Marlett and Fischer (2002) performed a study using colectomized rats fed 5% psyllium or 3.5% fraction B of psyllium compared to control colectomized rats. They observed that the viscous fraction B is the primary active component of psyllium seed husk that interferes with bile acid absorption and increases water holding capacity of colon contents, thus supporting the theory of dietary fiber and bile acid adsorption.

*Intestinal Glucose Transport.* Psyllium has been shown to slow the rate of monosaccharide absorption, in turn decreasing blood glucose concentrations, including the postprandial blood glucose increase (Blumenthal *et al.,* 2000). Glucose absorption decreased in type 2 diabetic patients when psyllium was consumed (Sierra *et al.,* 2002). Improvement in glucose tolerance produced by consumption of viscous fiber is more likely due to slower absorption of carbohydrate rather than decreased absorption. Intimate mixing, allowing physical interaction between food and fiber, seems to be important in slowing the rate of carbohydrate release and absorption (Sierra *et al.,* 2002). Absorption of glucose depends in part on intestinal contractions that ensure mixing of luminal contents and create convective currents to bring nutrients from the bulk phase towards the epithelium. In addition, the impact of psyllium on transit time in the small intestine may alter glucose contact time with absorptive surface (Cherbut *et al.,* 1994).

*Gastrointestinal Electrical Activity***.** Movement of digesta throughout the digestive tract depends on contractions (peristalsis) initiated by regular electrical impulses. Postprandial activity is defined as myoelectrical activity recorded between meal ingestion and the first phase-three activity following meal ingestion. Phase three of the migrating motor complex was defined as regular occurrences of spike bursts at a frequency of 10-12 cycles per minute. Rushes consist of high amplitude electrical activities (> 200 mV), and minute rhythms consisted of low amplitude electrical activities (<100 mV). In a study using human volunteers, researchers compared test meals containing glucose or glucose plus psyllium on small intestinal motility and blood glucose response (Cherbut *et al.*, 1994). Psyllium induced longer and faster (i.e., higher velocity) propagations of rushes and minute rhythms than the non-supplemented glucose meal. Psyllium reduced the occurrence of stationary activity and simultaneously increased the propagation length and velocity of propagated activity, therefore changing the patterns of propagated and stationary myoelectrical activity. These changes in postprandial activity could be related to physiochemical properties, including water holding capacity, and the increasing volume of gastrointestinal contents. Luminal volume is one of the numerous factors controlling gastrointestinal motility. Mechanoreceptors in the visceral wall are excited by distension, which modifies gastric and small intestinal motility (Cherbut *et al.*, 1994).

*Nutrient Absorption.* The gel matrix of psyllium may slow absorption of nutrients or bile acids by trapping them within the matrix, thereby slowing mixing and diffusion in the intestine. Psyllium may also slow the rate of enzymatic breakdown of dietary

constituents. In addition, polysaccharides have the ability to bind other polar molecules and ions. Ions may be released and absorbed as fiber is broken down in the colon (Oakenfull, 2001).

Viscous polysaccharides can cause delayed gastric emptying and slower transit through the small bowel, resulting in a reduced rate of nutrient absorption. Psyllium increases the viscosity of intestinal contents, and therefore decreases the rate of absorption of bile acids, glucose, and other nutrients and allowing their absorption along a greater length of the small intestine (Blackwood *et al.*, 2000). Polysaccharides with large hydrophobic surface areas have potentially important roles in binding of bile acids, carcinogens, and mutagens (Blackwood *et al.*, 2000). Psyllium also has been shown to interact with intestinal absorption of medications taken simultaneously, resulting in delayed intestinal absorption (Blumenthal *et al.*, 2000).

Viscosity is a factor affecting mineral absorption in rats; the viscous property of psyllium therefore is a factor in mineral absorption. For example, highly viscous psyllium appears to decrease absorption of calcium and magnesium (Asvarujanon *et al.*, 2004). Psyllium did not affect mineral or vitamin A or E concentrations in human type 2 diabetic patients (Sierra *et al.*, 2002). In African green monkeys, psyllium did not impair fat absorption (McCall *et al.*, 1992a).

*Intestinal Morphology*. Soluble fibers (including psyllium) are noted for their effect on the stomach and small intestine whereas insoluble fibers are noted for their effect on the large intestine, although some carbohydrates (such as psyllium) have an effect on both (Blackwood *et al.*, 2000). The arabinoxylan component of psyllium

stimulates microfloral growth and VFA formation. In addition, the arabinoxylan can generate low molecular weight materials that are especially effective substrates for VFA production. In turn, VFA production alters intestinal morphology and maintains a healthy digestive tract that can adsorb nutrients and molecules such as bile acids (Al-Assaf *et al.,* 2003).

Numerous studies have determined the effects of dietary psyllium inclusion on intestinal size, scale, and musculature. In rats fed psyllium, the length of the small intestine (Leng-Peschlow, 1991) and large intestine (Leng-Peschlow, 1991; Edwards *et al.,* 2003) were increased. Rats fed psyllium husks at a 4% inclusion rate had increased weights of small intestine, small intestinal mucosa, and cecum compared to a control group that did not receive psyllium (Schneeman and Richter, 1993). Colonic wet weight in rats increased with inclusion of psyllium in the diet (Edwards *et al.*. 1992). Leng-Peschlow (1991) observed that dietary psyllium increased tissue weight per 100 mm of length in middle and lower parts of the small intestine and colon of rats. Furthermore, mucosal thickness of the distal small intestine and villous height increased (Leng-Peschlow, 1991).

In rats fed psyllium at 4% inclusion, smooth muscle thickness was not impacted in the duodenum; however, smooth muscle thickness in the ileum was increased (Schneeman and Richter, 1993). In addition, the ratio of ileal and duodenal cells per villi was higher in the psyllium group than the control group, suggesting that the ileal cells were exposed to more nutrients. This greater smooth muscle thickness in the psyllium group could be indicative of some degree of muscle hypertrophy associated with the more viscous nature of these polysaccharides in the gut lumen. With age, absorptive

function in the proximal intestine appears to diminish. A benefit of viscous polysaccharides for maintaining gut function could be that from an early age, the ileum absorbs more nutrients, and thus the addition of fiber facilitates this age-related adaptation (Schneeman and Richter, 1993).

The swelling properties of fibers influence the mass of intestine; therefore, increasing volume of contents seems to be important factors in influencing mass of bowel wall. Psyllium has high water binding capacity, and swells early in gastrointestinal tract (Leng-Peschlow, 1991).

*Production of Volatile Fatty Acids.* The main end products of colonic fermentation are the VFA acetic, propionic, and butyric acids, and the gases carbon dioxide, hydrogen, and methane (Blackwood *et al.,* 2000). One key property of fraction B is its resistance to fermentation by intestinal microflora (Fischer *et al.*. 2004).

Cultures inoculated with microbial cells in fecal and colonic samples from monkeys fed psyllium exhibited higher viscosities than from those fed cellulose. This greater viscosity can be attributed to the hydrophilic properties of psyllium as discussed earlier. Increased VFA production in the culture containing psyllium was attributed to the high content of arabinoxylan (Costa *et al.,* 1989a).

Fermentation supports increased bacterial mass by producing VFA. Production of VFA decreases colonic pH (Edwards *et al.*, 2003). Total amounts of acetate increased in cecal contents and feces of rats fed psyllium (Leng-Peschlow, 1991). The VFA produced from psyllium fermentation stimulate mucosal proliferation and may contribute to structural and functional integrity of the mucosa (Leng-Peschlow, 1991). Fermentation

of psyllium and resultant production of VFA also may have important metabolic implications (Edwards *et al.*, 1992). Energy is salvaged in the large bowel from undigested carbohydrates through fermentation (Edwards *et al.*, 2003). The VFA are believed to be trophic agents for colonic mucosa, and may be used as a preferential energy source by the colonic epithelial cells (Edwards *et al.*. 1992). Total fecal VFA output increased with psyllium dose. Psyllium ingestion resulted in a higher fecal concentration of propionic acid. Propionic acid is thought to inhibit cholesterol synthesis in the liver (Edwards *et al.*. 1992). Fecal VFA concentration was higher in psyllium-fed monkeys (9.7% inclusion) than cellulose-fed monkeys, with no differences in fecal VFA profile (Costa *et al.*. 1989b). The same trend was exhibited in a culture system from the same monkeys (Costa *et al.*. 1989a). Colonic VFA concentration was higher in psyllium-fed monkeys than in cellulose-fed monkeys. Colonic contents of psyllium-fed monkeys also had a lower percentage of dry matter compared to the cellulose fed monkeys (Costa *et al.*. 1989b); this trend also was exhibited in a culture system using inoculant from the same monkeys (Costa *et al.*. 1989a).

The effect of dietary fiber on fecal bulking is due to the presence of indigestible residue and the water binding capacity of that residue; bacterial mass depends on amount of fermentable substrate in large intestine. Psyllium, as a highly soluble fiber, is digested to an extent of 70-90% by large intestinal microflora (Leng-Peschlow, 1991), with the remaining percentage resistant to colonic bacterial degradation (Washington *et al.*. 1998; Marlett *et al.*, 2000). Some laxation effects of dietary fiber may be due to the VFA produced by fermentation (Oakenfull, 2001).

In rats fed a diet containing fermentable fiber, it has been suggested that VFA increase daily epithelial cell production rate. This increased rate (3-4 times more epithelial cells produced per day) compared with the fiber-free diet could lead to an equivalent increase in epithelial cell loss, thereby increasing endogenous fecal nitrogen excretion (Sakata, 1987).

In canines, enterocytes oxidized more glucose, butyrate, and glutamine compared to colonocytes. The colonocytes preferred to oxidize butyrate and glucose over glutamine, and glutamine over propionate. However, if other substrates are limiting, canine enterocytes and colonocytes have the capability to oxidize propionate in large quantities (Beaulieu *et al.*, 2002).

In ruminants, VFA are not utilized well in the small intestine, and these tissues prefer to utilize glucose, glutamine, and ketones as primary energy sources. Butyrate, acetate, and propionate are the three most utilized VFA in the colon, similar to the rumen. Butyrate has been shown to increase growth of both rumen and colonic tissues. Glucose and glutamine can be utilized by colonocytes as energy substrates, but not with the same affinity as butyrate (Britton and Krehbiel, 1993). Butyrate is a preferred oxidative fuel of the colonic epithelium and has the most profound effect on colonocyte growth and differentiation. Instillation of VFA into the colonic lumen induces mucosal regeneration, as shown by increased weight, DNA content, and crypt length. These effects are predominantly a result of butyrate (Velazquez, 1996). In a study using adult sheep, Sakata and Tamate (1978) observed that there may be many stimulatory effects of butyrate on cell proliferation of the rumen epithelium. Sakata and Tamate (1978) noted

that rapid administration of butyrate into the rumen stimulated proliferation of the rumen epithelial cells.

Bartholome *et al*. (2004) observed an effect of butyrate on the intestinal epithelium in neonatal piglets. The piglets utilized in the study had undergone massive bowel resection and were maintained by total parenteral nutrition. Treatments consisted of no supplementation with VFA (control) or supplementation with a VFA mixture (36 mmol/L acetate, 15 mmol/L propionate, and 9 mmol/L butyrate), with 9 mmol/L butyrate, or with 60 mmol/L butyrate. Butyrate was the VFA responsible for intestinal growth because effects of 9 mmol/L butyrate were very similar to the effects noted with the mixture of acetate, propionate, and butyrate (Bartholome *et al.*, 2004). Effects observed were increased villus height in the duodenum, jejunum, and ileum, and consequently increases in villus surface area. Therefore, Bartholome *et al*. (2004) concluded that the supplementation of parenteral formulas with butyrate may aid in maximizing intestinal absorptive area in piglets.

*Intestinal Microbiota.* *Bifidobacterium* and *Lactobacillus* are two genera of bacteria that have been shown to be of benefit when administered to newborn calves and piglets (Abe et al., 1995). *Bifidobacterium* has been shown to be beneficial in humans, and may be a component of the intestinal flora of animals as well. The uses of *Bifidobacterium* and *Lactobacillus* as probiotics in calf feeds could provide favorable benefits when consumed by young animals. A series of studies evaluated probiotic use in neonatal calves and piglets (Abe et al., 1995). The two probiotics fed were *Bifidobacterium pseudolongum* and *Lactobacillus acidophilus*. The two probiotics were

fed separately along with a control group. The two groups receiving probiotics were not different from one another, but bodyweight gain increased and frequency of diarrhea decreased when probiotics were fed compared with the control groups. The conclusion reached was that the inclusion of probiotics may improve bodyweight gain, feed conversion, and health of livestock through establishment of beneficial gut microflora. These bacteria would then inhibit the growth of pathogenic bacteria in the intestine (Abe *et al.*, 1995). The potential for psyllium to act as a prebiotic by stimulating beneficial microbial populations in the gastrointestinal tract has not been well researched.

*Feed Intake.* Rats fed diets containing psyllium at 5% of diet tended to eat more food to account for dilution of energy by the fiber (Edwards *et al.*, 1992). Ingestion of increasing amounts of dietary fiber may lead to stomach distension, and viscous polysaccharides may slow the rate of gastric emptying (Oakenfull, 2001). Effects of psyllium on feed intake are not well characterized.

**Psyllium and Neonatal Dairy Calves**

Based on the literature discussed, psyllium could potentially have desirable effects in neonatal calves. Research involving psyllium inclusion in calf milk replacers is extremely limited. Current applications of psyllium to dairy calves appear to be confined mainly to treatments for diarrhea (scours).

Fettman (1992) postulated potential benefits of psyllium for treatment of neonatal calf diarrhea. These benefits included moderation of gastric emptying, which in turn would reduce the rate of glucose absorption and thereby result in increased efficiency of

glucose disposal via the insulin-mediated response. Theoretically, carbohydrate tolerance would be improved as well as an increase in efficiency of nutrient absorption. Furthermore, inclusion of psyllium could increase VFA production, specifically butyrate. In turn, butyrate may have a glucose-sparing effect in the lower intestinal tract. The glucose-sparing effect could occur by butyrate being more preferentially taken up into the TCA cycle, as opposed to pyruvate derived from glucose metabolism. In addition, the increase in VFA production could stimulate epithelial proliferation and morphological integrity (Fettman, 1992). Fettman's (1992) theories have been minimally tested in calves, but seem to have potential in light of the aforementioned research in monogastric species.

Naylor and Liebel (1995) examined the effects of psyllium in calves with scours. Using 10 calves in a cross-over design, they observed decreased glucose absorption in calves fed psyllium. Therefore, they concluded that the inclusion of psyllium did not improve glucose absorption and resulted in no other benefits (Naylor and Liebel, 1995).

A second study examined treatment of scours with solutions containing psyllium. Cebra *et al*. (1998) supplemented scouring calves with a commercial electrolyte solution either with or without psyllium for 3 days. Within 12 hours of consuming the electrolyte solution containing psyllium the calves' feces became gelatinous and viscous compared to the control calves. Furthermore, peak glucose and insulin values were lower on day 1 for the calves consuming psyllium. However, the researchers concluded that the inclusion of psyllium did not yield enough benefits to merit a recommendation for its use in treatments for neonatal calf scours (Cebra *et al.*, 1998).

Miller and DeGregorio (1996) performed two studies with varying rates of psyllium inclusion in neonatal dairy calves. They found that inclusion of psyllium resulted in a "protein sparing effect" as calculated by protein efficiency. Protein efficiency was calculated as body weight gained divided by crude protein consumed. Miller and DeGregorio (1996) concluded that inclusion of psyllium in calf milk replacers results in more efficient utilization of protein in milk replacer. Therefore, inclusion of psyllium could allow for lower protein content in calf milk replacer for the same efficiency of gain (Miller and DeGregorio, 1996).

## References


Abe, F., N. Ishibashi, and S. Shimamura. 1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. J. Dairy Sci. 78:2838-2846.

Al-Assaf, S., G. O. Phillips, P. A. Williams, S. Takigami, P. Dettmar, and M. Havler. 2003. Molecular weight, tertiary structure, water binding and colon behaviour of ispaghula husk fibre. Proc. Nutr. Soc. 62:211-216.

Asvarujanon, P., S. Ishizuka, and H. Hara. 2004. Inhibitory effects of psyllium on rat mineral absorption were abolished by reduction of viscosity with partial hydrolysis. Biosci. Biotechnol. Biochem. 68:1737-1742.

Bartholome, A. L., D. M. Albin, D. H. Baker, J. J. Holst, and K. A. Tappenden. 2004. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunoileal resection in neonatal piglets. J. Paren. Ent. Nutr. 28:210-223.

Beaulieu, A.D., J. K. Drackley, T. R. Overton, and L. S. Emmert. 2002. Isolated canine and murine intestinal cells exhibit a different pattern of fuel utilization for oxidative metabolism. J. Anim. Sci. 80:1223-1232.

Blackwood, A. D., J. Salter, P. W. Dettmar, and M. F. Chaplin. 2000. Dietary fibre, physicochemical properties and their relationship to health. J. Royal Soc. Health 120:242-247.

Blumenthal, M. A. Goldberg, J. Brinkman. 2000. Herbal Medicine: Expanded Commision E Monographs. Integrative Medicine Communications. Newton, MA.

Britton, R., and C. Krehbiel. 1993. Nutrient metabolism by gut tissues. J. Dairy Sci. 76:2125-2131.

Cebra, M. L., F. B. Garry, C. K. Cebra, R. Adams, J. P. McCann, and M. Fettman. 1998. Treatment of neonatal calf diarrhea with an oral electrolyte solution supplemented with psyllium mucilloid. J. Vet. Intern. Med. 12:449-455.

Cherbut, C., S. B. Des Varannes, M. Schnee, M. Rival, J. P. Galmiche, and J. Delort-Laval. 1994. Involvement of small intestinal motility in blood glucose response to dietary fibre in man. Br. J. Nutr. 71:675-685.

Church, D. C. 1988. The Ruminant Animal: Digestive Physiology and Nutrition. Prentice-Hall, Englewood Cliffs, NJ.

Costa, M. A., T. Mehta, and J. R. Males. 1989a. Effect of dietary cellulose and psyllium husk on monkey colonic microbial metabolism in continuous culture. J. Nutr. 119:979-985.

Costa, M. A., T. Mehta, and J. R. Males. 1989b. Effects of dietary cellulose, psyllium husk, and cholesterol level on fecal and colonic microbial metabolim in monkeys. J. Nutr. 119:986-992.

Davis, C. L., and J. K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State University Press, Ames.

Dikeman, C. L., M. R. Murphy, and G. C. Fahey. 2006. Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. J. Nutr. 136: 913-919, 2006.

Edwards, C. A., J. Bowen, W. G. Brydon, and M. A. Eastwood. 1992. The effects of ispaghula on rat caecal fermentation and stool output. Br. J. Nutr. 68:473-482.

Edwards, S., M. F. Chaplin, A. D. Blackwood, and P. W. Dettmar. 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. Proc. Nutr. Soc. 62:217-222.

Fettman, M. 1992. Potential Benefits of Psyllium Mucilloid Supplementation of Oral Replacement Formulas for Neonatal Calf Scours. The Compendium, North American Edition, Food Animal 14:247-255.

Fischer, M. H., N. Yu, G. R. Gray, J. Ralph, L. Anderson, and J. A. Marlett. 2004. The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk). Carb. Res. 339:2009-2017.

Garrett, R. H., and C. M. Grisham. 1999. Biochemistry. Harcourt, Inc., Orlando, FL. United States.

Heinrichs, A. J. 2005. Rumen development in the dairy calf. Adv. Dairy Tech. 17:179-187.

Leng-Peschlow, E. 1991. *Plantago ovata* seeds as dietary fibre supplement: physiological and metabolic effects in rats. Br. J. Nutr. 66:331-349.

Marlett, J. A., and M. H. Fischer. 2002. A poorly fermented gel from psyllium seed husk increases excreta moisture and bile acid excretion in rats. J. Nutr. 132:2638-2643.

Marlett, J. A., and M. H. Fischer. 2003. The active fraction of psyllium seed husk. Proc. Nutr. Soc. 62:207-209.

Marlett, J. A., T. M. Kajs, and M. H. Fischer. 2000. An unfermented gel component of psyllium seed husk promotes laxation as a lubricant in humans. Am. J. Clin. Nutr. 72:784-789.

McCall, M. R., T. Mehta, C. W. Leathers, and D. M. Foster. 1992. Psyllium husk I: effect on plasma lipoproteins, cholesterol metabolism, and atherosclerosis in African green monkeys. Am. J. Clin. Nutr. 56:376-384.

McRorie, J., S. Pepple, and C. Rudolph. 1998. Effects of fiber laxatives and calcium docusate on regional water content and viscosity of digesta in the large intestine of the pig. Dig. Dis. Sci. 43:738-745.

Miller, B., and R. DeGregorio. 1996. Method of Making an Animal Feed Having a Stable Suspension of Psyllium. Patent no. 5571542, 1-6 Land O'Lakes, Inc., Arden Hills, MN.

Morris, E. R. 2001. Assembly and Rheology of Non-Starch Polysaccharides. In: B. V. McCleary and L. Prosky (Eds.) Advanced Dietary Fibre Technology. pp. 30-41. Blackwell Science Ltd, Oxford.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, D. C.

Naylor, J. M., and T. Liebel. 1995. Effect of psyllium on plasma concentration of glucose, breath hydrogen concentration, and fecal composition in calves with diarrhea treated orally with electrolyte solutions. Am. J. Vet. Res. 56:56-59.

Oakenfull, D. 2001. Physicochemical properties of Dietary Fiber: Overview. In: S. S. Cho and M. L. Dreher (Eds.) Handbook of Dietary Fiber. pp. 195-206. Marcel Dekker, Inc., New York.

Russel, J., and P. Bass. 1985. Canine gastric emptying of fiber meals: influence of meal viscosity and antroduodenal motility. Am. J. Physiol. 249:G662-G667.

Sakata, T. 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes, and luminal trophic factors. Br. J. Nutr. 58:95-103.

Sakata, T., and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61:1109-1113.

Schneeman, B. O., and D. Richter. 1993. Changes in plasma and hepatic lipids, small intestinal histology and pancreatic enzyme activity due to aging and dietary fiber in rats. J. Nutr. 123:1328-1337.

Sierra, M., J. J. Garcia, N. Fernández, M. J. Diez, A. P. Calle, and Farmafibra Group. 2002. Therapeutic effects of psyllium in type 2 diabetic patients. Eur. J. Clin. Nutr. 56:830-842.

Sutton, J. D., A. D. McGilliard, and N. L. Jacobson. 1963. Functional development of rumen mucosa. I. Absorptive ability. J. Dairy Sci. 46:426.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Tomlin, J., and N. W. Read. 1988. The relation between bacterial degradation of viscous polysaccharides and stool output in human beings. Br. J. Nutr. 60:467-475.

Vazquez-Anon, M., A. J. Heinrichs, J. M. Aldrich, and G. A. Varga. 1993. Postweaning age effects on rumen fermentation end-products and digesta kinetics in calves weaned at 5 weeks of age. J. Dairy Sci. 76:2742-2748.

Velázquez, O. C., D. Zhou, R. Seto, A. Jabbar, J. Choi, H. Lederer, and J. Rombeau. 1996. In vivo crypt surface hyperproliferation is decreased by butyrate and increased by deoxycholate in normal rat colon: associated in vivo effects on c-Fos and c-Jun expression. J. Parenter. Enteral Nutr. 20:243-250.

Warner, R. G., W. P. Flatt, and J. K. Loosli. 1956. Dietary factors influencing the development of the ruminant stomach. Agric. Food Chem. 4:788-792.

Washington, N., M. Harris, A. Musselwhite, and R. C. Spiller. 1998. Moderation of lactulose-induced diarrhea by psyllium: effects on motility and fermentation. Am. J. Clin. Nutr. 67:317-321.

# Chapter 2

## Effects of inclusion of psyllium in milk replacer on growth, rate of passage, digesta viscosity, nutrient digestibilities, and blood parameters in neonatal dairy calves

### Introduction

In recent years, more attention has been focused on improving the health, growth, and development of neonatal dairy calves. A major challenge to young calves is gut health. According to the National Animal Health Monitoring Systems Dairy 2007 Report (National Animal Health Monitoring System, 2007), 56.5% of unweaned heifer deaths can be attributed to scours (diarrhea) or other digestive challenges. Psyllium has been shown in other species to ameliorate diarrhea (Washington *et al.* 1998; Blumenthal *et al.* 2000, Marlett *et al.* 2000) and decrease passage time during cases of constipation (Blumenthal *et al.*, 2000; Al-Assaf *et al.* 2003). Dietary fiber has been shown to limit the rate of release of nutrients through increasing the viscosity of the digesta, therefore reducing the rate of nutrient transport to the epithelium (Morris, 2001) and allowing nutrient absorption along a greater length of the small intestine (Blackwood *et al.*, 2000). In dogs, inclusion of psyllium at 3% slowed digesta outflow from the stomach (Russell and Bass, 1985). Psyllium inclusion increases the viscosity of intestinal contents, thereby reducing the rate of absorption of glucose and other nutrients (Blackwood *et al.*, 2000). Psyllium has been shown to decrease the post-prandial increase of blood glucose concentration (Blumenthal *et al.*, 2000). If these effects occurred in young calves supplemented with psyllium it might translate to improved health and greater growth efficiency.

The purpose of this research was to determine the impact of psyllium inclusion in milk replacer on feed intake, water intake, growth, illness, digesta viscosity, rate of passage, nutrient digestibilities, and blood components in neonatal dairy calves.

## Materials and Methods

### Animal Care

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol number 04083).

The study occurred in two periods, the first (May 2004) using 12 calves and the second (June 2004) using 22 calves. Male Holstein calves were purchased from Stone Ridge Dairy (Bellflower, IL) at less than 36 h of age.

At the beginning of the trial period, daily late afternoon trips were made to Stone Ridge Dairy to select and purchase calves. At Stone Ridge Dairy, all male Holstein calves less than 36 h of age in the calf pack were evaluated for potential purchase by trial personnel. The evaluation included body temperature, heart and lung auscultation, hydration status, mobility, navel status, and overall thriftiness. Also, the calves must have received at least one feeding of colostrum by Stone Ridge personnel. Calves deemed satisfactory were then transported to the University of Illinois Dairy Nutrition Field Laboratory (ca. 42 km).

Upon arrival, each calf received an eartag in the left ear for identification purposes. Calves were then measured for withers height, body length, heart girth circumference, and body weight. Ear notches were taken from each calf and placed in formalin for determination of persistently infected bovine viral diarrhea (PI-BVD) status.

Each calf was vaccinated against infectious bovine rhinotracheitis virus and parainfluenza-3 intranasally (2 ml of TSV-2; Pfizer Animal Health, Exton, PA). In addition, calves received 15 mL of Quatracon-2X Antiserum (Boehringer Ingleheim Vetmedica, Inc.; St. Joseph, MO) subcutaneously in the neck. Each calf also received 2 mL of Excenel (Pfizer Animal Health; Exton, PA) subcutaneously in the neck for the first 3 d of the study. After entry injections, the calves were moved to southward-facing individual calf hutches (Calf-Tel; Hampel Corp., Germantown, WI) on 15 to 20 cm of crushed limestone located on the south side of the Dairy Nutrition Field Laboratory. No bedding was used, to minimize ingestion of bedding material that might confound treatment inferences.

**Assignment to Treatments**

Calves were blocked by pairs based upon birth date, body weight, and total protein score and then randomly assigned within pair to each of the dietary treatments. These pairs of calves were then randomly assigned to harvest week. Of the 34 calves assigned to treatments, 1 calf died at 2 d of age. Necropsy results (University of Illinois College of Veterinary Medicine, Urbana, IL) indicated that the likely cause of mortality was related to birth trauma. All other calves completed the trial in good health.

**Feeding**

On the night of arrival, each calf was offered colostrum for ad libitum intake. On the morning following arrival, each calf again was offered colostrum. Treatment feedings were administered at 0600 and 1800 h. Calves were fed milk replacers reconstituted to 12.5% dry matter (DM) at a rate of 12% of bodyweight (BW) daily, adjusted weekly as calves grew. Milk replacers (Land O'Lakes Animal Milk Products

Co., Arden Hills, MN) without (CON) or with 1.1% psyllium (PSY) were formulated to contain 22% protein and 20% fat, and contained only milk proteins. Neither milk replacer contained growth-promoting antibiotics. Water was available to calves for ad libitum consumption, with fresh warm water provided twice daily after each milk replacer feeding. No other feeds were offered.

**Health Monitoring**

Calves were observed at least twice daily for general health, including appearance (alertness), appetite (ability to consume feed), and fecal scores. Fecal scores were recorded daily, using the following guidelines: 1 = dry, hard; 2 = soft, formed; 3 = pudding-like; 4 = mix of liquids with some solids; and 5 = liquid. If the calf's hydration status was low (skin tenting evident), 500 mL of Lactated Ringers Solution (Abbott Laboratories; Abbott Park, IL) was administered subcutaneously. If a calf appeared unhealthy, 3 mL of Excenel (Pfizer, Exton, PA) was administered subcutaneously for a period of 3 d. If body temperature was over 39° C, 1 mL of Banamine (Schering-Plough Animal Health; Union, NJ) was administered subcutaneously. Calves received a booster of TSV-2 at 2 wk of age.

**Body Growth Measurements**

Calves were weighed weekly prior to the evening feeding on the same day each week. Growth measurements including heart girth, withers height, body length, and heart girth were made at the same time.

**Blood Sampling**

Blood samples were collected weekly 1 h before feeding and 3 h post-feeding on the same day that body measurements were made. Samples were obtained via jugular

venipuncture using 20-gauge needles (Becton Dickinson; Franklin Lakes, NJ) into 10-mL vacutainer tubes containing sodium heparin (Becton Dickinson; Franklin Lakes, NJ). Tubes were placed on ice and then centrifuged within 2 h of collection at 1300 rpm for 15 min in an Eppendorf model 5804 centrifuge with swinging buckets (Brinkmann Instruments; Westbury, NY). After centrifugation, the plasma was removed and aliquots placed into 5-mL Falcon tubes (Becton Dickinson; Franklin Lakes, NJ) at -20$^{o}$C until analysis.

Aliquots of pre- and post-prandial plasma were analyzed for concentrations of NEFA (kit number 990-75409; Wako Pure Chemical Industries, Ltd., Osaka 541, Japan) with procedural modifications by Johnson and Peters (1993). Concentrations of glucose, total protein, cholesterol, β-hydroxybutyrate (BHBA), and urea nitrogen were determined by enzymatic procedures in an auto-analyzer (University of Illinois Clinical Pathology Laboratory, Urbana, IL).

**Calf Harvest Procedure**

Pairs of calves were harvested for analysis weekly. For period 1, 2 calves were euthanized after arrival before experimental treatments began (week zero) for baseline measurements. For the remaining weeks, one pair of calves was euthanized weekly, with the exception of week 4. In week 4 of period 1, 4 calves were euthanized. For period 2, 4 baseline calves were euthanized at week zero, and 2 pairs were euthanized during weeks 1, 2, and 3. For week 4, 5 calves were harvested due to the 1 mortality.

Calves were transported to the University of Illinois College of Veterinary Medicine at 0630 h. Calves were not fed their morning milk replacer prior to euthanasia. The harvest procedure consisted of the calf being administered Xylazine HCl

intramuscularly (50 mg/mL; Fort Dodge Animal Health; Fort Dodge, IA). Once the calf was sedated, it was euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus; Veterinary Laboratories Inc., Lenexa, KS) and then exsanguinated. This method was utilized instead of captive bolt stunning to minimize epithelial sloughing, which potentially could negatively impact measurements of ion transport and histomorphological analyses (Shirazi-Beechey *et al.*; 1989).

When the calf was declared dead, the veterinarian opened the body cavity. The gastrointestinal tract was ligated at the caudal esophagus and rectum and removed as rapidly as possible without damaging the tract. To prevent movement of digesta between compartments, the different segments of the gastrointestinal tract were identified and ligated. The gastrointestinal tract was then divided into three portions: the stomach portion consisting of rumen, reticulum, omasum, and abomasum; the small intestine, consisting of duodenum, jejunum, and ileum; and the colon. Each portion was processed by the respective team of personnel for tissue and digesta collection.

**Digesta Collection**

Digesta was removed from the gastrointestinal tract and placed into Whirlpack bags (Nasco; Fort Atkinson, WI). Aliquots were removed at the laboratory for DM determination, and samples for viscosity measurement were stored at -20°C until further analysis. The DM of the digesta was determined by drying an aliquot in an oven at 110ºC for 24 h.

**Total Fecal Collection: Digestibility and Rate of Passage**

To determine rate of passage and digestibility, calves to be harvested at weeks 2, 3, and 4 underwent total fecal collections for a period of 5 d. Calves were fitted with

canvas sheep fecal bags, modified slightly to account for differences in body structure. These canvas bags were lined with a plastic bag to obtain the feces excreted by the calf.

To determine the rate of passage, calves were dosed with Co-EDTA. The Co-EDTA was prepared according to the methods of Uden *et al*. (1980). The solution of Co-EDTA (13.8% cobalt) was made by dissolving the dry material in distilled water at a rate of 17.05 g per L of water. This solution was then mixed with the milk replacer fed to the calf at a rate of 10 mL per L of milk replacer. Calves consumed this marker for a period of 16 feedings, at which point marker administration ceased and the rate of passage portion of the total collection began.

The first 48 h of each total fecal collection period involved fecal collections every 12 h. After the last dose of Co-EDTA was consumed within the milk replacer, collection times shifted to every 6 h for the remaining 72 h of collection. When feces were obtained, the plastic bag was placed within a second plastic bag and stored at -20ºC until further analysis.

***Fecal Dry Weights.*** Feces were dried individually in a forced-air oven at 60ºC until constant weights were achieved. Dried feces from the first 48 h of collection were composited, while feces from the rate of passage portion were maintained individually. The dried feces were then ground through a 1-mm screen in a Wiley Mill (Thomas-Wiley; Swedesboro, NJ).

***Sampling and Analysis of Milk Replacers and Feces.*** Each milk replacer was sampled weekly. Subsamples were combined by treatment and period, and stored at -20° C until analysis could occur concurrently with the fecal analyses to determine apparent digestibility of DM, ash, crude protein, energy, and long-chain fatty acids. One half of all

dried samples of feces (48-h composite and rate of passage samples) were composited to provide samples for analysis of absolute DM , crude protein, gross energy, long-chain fatty acids, and ash.

Absolute DM was determined in duplicate utilizing the AOAC (1975) method. Ash content was determined by combusting duplicate samples in a muffle furnace for 24 h at 600ºC. Crude protein was analyzed in duplicate with the Leco Nitrogen/Protein Determinator (model FP-2000; Leco Corporation, St. Joseph, MI) using Official Method 992.15 (AOAC, 2000). Gross energy was determined in duplicate via bomb calorimetry (1261 Isoperibol Calorimeter; Parr Instrument Co., Moline, IL; Parr Instrument Manuals). Long-chain fatty acids were determined by gas chromatography (Shimadzu GC 17-A; Shimadzu Scientific Instruments, Inc.; Columbia, MD) of methyl esters formed by acid-catalyzed transesterification using the methods of Sukhija and Palmquist (1988), as described by Beaulieu et al. (2002).

Apparent digestibilities of DM, ash, crude protein, gross energy, and long-chain fatty acids were calculated as: ((nutrient consumed − nutrient in feces)/nutrient consumed) × 100.

**Rate of Passage Analyses**

To determine rate of passage, concentrations of cobalt were measured in the feces voided from the calves once cobalt administration ceased. Cobalt analysis followed the methods of Hart and Polan (1984). Briefly, 20 mL of 0.05 *M* EDTA solution was combined with 0.2 g of dried feces, shaken for 30 min, and filtered through Whatman #4 filter paper (Whatman International Ltd.; Florham Park, NJ).

Samples were then quantified by atomic absorption spectroscopy (air and acetylene flame; Perkin-Elmer, Norwalk, CT). The percent Co in each sample was calculated as:

$$\% \text{ Co} = \frac{\text{ppm} \times \text{dilution factor}}{\text{Sample weight (g)} \times 1{,}000{,}000} \times 100$$

The model $y(t) = y(0) \cdot \exp(-kt)$ was utilized to describe the disappearance of cobalt, where $y$ is the amount of marker present at time $t$ and $k$ is the rate constant. The mean retention time (MRT) was then calculated as the reciprocal of $k$ (Faichney, 1993).

**Viscosity**

Viscosity of digesta samples was determined utilizing methods from Dikeman (2005). Samples were allowed to warm to room temperature (23°C) and gently mixed with a metal spatula for 30 sec to homogenize the samples. Aliquots (2 mL) of rumen, abomasal, and jejunal fluid were removed and used for viscosity measurement. Samples from the rumen, abomasum, and jejunum were analyzed in duplicate using a digital Brookfield viscometer (LV-DV-II+) with a Wells/Brookfield cone and plate extension, either the CP-40 or CP-41. Colon samples were too viscous to obtain 2-mL aliquots. Therefore, a Brookfield LV spindle set (LV-1; LV-2; LV-3) was utilized with digesta being placed into 100-mL beakers with a diameter of 5 cm. The data were collected by Wingather Data software collection program. Samples were analyzed at six different speeds (0.3, 0.5. 1.0, 1.5, 2.0, and 3.0 rpm), and viscosity was calculated as a function of rpm over rpm range.

**Statistical Analysis**

Statistical analyses were conducted utilizing the Proc Mixed procedure of SAS. The data were analyzed as a randomized complete block design (SAS version 9.1; SAS Institute Inc.; Cary, NC), with period as a random effect. Effects of dietary treatment, week, and the interaction of diet and week were included in the model as fixed effects. Baseline calves were not included in the analysis, but means and standard errors are shown for comparison. Significant differences were declared at $P < 0.05$, and trends toward significant effects were noted at $P < 0.15$.

## Results and Discussion

The nutrient composition of the two diets is exhibited in Table 2.1. The milk replacers were formulated to contain 22% CP and 20% fat (Land O'Lakes, Arden Hills, MN). The PSY milk replacer was produced by addition of 1.1% psyllium to the CON milk replacer. This inclusion of psyllium resulted in a slight dilution effect, which likely explains why the nutrient values for all ingredients except lactose and manganese were numerically lower in PSY than in CON.

Water intakes did not differ significantly between treatments (Table 2.2). Intakes of DM, protein, and metabolizable energy (ME) also did not differ between diets. Water, DM, protein, and ME intakes increased from wk 1 to 4 ($P < 0.01$) as expected (data not shown), but the diet × week interaction was not significant.

The diet × week interaction was significant for body length (Table 2.3). Body length tended to increase ($P < 0.08$) from wk 1 to 4 as expected, but increases during wk 1 and 2 were greater for calves fed CON than for those fed PSY. Heart girth increased (*P*

< 0.01) from wk 1 to 4, but effects of diet and the diet × week interaction were not significant. Withers height was not affected by dietary treatment or age. Body weights increased with age of the calves as expected, but dietary treatments did not affect BW. Inclusion of psyllium in the diet, therefore, had minimal impact on growth parameters in this study. The ADG of BW was lower than those predicted by the NRC (2001) model (Table 2.3). Possible reasons for this finding include the young age of the calves, the fact that calves had been transported, the degree of heat stress on the calves, and the impact of experimental procedures performed on the calves.

Inclusion of psyllium in the calf milk replacer increased ($P < 0.005$) viscosities of abomasal and colon digesta (Table 2.4). Ruminal and jejunal digesta viscosities did not differ between treatments. Rumen viscosity increased from wk 1 to 4 ($P < 0.08$), but the diet × week interaction was not significant. The lack of difference in rumen viscosity is most likely attributable to the fact that the milk replacers mostly bypassed the rumen via the reticular groove in these pre-ruminant calves. Therefore, minimal milk replacer would enter the rumen and contribute to differences in rumen viscosity.

Given the viscous properties of psyllium, it would be expected that the digesta from the PSY calves would exhibit greater viscosity throughout the gastrointestinal tract, as was seen in abomasal and colonic digesta. The lack of difference in jejunal viscosity (Table 2.4) is intriguing. It is possible that pH or digestive secretions may have caused the lack of effect, but neither pH nor digestive secretions were measured in this study. In pre-ruminant calves, the pH of digesta has been reported to range from 2.5 to 5.5 in the proximal small intestine, increasing as the digesta approaches the distal ileum (Guilloteau and Zabielski, 2005). However, the pH of abomasal digesta in pre-ruminant calves has

been reported to range from 2.5 to 5.5 (Guilloteau and Zabielski, 2005) or from 1.5 to 6.0 (Davis and Drackley, 1998). This broader range for abomasal pH than jejunal pH challenges the potential effect of pH on the lack of viscosity differences in jejunal digesta. It has been shown that the pH swings of the abomasal digesta are related to meal feeding, with lower pH values pre-prandially and higher values post-prandially (Davis and Drackley, 1998).

A trend ($P < 0.11$) was observed for increased mean retention time for calves fed the PSY milk replacer (Table 2.5). This increase in mean retention time for the PSY calves could be attributed to the increased viscosity of the digesta delaying gastric emptying, as observed in studies with other species (Russell and Bass, 1985; Washington *et al.*, 1998).

No significant differences were observed between treatments in DM content of rumen digesta (Table 2.6), probably because little milk replacer entered the rumen. Differences between diets also were not significant for digesta DM content of the abomasum and jejunum, although means were numerically lower for calves fed PSY. The digesta in the proximal colon ($P < 0.02$) and distal colon ($P < 0.02$) of calves fed PSY exhibited a lower DM content than in the CON calves, most likely due to the water-holding capacity of psyllium. These data are similar to those of McRorie *et al*. (1998), who found that water content of the colonic digesta in pigs supplemented with psyllium was significantly increased in the 9 most-distal bowel segments of the 13 segments evaluated. They concluded that psyllium was able to resist the dehydrational effects of the distal colon, resulting in softer digesta and feces.

The PSY calves produced more fecal DM output than CON calves ($P < 0.03$; Table 2.7). Increased fecal output with dietary psyllium also has been observed in rats (Leng-Peschlow, 1991; Edwards *et al.,* 1992; Asvarujanon *et al.,* 2004). Fecal DM content was significantly lower for the PSY calves ($P < 0.01$). Similar to the colonic digesta, lower fecal DM likely reflects the greater water-holding capacity of psyllium increasing the fecal water content as observed in monkeys fed psyllium (Costa *et al.,* 1989). Furthermore, a gel-like quality was observed in the feces of PSY calves, supporting similar observations in monkeys (McCall *et al.*, 1992), humans (Marlett *et al.,* 2000), and calves (Cebra *et al.,* 1998).

The apparent digestibility of DM was greater ($P < 0.027$) for the CON calves (Table 2.7). Lower apparent digestibility of DM for calves fed PSY could be a result of the dilution effect of psyllium inclusion, which as a fermentable dietary fiber source is still less digestible than the other ingredients in milk replacer. In addition, a significant treatment × week interaction ($P < 0.035$) was observed. Differences between treatments were greatest during wk 2 and least during wk 3. Similar dietary effects and interactions between dietary treatment and week were also noted for digestibilities of energy, ash, and CP (Table 2.7).

The CON calves tended to have an increased ash digestibility when compared to the PSY calves, particularly during wk 2 and 4 (treatment × week interaction, $P < 0.06$). In rats, it has been shown that increased viscosity can decrease mineral absorption (Asvarujanon *et al.,* 2004). The viscous nature of psyllium allows for entrapment of minerals within its matrix, thereby potentially decreasing absorption; however, this is an area that has not been extensively researched. Although psyllium has been shown to

decrease the rate of nutrient absorption (Blackwood *et al.*, 2000), effects on the extent of absorption are not fully understood.

No significant effects of treatment ($P \geq 0.63$) were observed for digestibilities of the long-chain fatty acids C16:0, C18:0, C18:1*cis*, or total fatty acids (Table 2.7). However, an effect of week was noted for C16:0 ($P < 0.03$) and C18:0 ($P < 0.03$) because digestibilities increased with age. No diet × week interactions were noted for fatty acid digestibilities.

Psyllium has been used in humans to slow the rate of glucose absorption and to blunt the post-prandial increase in blood glucose (Blumenthal *et al.*, 2000, Sierra *et al.*, 2002). In our study, there was no difference in post-prandial glucose concentrations between the PSY and CON treatments (Table 2.9). However, there was a tendency ($P < 0.064$) for the PSY calves to have higher blood glucose concentration pre-prandially (i.e., 10 h post-prandial, Table 2.8). This finding could be the result of the viscous nature of psyllium slowing the rate of glucose absorption over a longer period of time. It is also possible that the CON calves had a higher insulin response to dispose of elevated glucose concentrations post-prandially, but blood insulin concentrations were not measured in this study.

Concentrations of NEFA, BHBA, cholesterol, urea N, and total protein were not different between treatments either pre-prandially ($P \geq 0.28$; Table 2.8) or post-prandially ($P \geq 0.44$; Table 2.9). However, an effect of week (i.e., age of calves) was observed for pre-prandial values of NEFA (decrease, $P < 0.01$), BHBA (increase, $P < 0.08$), and cholesterol (increase, $P < 0.001$). Furthermore, an effect of week was also observed for post-prandial values of NEFA (decrease, $P < 0.01$), BHBA (increase, $P < 0.001$),

cholesterol (increase, $P < 0.001$) and total protein (decrease, $P < 0.02$). A tendency toward a diet × week interaction ($P < 0.12$) was detected for cholesterol in pre-prandial plasma, but the interpretation is unclear. No significant treatment × week interactions were observed ($P \geq 0.18$) in post-prandial plasma samples.

## Conclusions

The moderation of blood glucose concentrations with psyllium in the diet is an interesting finding to consider in neonatal dairy calves, particularly as it pertains to efficiency of growth. Whether these effects would be observed in calves also fed starters is an area for future research.

The increase in viscosity of digesta and increase in MRT could potentially be of benefit to neonatal dairy calves raised in environments where infectious challenges were more prevalent or once calf starter consumption begins. Altered digesta flow could potentially allow for increased digestibility of the diet, despite the fact that minimal differences in digestibility of milk replacer were observed in this trial. Although psyllium did not affect growth or health of calves in this study, the physiological changes observed suggest that effects of psyllium supplementation should be tested in large numbers of calves. It would also be of interest to evaluate digestibilities and growth of dairy calves fed larger amounts of milk replacer with calf starter, as opposed to this trial in which only limited amounts of milk replacer were fed. Therefore, psyllium use is potentially of benefit in the field provided it is economically feasible, and should be further researched in a typical dairy calf raising situation.

## References


AOAC. (2000) Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC. (1975) Official Methods of Analysis. 12th ed. Association of Official Analytical Chemists, Washington, DC.

Al-Assaf, S., G. O. Phillips, P. A. Williams, S. Takigami, P. Dettmar, and M. Havler. 2003. Molecular weight, tertiary structure, water binding and colon behaviour of ispaghula husk fibre. Proc. Nutr. Soc. 62:211-216.

Asvarujanon, P., S. Ishizuka, and H. Hara. 2004. Inhibitory effects of psyllium on rat mineral absorption were abolished by reduction of viscosity with partial hydrolysis. Biosci. Biotechnol. Biochem. 68:1737-1742.

Beaulieu, A. D., J. K. Drackley, and N. R. Merchen. 2002. Concentrations of conjugated linoleic acid (*cis*-9, *trans*-11-octadecadienoic acid) are not increased in tissue lipids of cattle fed a high-concentrate diet supplemented with soybean oil. J. Anim. Sci. 80:847-861.

Blackwood, A. D., J. Salter, P. W. Dettmar, and M. F. Chaplin. 2000. Dietary fibre, physicochemical properties and their relationship to health. J. Royal Soc. Health 120:242-247.

Blumenthal, M. A. Goldberg, J. Brinkman. 2000. Herbal Medicine: Expanded Commision E Monographs. Integrative Medicine Communications. Newton, MA.

Cebra, M. L., F. B. Garry, C. K. Cebra, R. Adams, J. P. McCann, and M. Fettman. 1998. Treatment of neonatal calf diarrhea with an oral electrolyte solution supplemented with psyllium mucilloid. J. Vet. Intern. Med. 12:449-455.

Costa, M. A., T. Mehta, and J. R. Males. 1989. Effects of dietary cellulose, psyllium husk, and cholesterol level on fecal and colonic microbial metabolim in monkeys. J. Nutr. 119:986-992.

Davis, C. L., and J. K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State University Press, Ames.

Dikeman, C. L. 2005. Factors affecting viscosity of select dietary fibers, complete food matrices, and canine ileal digesta. Ph.D. Diss., Univ. Illinois, Urbana.

Edwards, C. A., J. Bowen, W. G. Brydon, and M. A. Eastwood. 1992. The effects of ispaghula on rat caecal fermentation and stool output. Br. J. Nutr. 68:473-482.

Faichney, G. J. 1993. Digesta Flow. Page 70 in Quantitative Aspects of Ruminant Digestion and Metabolism. J. M. Forbes and J. France, ed. University Press, London.

Guilloteau, P. and R. Zabielski. 2005. Digestive secretions in preruminant and ruminant calves and some aspects of their regulation. Pages 159-189 in Calf and Heifer Rearing. P. Garnsworthy, ed. Nottingham University Press, Nottingham, United Kingdom.

Hart, S. P., and C. E. Polan. 1984. Simultaneous extraction and determination of ytterbium and cobalt ethylenediaminetetraacetate complex in feces. J. Dairy Sci. 677: 888-892.

Johnson, M. M. and J. P. Peters. 1993. Technical Note: An improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci. 71:753-756.

Leng-Peschlow, E. 1991. *Plantago ovata* seeds as dietary fibre supplement: physiological and metabolic effects in rats. Br. J. Nutr. 66:331-349.

Marlett, J. A., T. M. Kajs, and M. H. Fischer. 2000. An unfermented gel component of psyllium seed husk promotes laxation as a lubricant in humans. Am. J. Clin. Nutr. 72:784-789.

McCall, M. R., T. Mehta, C. W. Leathers, and D. M. Foster. 1992. Psyllium husk I: effect on plasma lipoproteins, cholesterol metabolism, and atherosclerosis in African green monkeys. Am. J. Clin. Nutr. 56:376-384.

McRorie, J., S. Pepple, and C. Rudolph. 1998. Effects of fiber laxatives and calcium docusate on regional water content and viscosity of digesta in the large intestine of the pig. Dig. Dis. Sci. 43:738-745.

Morris, E. R. 2001. Assembly and Rheology of Non-Starch Polysaccharides. In: B. V. McCleary and L. Prosky (Eds.) Advanced Dietary Fibre Technology. pp. 30-41. Blackwell Science Ltd, Oxford.

National Animal Health Monitoring System. 2007. *Part 1: Reference of Dairy Cattle Health and Management Practices in the United States, 2007*. Ft. Collins, CO: USDA:APHIS:VS:CEAH

National Research Council. 2001. *7th Revised Editon: Nutrient Requirements of Dairy Cattle.* National Academy Press. Washington, D.C.

Russell, J., and P. Bass. 1985. Canine gastric emptying of fiber meals: influence of meal viscosity and antroduodenal motility. Am. J. Physiol. 249:G662-G667.

Sierra, M., J. J. Garcia, N. Fernández, M. J. Diez, A. P. Calle, and Farmafibra Group. 2002. Therapeutic effects of psyllium in type 2 diabetic patients. Eur. J. Clin. Nutr. 56:830-842.

Shirazi-Beechey, S. P., R. B. Kemp, J. Dyer, and R. B. Beechey. 1989. Changes in the functions of the intestinal brush border membrane during the development of the ruminant habit in lambs. Comp. Biochem. Physiol. 94B:801-806.

Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid contend and composition of feed stuffs and feces. J. Agric. Food Chem. 36:1202.

Udén, P., P. E. Colucci, and P. J. Van Soest. 1980. Investigation of chromium, cerium, and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31:625-632.

Washington, N., M. Harris, A. Musselwhite, and R. C. Spiller. 1998. Moderation of lactulose-induced diarrhea by psyllium: effects on motility and fermentation. Am. J. Clin. Nutr. 67:317-321.

**Table 2.1** Composition of milk replacers.[1]

| Nutrient | Control (CON) | Psyllium (PSY) |
|---|---|---|
| Dry matter, % | 96.4 | 96.2 |
| Crude protein, % | 21.8 | 21.6 |
| Crude fiber, % | 1.65 | 0.85 |
| Lactose, % | 33.0 | 34.0 |
| Crude fat, % | 21.00 | 19.95 |
| Ash, % [2] | 11.8 | 11.7 |
| TDN, % | 106.0 | 104.5 |
| Gross energy, (kcal/kg)[3] | 4844 | 4822 |
| $NE_L$, (Mcal/kg) | 2.73 | 2.70 |
| $NE_M$, (Mcal/kg) | 2.99 | 2.95 |
| $NE_G$, (Mcal/kg) | 2.16 | 2.12 |
| Calcium, % | 0.81 | 0.78 |
| Phosphorus, % | 0.74 | 0.72 |
| Magnesium, % | 0.13 | 0.13 |
| Potassium, % | 2.24 | 2.16 |
| Sodium, % | 1.24 | 1.20 |
| Iron, mg/kg | 101 | 68 |
| Zinc, mg/kg | 24 | 18 |
| Copper, mg/kg | <1 | <1 |
| Manganese, mg/kg | 21 | 22 |
| Molybdenum, mg/kg | 0.50 | 0.45 |
| Psyllium, % [4] | 0.00 | 1.10 |

[1] Determined by Dairy One (Ithaca, NY) unless otherwise noted. All values except dry matter (% as fed) are expressed on a dry matter basis.

[2] Determined according to AOAC (1975).

[3] Determined by bomb calorimetry (Parr Instrument Co., Moline, IL).

[4] As reported by manufacturer (Land O'Lakes Animal Milk Products; Arden Hills, MN).

**Table 2.2** Least squares means and associated standard errors for water, dry matter, and nutrient intakes.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Intake | CON | PSY | SE | T | W | T*W |
| Water, L | | | | | | |
| Morning | 2.15 | 2.16 | 0.19 | 0.99 | <.0001 | 0.44 |
| Night | 1.21 | 1.58 | 0.21 | 0.12 | 0.01 | 0.30 |
| Daily | 3.32 | 3.47 | 0.33 | 0.75 | <.0001 | 0.28 |
| DMI, g/d | 712 | 732 | 21.7 | 0.31 | 0.001 | 0.98 |
| Protein, g/d | 155 | 158 | 4.7 | 0.50 | 0.013 | 0.98 |
| ME, kcal/d | 3175 | 3105 | 95 | 0.39 | 0.013 | 0.98 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON = Control diet

[3] PSY = Psyllium-supplemented diet

**Table 2.3** Least squares means and associated standard errors for average daily growth.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | T | W | T*W |
| Body length, cm/d | 0.20 | 0.12 | 0.05 | 0.17 | 0.08 | 0.04 |
| Heart girth, cm/d | 0.19 | 0.17 | 0.04 | 0.76 | 0.01 | 0.94 |
| Withers height, cm/d | 0.22 | 0.19 | 0.05 | 0.71 | 0.85 | 0.69 |
| Body weight, kg/d | 0.29 | 0.27 | 0.04 | 0.66 | <.001 | 0.82 |
| Predicted BW gain[4], kg/d | 0.54 | 0.55 | --- | | | |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON=Control diet

[3] PSY= Psyllium-supplemented diet

[4] Predicted average daily gain according to NRC (2001). Not analyzed statistically, but shown for information purposes.

**Table 2.4** Least squares means and associated standard errors (centipoise, cP) for viscosity of digesta.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | T | W | T*W |
| Rumen | 10.17 | 8.80 | 0.98 | 0.33 | 0.08 | 0.25 |
| Abomasum | 18.10 | 34.11 | 6.88 | 0.004 | 0.28 | 0.77 |
| Jejunum[4] | 21.44 | 19.91 | 4.81 | 0.69 | 0.36 | 0.54 |
| Colon[4] | 3.67 | 4.74 | 0.14 | <0.001 | 0.16 | 0.24 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON=Control diet

[3] PSY= Psyllium-supplemented diet

[4] Data were $Log_{10}$ transformed for statistical analysis, but back-transformed means are shown for ease of interpretation.

**Table 2.5** Least squares means and associated standard errors for mean digesta retention time in the total tract.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Location | CON[2] | PSY[3] | SE | T | W | T*W |
| Mean retention time, h | 8.45 | 9.71 | 0.59 | 0.106 | 0.93 | 0.76 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON=Control diet.

[3] PSY= Psyllium-supplemented diet

**Table 2.6.** Least squares means and associated standard errors for weekly dry matter contents (%) of gastrointestinal tract digesta.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Location | CON[2] | PSY[3] | SE | T | W | T*W |
| Rumen | 1.5 | 1.7 | 0.00 | 0.52 | 0.18 | 0.32 |
| Abomasum | 3.8 | 2.7 | 0.01 | 0.28 | 0.30 | 0.52 |
| Jejunum | 8.2 | 7.6 | 0.01 | 0.55 | 0.38 | 0.62 |
| Proximal colon | 17.3 | 13.8 | 0.01 | 0.02 | 0.74 | 0.84 |
| Distal colon | 20.9 | 17.8 | 0.01 | 0.05 | 0.10 | 0.65 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction
[2] CON=Control diet
[3] PSY= Psyllium-supplemented diet

**Table 2.7.** Least squares means and associated standard errors for total tract apparent digestibilities.

| | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| 5-d Total fecal output, g DM | 184 | 257 | 27.8 | 213 | 243 | 27.8 | 196 | 258 | 24.1 | 198 | 253 | 15.4 | 0.023 | 0.96 | 0.72 |
| Fecal DM, % | 21.8 | 16.6 | 1.7 | 18.7 | 16.2 | 1.7 | 21.2 | 16.9 | 1.5 | 20.6 | 16.5 | 0.01 | 0.007 | 0.48 | 0.71 |
| Digestibilities, % | | | | | | | | | | | | | | | |
| DM | 94.1[a] | 91.2[b] | 0.7 | 93.5 | 94.3 | 0.7 | 94.6[c] | 93.0[d] | 0.6 | 94.1 | 92.8 | 0.4 | 0.027 | 0.132 | 0.035 |
| Energy | 94.9[e] | 92.6[f] | 0.7 | 94.2 | 95.2 | 0.7 | 95.8[g] | 94.5[h] | 0.6 | 95.0 | 94.1 | 0.4 | 0.087 | 0.081 | 0.054 |
| Ash | 87.8[i] | 82.5[j] | 1.3 | 87.8 | 89 | 1.3 | 86.1[k] | 83.9[l] | 1.1 | 87.2 | 85.2 | 0.7 | 0.068 | 0.021 | 0.058 |
| CP | 86.1[m] | 82.7[n] | 1.7 | 83.5[o] | 88.4[p] | 1.7 | 88.5 | 87.7 | 1.5 | 86.3 | 86 | 1.1 | 0.84 | 0.057 | 0.049 |
| C16:0 | 99.0 | 98.6 | 0.22 | 99.2 | 99.6 | 0.22 | 99.5 | 99.2 | 0.19 | 99.2 | 99.1 | 0.12 | 0.63 | 0.029 | 0.21 |
| C18:0 | 96.4 | 95.8 | 0.79 | 98.9 | 97.5 | 0.79 | 98.5 | 97.8 | 0.69 | 97.5 | 97.5 | 0.44 | 0.95 | 0.028 | 0.31 |
| C18:1*cis* | 99.8 | 99.8 | 0.48 | 99.8 | 99.9 | 0.48 | 99.9 | 99.8 | 0.48 | 99.8 | 99.8 | 0.03 | 0.92 | 0.21 | 0.17 |
| Total fatty acids | 99.1 | 99.9 | 0.19 | 99.3 | 99.7 | 0.19 | 99.5 | 99.4 | 0.16 | 99.3 | 99.3 | 0.1 | 0.93 | 0.027 | 0.23 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction
[2] CON=Control diet
[3] PSY= Psyllium-supplemented diet
[a,b] Subcolumn means within row and week category with different superscripts differ (*P* <0.01).
[c,d,g,h] Subcolumn means within row and week category with different superscripts differ (*P* <0.10).
[e,f,o,p] Subcolumn means within row and week category with different superscripts differ (*P* <0.05).
[i,j] Subcolumn means within row and week category with different superscripts differ (*P* <0.02).
[k,l] Subcolumn means within row and week category with different superscripts differ (*P* <0.20).
[m,n] Subcolumn means within row and week category with different superscripts differ (*P* <0.15).

**Table 2.8** Least squares means and associated standard errors for weekly pre-prandial blood constituents.

| | Week 0 | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON | PSY | SE | T | W | T*W |
| Glucose, mg/dL | 102.6 | 3.2 | 94.6 | 99.2 | 4.2 | 0.06 | 1.00 | 0.67 |
| NEFA | 395 | 29.5 | 244 | 231 | 22 | 0.52 | 0.01 | 0.66 |
| BHBA, mmol/L | 0.06 | 0.01 | 0.06 | 0.06 | 0.01 | 0.80 | 0.08 | 0.96 |
| Cholesterol, mg/dL | 27.9 | 1.6 | 75.1 | 71.6 | 2.5 | 0.28 | <.001 | 0.12 |
| Urea N, mg/dL | 12.6 | 0.94 | 6.3 | 6.1 | 0.54 | 0.68 | 0.85 | 0.81 |
| Total protein, mg/dL | 5.77 | 0.11 | 5.73 | 5.6 | 0.23 | 0.36 | 0.40 | 0.69 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet.

[4] PSY= Psyllium-supplemented diet

**Table 2.9** Least squares means and associated standard errors for weekly post-prandial blood constituents.

| | Week 0 | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON | PSY | SE | T | W | T*W |
| Glucose, mg/dL | 103.6 | 4.23 | 102.5 | 105.7 | 2.8 | 0.42 | 0.073 | 0.87 |
| NEFA | 261 | 22.4 | 191 | 185 | 12.6 | 0.73 | 0.013 | 0.52 |
| BHBA, mmol/L | 0.05 | 0.01 | 0.02 | 0.02 | 0.01 | 0.45 | <0.001 | 0.74 |
| Cholesterol, mg/dL | 26.4 | 1.3 | 67.9 | 68.1 | 3.0 | 0.96 | <0.001 | 0.18 |
| Urea N, mg/dL | 12.0 | 0.98 | 6.2 | 5.8 | 0.73 | 0.44 | 0.89 | 0.64 |
| Total protein, mg/dL | 5.10 | 0.43 | 5.13 | 5.10 | 0.61 | 0.99 | 0.018 | 0.76 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction
[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.
[3] CON=Control diet
[4] PSY= Psyllium-supplemented diet

# CHAPTER 3

## Effects of inclusion of psyllium in milk replacer on volatile fatty acid concentrations, microbial populations, and gastrointestinal size in neonatal dairy calves

### Introduction

Psyllium has been studied in many species for its potential effects on digestive function, rate of passage, nutrient absorption, and intestinal morphology when added to the diet. Neonatal dairy calves are subject to development of scours (diarrhea) and other gastrointestinal upsets, which contribute to the high rates of morbidity and mortality (National Animal Health Monitoring System, 2007). The impact of psyllium on digestive function, rate of passage, and metabolism in young calves is not known.

As a fermentable fiber, psyllium would be expected to favor intestinal anaerobic bacteria with the capacity to ferment it. Increased fermentation in the lower tract results in greater concentrations of VFA, which in turn increase mass of the gastrointestinal tract in other species such as rats (Leng-Peschlow, 1991; Edwards *et al.* 1992; Schneeman and Richter, 1993; Edwards *et al.* 2003). A larger gastrointestinal tract with a more stable population of desirable bacterial species might improve growth and health in young dairy calves.

Little is known about the effects of supplementing psyllium to the diet of young calves. The purpose of this portion of the study was to determine the effects of inclusion of psyllium in milk replacers on VFA concentrations and microbial populations throughout the gastrointestinal tract, as well as on size and scale of gastrointestinal tissues in neonatal dairy calves.

## Materials and Methods

### Animal Care

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol number 04083).

The study was conducted during two periods, the first (May 2004) using 12 calves and the second (June 2004) using 22 calves. Male Holstein calves were purchased from Stone Ridge Dairy (Bellflower, IL) at less than 36 h of age.

At the beginning of the trial period, daily late afternoon trips were made to Stone Ridge Dairy to select and purchase calves. At Stone Ridge Dairy, all male Holstein calves less than 36 h of age in the calf pack were evaluated for potential purchase by trial personnel. The evaluation included body temperature, heart and lung auscultation, hydration status, mobility, navel status, and overall thriftiness. Also, the calves must have received at least one feeding of colostrum by Stone Ridge personnel. Calves deemed satisfactory were then transported to the University of Illinois Dairy Nutrition Field Laboratory.

Upon arrival, each calf received an eartag in the left ear for identification purposes. Calves were then measured for withers height, body length, heart girth circumference, and body weight. Ear notches were taken from each calf and placed in formalin for determination of persistently infected bovine viral diarrhea (PI-BVD) status. Each calf was vaccinated against infectious bovine rhinotracheitis virus and parainfluenza-3 intranasally (2 ml of TSV-2; Pfizer Animal Health, Exton, PA). In addition, calves received 15 mL of Quatracon-2X™ Antiserum (Boehringer Ingleheim

Vetmedica, Inc.; St. Joseph, MO) subcutaneously in the neck. Each calf also received 2 mL of Excenel (Pfizer Animal Health; Exton, PA) subcutaneously in the neck for the first 3 d of the study. After entry injections, the calves were moved to southward-facing individual calf hutches (Calf-Tel; Hampel Corp., Germantown, WI) on 15 to 20 cm of crushed limestone located on the south side of the Dairy Nutrition Field Laboratory. No bedding was used, to minimize ingestion of bedding material that might confound treatment inferences.

**Assignment to Treatments**

Calves were blocked by pairs based upon birth date, body weight, and total protein score and then randomly assigned within pair to each of the dietary treatments. These pairs of calves were then randomly assigned to harvest week. Of the 34 calves assigned to treatments, 1 calf died at 2 d of age. Necropsy results (University Of Illinois College of Veterinary Medicine, Urbana, IL) indicated that the cause of mortality was likely related to birth trauma. All other calves completed the trial in good health.

**Feeding**

On the night of arrival, each calf was offered colostrum for ad libitum intake. On the morning following arrival, each calf again was offered colostrum. Treatment feedings were administered at 0600 and 1800 h. Calves were fed milk replacers reconstituted to 12.5% DM at a rate of 12% of BW daily, adjusted weekly as calves grew. Milk replacers (Land O'Lakes Animal Milk Products Co.; Arden Hills, MN) with or without a 1.1% inclusion of psyllium were formulated to contain 22% protein and 20% fat, and contained only milk proteins. Neither milk replacer contained growth-promoting

antibiotics. Water was available to calves for ad libitum consumption, with fresh warm water provided twice daily after each milk replacer feeding. No other feeds were offered.

**Health Monitoring**

Calves were observed at least twice daily for general health, including appearance (alertness), appetite (ability to consume feed), and fecal scores. Fecal scores were recorded daily, using the following guidelines: 1 = dry, hard; 2 = soft, formed; 3 = pudding-like; 4 = mix of liquids with some solids; and 5 = liquid. If the calf's hydration status was low (skin tenting evident), 500 mL of Lactated Ringers Solution (Abbott Laboratories; Abbott Park, IL) was administered subcutaneously. If a calf appeared unhealthy, 3 mL of Excenel (Pfizer, Exton, PA) was administered subcutaneously for a period of 3 d. If body temperature was over 39° C, 1 mL of Banamine (Schering-Plough Animal Health; Union, NJ) was administered subcutaneously. Calves received a booster of TSV-2 at 2 wk of age.

**Calf Harvest Procedure**

Pairs of calves were harvested weekly for analysis. For period 1, two calves were euthanized after arrival before experimental treatments began (wk 0) for baseline measurements. For the remaining weeks, one pair of calves was euthanized weekly, with the exception of wk 4. In wk 4 of period 1, four calves were euthanized. For period 2, four baseline calves were euthanized at wk 0, and two pairs were euthanized during wk 1, 2, and 3. For wk 4, five calves were harvested due to the one mortality.

Calves were transported to the University of Illinois College of Veterinary Medicine at 0630 h. Calves were not given their morning milk replacer prior to euthanasia. The harvest procedure consisted of the calf being administered Xylazine HCl

intramuscularly (50 mg/mL; Fort Dodge Animal Health; Fort Dodge, IA). Once the calf was sedated, it was euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus; Veterinary Laboratories Inc., Lenexa, KS) and then exsanguinated.

When the calf was declared dead, the veterinarian opened the body cavity. The gastrointestinal tract was ligated at the caudal esophagus and rectum and removed as rapidly as possible without damaging the tract. To prevent movement of digesta between compartments, the different segments of the gastrointestinal tract were identified and ligated. The gastrointestinal tract was then divided into three portions: the stomach portion consisting of rumen, reticulum, omasum, and abomasum; the small intestine, consisting of duodenum, jejunum, and ileum; and the colon. Each portion was processed by the respective team of personnel for tissue and digesta collection.

**Digesta Collection**

Individual aliquots of digesta from the rumen, jejunum, and colon were placed into pre-weighed Cary-Blair transport media containers (Meridian Diagnostics; Cincinnati, OH). The medium in the Cary-Blair allows for stabilization of anaerobic microorganisms prior to laboratory enumeration for *Lactobacillus, Bifidobacteria*, *Clostridium perfringens*, and *E. coli*.

For VFA sample collection, approximately 1 to 5 mL of digesta was placed into a pre-weighed Nalgene bottle (Nalge Nunc International; Rochester, NY) containing 10 mL of 2 *N* HCl. The bottles were then weighed again to determine amount of digesta placed into each bottle. The samples were then stored at -20°C until analysis.

After all digesta had been removed from the gastrointestinal tract, each section was rinsed. Wet weights and lengths were measured and recorded for calculation of gastrointestinal size and scale parameters.

**Microbial Analyses**

The procedure for enumeration of *Bifidobacterium spp.* was that of Muñoa and Pares (1988), utilizing Bifidobacteria agar (Difco Laboratories; Detroit, MI). *Lactobacillus spp.* were cultured on Rogosa SL agar (Difco Laboratories; Detroit, MI). *Clostridium perfringens* was cultured on Clostridia agar (Sigma-Aldrich; St. Louis, MO), utilizing the Food and Drug Administration Method #196 (1992). *E. coli* were cultured on EMB agar (Difco Laboratories; Detroit, MI).

When digesta samples arrived at the laboratory, serial dilutions ($10^{-1}$ through $10^{-8}$) in anaerobic diluent were made from the Cary-Blair media containing digesta according to the methods of Bryant and Burkey (1953). Droplets of five appropriate dilutions were then inoculated onto their respective petri dishes for each of the four agars to maximize counting precision of the microbial colonies. Each petri dish received 7 droplets of 10 μL each. After droplets had been adsorbed into the sterile agar, plates for *Bifidobacteria*, *Lactobacillus*, and *C. perfringens* were inverted and incubated anaerobically (95% $CO_2$ and 5%$H_2$) at 39°C. *E. coli* plates were incubated aerobically at 37°C. Colonies were counted after 24 to 48 h of incubation to determine colony forming units (CFU) per gram of sample. Plates exhibiting 4 to 20 colonies per droplet were counted and averaged. A CFU was defined as a distinct colony measuring at least 1 mm in diameter. Colony forming units per gram sample (DM basis) were calculated as:

$$\text{CFU/g} = \frac{\text{(mean CFU) x (dilution) x (diluent dilution)}}{\text{(g sample, DM basis) x (mL in droplet on culture plate)}}$$

**Volatile Fatty Acid Analyses**

Samples of digesta collected from the rumen, abomasum, jejunum, proximal colon, and distal colon were prepared for analysis of volatile fatty acid (VFA) concentrations, using the methods of Erwin *et al*. (1961) with modifications. Briefly, an initial centrifugation (500 × *g*) of the samples was performed to remove particulate matter, using a Beckman centrifuge at 13,000 rpm with a JA-14 rotor. Aliquots (4 mL) of the samples acidified in HCl were mixed with 1.0 mL of 250 g/L *m*-phosphoric acid, precipitated at room temperature for 30 min, and centrifuged at 20,000 × *g*. The supernatant was decanted and frozen overnight at -20°C in microcentrifuge tubes. The supernatant was thawed and the microcentrifuge tubes were centrifuged at 13,000 × *g* for 15 min using an Eppendorf 5804 centrifuge with an F-4S-30-11 rotor (Brinkmann Instruments; Westbury, NY). Samples were centrifuged with centrifugal filters (Centricon 3000 MC; Millipore, Billerica, MA) to remove remaining particulate matter. The supernatants were then placed in gas chromatography vials (SUN-SRI; Duluth, GA) that were capped and refrigerated until concentrations of VFA were determined.

Concentrations of VFA in the supernatants were determined by gas-liquid chromatography. Briefly, concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were determined using a Hewlett-Packard 5890A Series II gas chromatograph (Palo Alto, CA) fitted with a glass column (180 cm × 4 mm i.d.) packed

with 10% SP-1200/1% $H_3PO_4$ on 80/100 mesh Chromosorb W AW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were 125, 175, and 180ºC, respectively. Concentrations of VFA were corrected for quantities of VFA measured in reagent blank tubes analyzed concurrently.

**Statistical Analysis**

Statistical analyses were conducted utilizing the Proc Mixed procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC). The data were analyzed as a randomized complete block design, with period as a random effect. Effects of diet, week, and the interaction of diet and week were included in the model as fixed effects. Baseline calves were not included in the analysis, but means and standard errors are shown for comparison. Significant differences were declared at $P < 0.05$, and trends toward significant effects were noted at $P < 0.10$.

## Results and Discussion

**VFA Concentrations and Profiles**

Concentrations of VFA in rumen contents did not differ significantly between treatments (Table 3.1). The treatment × week interaction approached significance for concentrations of propionate and butyrate. Concentrations of these VFA were higher for PSY calves in wk 1, but higher in CON calves during wk 2. Due to the small numbers of calves measured at each age, additional research would be necessary to determine whether these changes were repeatable and of significance to rumen development.

The molar percentage of butyrate in the rumen was greater ($P < 0.05$) for PSY calves than for CON calves. An interaction of treatment × week occurred for rumen butyrate percentages ($P < 0.04$), with butyrate percentage being higher in PSY except in wk 2. The greater relative abundance of butyrate could be of interest as butyrate is the VFA most responsible for stimulation of rumen epithelial development (Heinrichs, 2005). Although most milk replacer should have bypassed the rumen, small amounts can enter through incomplete closure of the reticular groove. The presence of the fermentable psyllium for PSY calves may have led to greater butyrate formation.

Total VFA concentrations (mMol/L) were low in the abomasum, and few significant differences in concentrations were observed between treatments (Table 3.2). A significant treatment × week interaction was noted for abomasal acetate percentage (moles per hundred moles of total VFA; $P < 0.02$), with PSY being higher at wk 2 but CON being higher at wk 1, 3, and 4. A significant treatment × week interaction was observed for abomasal propionate percentage ( $P < 0.01$), with PSY being higher at wk 1 and 4 and CON being higher at wk 2 and 3. Abomasal butyrate percentages tended ($P < 0.07$) to be higher in the PSY calves than in CON calves. Furthermore, the percentage of isovalerate was greater ($P < 0.03$) for the PSY calves.

Concentrations of VFA also were low in jejunal contents (Table 3.3). The PSY calves tended ($P < 0.06$) to exhibit higher jejunal acetate concentrations and concentrations decreased with age (Table 3.3). A tendency for an effect of week ($P < 0.06$) was detected for jejunal butyrate concentrations, which increased from wk 1 to wk 2 and then decreased during wk 2 to 4. The concentration of valerate was greater for PSY than for CON, and the treatment × week interaction was significant; valerate was greater

for PSY calves for all weeks except wk 4. The total concentration of VFA in the jejunum was very low compared with those in the rumen, but tended ($P < 0.07$) to be higher for calves fed PSY. This effect indicates that bacteria within the jejunum may have been able to ferment a portion of the psyllium during its passage through the small intestine.

The molar percentages of isobutyrate, butyrate, isovalerate, and valerate all exhibited a significant effect of week ($P < 0.01$), but no significant effect of treatment was observed. The small changes with age may reflect alterations in relative populations of microbial species within the small intestine but are of unknown importance.

In digesta from the proximal colon (Table 3.4), the PSY calves had higher concentrations of acetate ($P < 0.001$), propionate ($P < 0.001$), isobutyrate ($P < 0.03$), and butyrate ($P <0.02$). The PSY calves also tended ($P < 0.06$) to have higher concentrations of valerate but isovalerate did not differ. Therefore, total VFA concentrations also were significantly higher for the PSY calves compared to the CON calves ($P < 0.001$). A significant effect of week was also detected ($P < 0.003$) for individual and total VFA concentrations. The concentration of VFA generally increased with greater age of the calves. Also, a significant treatment × week effect was noted for butyrate ($P < 0.03$), as differences between treatments became larger as the calves age grew older, possibly due to an increase in fermentation.

A tendency ($P <0.10$) was noted for the PSY calves to have a higher percentage of propionate in digesta from the proximal colon (Table 3.4). The CON calves had significantly higher ($P <0.05$) percentages of isovalerate in the proximal colon.

In digesta from the distal colon, PSY calves had higher concentrations of acetate ($P < 0.01$), propionate ($P < 0.01$), and isobutyrate ($P < 0.05$) than CON calves, and

tended ($P < 0.09$) to have higher concentrations of valerate (Table 3.5). The total concentration of VFA in the distal colon was higher for the PSY calves ($P < 0.001$) than for the CON calves, which would therefore indicate increased fermentation in the distal colon of calves fed the PSY treatment. No significant differences were noted between CON and PSY treatments for molar percentages of VFA in the distal colon.

Overall, the predominant effect of psyllium inclusion in the milk replacer on VFA concentrations was in the lower gastrointestinal tract as indicated by the higher total VFA concentrations in the jejunum, proximal colon, and distal colon. Greater lower gut fermentation is logical because psyllium is a fiber that can be fermented by intestinal bacteria as seen in humans (Edwards *et al.,* 1992) and monkeys (Costa *et al.,* 1989b). Acetate concentrations were increased in the jejunum, proximal colon, and distal colon, similar to observations in cecal contents and feces of rats fed psyllium (Leng-Peschlow, 1991). Propionate concentrations were increased in the proximal colon and distal colon. In rats, fecal concentration of propionate was increased when psyllium was fed (Edwards *et al.*, 1992).

The molar percentages of VFA in the jejunum or distal colon were not affected by inclusion of psyllium. However, there was a tendency in the proximal colon for the PSY calves to exhibit higher percentages of propionate, and for the CON calves to exhibit higher percentages of isovalerate. In monkeys no differences in the fecal VFA profile were observed when psyllium was fed (Costa *et al.*, 1989b) or in a culture system utilizing the same monkeys (Costa *et al.*, 1989a).

**Bacterial Populations in the Digestive Tract**

Ruminal bifidobacteria concentrations were significantly higher ($P < 0.05$) for the PSY calves than for the CON calves (Table 3.6). Counts did not differ between diets in jejunum or colon. No differences were detected between CON and PSY treatments for *Clostridium perfringens* concentrations in the rumen, jejunum, or colon. Counts generally increased with age in the colon ($P < 0.01$) regardless of treatment. A weak tendency ($P < 0.11$) was observed for the CON calves to have higher counts of *E. coli* present in the rumen. No other differences were noted between CON and PSY treatments. Lactobacilli counts in the rumen were higher for the PSY calves ($P < 0.02$) when compared to the CON calves. There was also a significant effect of week ($P < 0.001$) observed for both rumen and jejunal lactobacilli concentrations, with counts higher in calves after wk 1.

Effects of psyllium inclusion on bacterial counts were minimal. The inclusion of psyllium in the milk replacer increased rumen bifidobacteria and lactobacilli concentrations. These data potentially indicate an effect of psyllium inclusion as a prebiotic due to small amounts of milk replacer entering the rumen through incomplete closure of the reticular groove. However, no significant differences in bifidobacteria and lactobacilli concentrations were observed in the jejunum or colon.

**Gastrointestinal Tract Size and Scale**

A tendency was noted ($P < 0.09$) for the PSY calves to have heavier reticulo-rumens (Table 3.7). Furthermore, a tendency for an effect of week ($P < 0.07$) was detected for reticulo-rumen length, as well as a significant ($P < 0.03$) effect of week on

reticulo-rumen weight. In addition, a tendency for an effect of week ($P < 0.08$) was noted for density (g/cm) of reticulo-rumen tissue.

No significant differences were noted between PSY and CON treatments for omasal growth parameters (Table 3.8). There was a significant effect of week ($P < 0.02$) for abomasal length and for abomasal density per kg BW ($P < 0.003$; Table 3.9). There was a tendency for an effect of week ($P < 0.09$) on g abomasum per kg BW, indicating that the abomasum decreased in size relative to the rest of the body as calves grew.

Calves fed PSY had heavier ($P < 0.03$) duodenal weights (Table 3.10). Furthermore, PSY calves had more duodenal tissue per kg BW ($P < 0.02$), and a tendency for more dense (g/cm) duodenal tissue ($P < 0.06$). Also, PSY calves tended ($P < 0.06$) to exhibit a higher tissue density (g/cm) per kg BW.

The CON calves had longer jejunums than the PSY calves (Table 3.11), although this difference did not achieve statistical significance ($P < 0.09$). However, the PSY calves tended ($P < 0.12$) to have heavier jejunums, which resulted in greater ($P < 0.056$) mass of jejunum per unit BW. Furthermore, the PSY calves exhibited significantly ($P < 0.001$) more dense jejunal tissue, which also carried through to significance ($P < 0.001$) for greater tissue density per unit of calf BW. However, the CON calves exhibited greater ($P < 0.01$) length of jejunal tissue per unit of calf BW.

The PSY calves had more dense ($P < 0.04$) ileal tissue than the CON calves (Table 3.12). As a result, the PSY calves tended ($P < 0.08$) to have greater density of ileal tissue per kg BW.

The effect of treatment was significant ($P < 0.04$) for colon weight with the PSY calves having heavier colons than CON calves (Table 3.13). This resulted in PSY calves

having greater grams of colon per kilogram calf BW ($P < 0.01$). The PSY calves also tended ($P < 0.06$) to have greater density of colon tissue than the CON calves, resulting in the PSY calves having more dense colon tissue per unit of calf BW ($P < 0.04$).

The inclusion of psyllium in milk replacer did not increase lengths of the duodenum ($P < 0.90$), jejunum ($P < 0.12$), ileum ($P < 0.51$), or colon ($P < 0.51$). These data contrast with the effects observed in rats, where lengths of the small intestine (Leng-Peschlow, 1991) and large intestine (Leng-Peschlow, 1991; Edwards *et al.*, 1992) were increased when psyllium was included in the diet. However, tissue wet weights were increased for the PSY calves in the duodenum ($P < 0.03$), jejunum ($P < 0.12$), and colon ($P < 0.04$). Rats fed psyllium also were reported to have greater small intestine (Schneeman and Richter, 1993) and colon (Edwards *et al.*, 1992) weights. The increased tissue density of duodenum ($P < 0.06$), jejunum ($P < 0.01$), ileum ($P < 0.04$), and colon ($P < 0.06$) for calves consuming psyllium was similar to the increased tissue density of small intestine and colon observed in rats (Leng-Peschlow, 1991).

## Conclusions

The increase in size and scale of the gastrointestinal tract could be of benefit to the neonatal dairy calf from a standpoint of potential greater absorptive capacities of the tissue, and greater tissue resistance to disease intrusion into the bloodstream. The inclusion of a fermentable fiber could also potentially "set-up" the path to allow more rapid gastrointestinal growth once calf starter ingestion begins. However, more gastrointestinal tissue may increase dietary nutrient requirements for maintenance of these highly metabolically active tissues.

The lack of significant microbial changes in the gastrointestinal tract despite the increase in VFA could be due to subtle differences or a small sample size with an assay with a larger margin of error compared to other assays utilized in this study. This is an area that merits further research with a larger sample size to determine potential effects of inclusion of psyllium in milk replacers on microbial populations of the gastrointestinal system of the neonatal dairy calf.

The lack of differences in ADG for calves fed PSY (see Chapter 2) could be attributed to the fact that these calves were limit fed and were not fed a more biologically normal diet to allow for maximal ADG and feed intakes. A future trial should be done with the use of calf starter, as that is where there could be more benefit due to potential synergisms.

## References


Bryant, M. P. and L. A. Burkey. 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 36:205-217.

Costa, M. A., T. Mehta, and J. R. Males. 1989a. Effect of dietary cellulose and psyllium husk on monkey colonic microbial metabolism in continuous culture. J. Nutr. 119:979-985.

Costa, M. A., T. Mehta, and J. R. Males. 1989b. Effects of dietary cellulose, psyllium husk, and cholesterol level on fecal and colonic microbial metabolim in monkeys. J. Nutr. 119:986-992.

Edwards, C. A., J. Bowen, W. G. Brydon, and M. A. Eastwood. 1992. The effects of ispaghula on rat caecal fermentation and stool output. Br. J. Nutr. 68:473-482.

Edwards, S., M. F. Chaplin, A. D. Blackwood, and P. W. Dettmar. 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. Proc. Nutr. Soc. 62:217-222.

Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. 44:1768-1771.

Heinrichs, A. J. 2005. Rumen development in the dairy calf. Adv. Dairy Tech. 17:179-187.

Leng-Peschlow, E. 1991. *Plantago ovata* seeds as dietary fibre supplement: physiological and metabolic effects in rats. Br. J. Nutr. 66:331-349.

Lesmeister, K. E., P. R. Tozer, and A. J. Heinrichs. 2004. Development and analysis of a rumen tissue sampling procedure. J. Dairy Sci. 87:1336-1344.

Muñoa, F. J. and R. Pares. 1988. Selective medium for isolation and enumeration of *Bifidobacterium* spp. Applied and Environmental Microbiology 1988:1715-1718.

National Animal Health Monitoring System. 2007. *Part 1: Reference of Dairy Cattle Health and Management Practices in the United States, 2007*. Ft. Collins, CO: USDA:APHIS:VS:CEAH

Schneeman, B. O., and D. Richter. 1993. Changes in plasma and hepatic lipids, small intestinal histology and pancreatic enzyme activity due to aging and dietary fiber in rats. J. Nutr. 123:1328-1337.

**Table 3.1.** Least squares means and associated standard errors for weekly concentrations (mMol/L) and molar percentages of VFA in ruminal digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Acetate | | | | | | | | | | | | | | | | | | | | |
| concentration | 21.94 | 3.32 | 22.73 | 32.03 | 6.11 | 40.11 | 36.11 | 6.11 | 31.43 | 44.02 | 6.11 | 40.47 | 32.63 | 5.31 | 33.68 | 36.20 | 3.08 | 0.55 | 0.26 | 0.23 |
| percentage | 73.34 | 3.36 | 68.94[c] | 61.93[d] | 3.56 | 58.34[e] | 68.73[f] | 3.56 | 70.65 | 71.26 | 3.56 | 71.87 | 68.11 | 3.14 | 67.45 | 67.51 | 2.09 | 0.98 | 0.09 | 0.07 |
| Propionate | | | | | | | | | | | | | | | | | | | | |
| concentration | 4.51 | 1.16 | 7.09[a] | 13.22[b] | 2.45 | 18.24[a] | 11.77[b] | 2.45 | 8.93 | 11.91 | 2.45 | 11.27 | 9.56 | 2.12 | 11.38 | 11.61 | 1.18 | 0.89 | 0.17 | 0.09 |
| percentage | 15.64 | 3.42 | 22.18 | 24.8 | 2.65 | 26.69 | 24.58 | 2.65 | 20.65 | 19.98 | 2.65 | 19.52 | 21.07 | 2.45 | 22.26 | 22.61 | 1.99 | 0.8 | 0.03 | 0.62 |
| Isobutyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.50 | 0.18 | 0.51 | 1.52 | 0.40 | 1.47 | 1.03 | 0.40 | 0.84 | 1.11 | 0.40 | 1.15 | 1.03 | 0.34 | 0.99 | 1.17 | 0.19 | 0.51 | 0.91 | 0.32 |
| percentage | 1.55 | 0.64 | 1.56 | 2.7 | 0.38 | 1.79 | 1.73 | 0.38 | 1.9 | 1.77 | 0.38 | 1.97 | 2.25 | 0.33 | 2.11 | 1.81 | 0.19 | 0.25 | 0.67 | 0.36 |
| Butyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 1.01 | 0.2 | 0.66[a] | 2.51[b] | 0.53 | 2.00[c] | 0.76[d] | 0.53 | 1.05 | 1.82 | 0.53 | 1.44 | 1.35 | 0.46 | 1.29 | 1.61 | 0.26 | 0.39 | 0.98 | 0.06 |
| percentage | 3.60 | 0.11 | 2.14[g] | 4.60[h] | 0.69 | 2.56 | 1.52 | 0.69 | 2.49 | 3.03 | 0.69 | 2.46 | 3.71 | 0.63 | 2.41 | 3.21 | 0.49 | 0.05 | 0.12 | 0.04 |
| Isovalerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.36 | 0.09 | 0.41 | 1.9 | 0.56 | 1.83 | 1.11 | 0.56 | 0.8 | 1.16 | 0.56 | 1.21 | 1.03 | 0.49 | 1.30 | 1.06 | 0.27 | 0.55 | 0.84 | 0.27 |
| percentage | 1.29 | 0.31 | 1.27 | 3.33 | 0.59 | 2.06 | 1.80 | 0.59 | 1.80 | 1.87 | 0.59 | 2.06 | 2.69 | 0.51 | 1.80 | 2.42 | 0.29 | 0.14 | 0.71 | 0.25 |
| Valerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 1.56 | 0.64 | 1.36 | 1.57 | 2.8 | 9.93 | 1.10 | 2.8 | 1.26 | 1.41 | 2.8 | 1.21 | 1.07 | 2.43 | 3.44 | 1.29 | 1.36 | 0.27 | 0.34 | 0.30 |
| percentage | 5.41 | 2.22 | 4.22 | 2.95 | 1.99 | 8.89 | 1.96 | 1.99 | 2.83 | 2.41 | 1.99 | 2.12 | 2.36 | 1.72 | 4.52 | 2.41 | 1.05 | 0.17 | 0.44 | 0.33 |
| Total VFA concentration | 29.62 | 4.04 | 32.89 | 52.88 | 11.43 | 73.71 | 52.02 | 11.43 | 44.45 | 61.56 | 11.43 | 56.74 | 47.26 | 9.9 | 51.95 | 53.43 | 5.53 | 0.85 | 0.41 | 0.21 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction
[2] Baseline values are shown for comparison but were not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( P < 0.10).

[c,d] Subcolumn means within row and week category with different superscripts differ ( P < 0.15).

[e,f] Subcolumn means within row and week category with different superscripts differ ( P < 0.05).

[g,h] Subcolumn means within row and week category with different superscripts differ ( P < 0.01).

**Table 3.2.** Least squares means and associated standard errors for weekly concentrations (mMol/L) and molar percentages of VFA in abomasal digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Acetate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.45 | 0.10 | 5.17 | 8.03 | 1.78 | 8.53 | 2.37 | 1.78 | 2.78 | 2.85 | 1.78 | 3.11 | 3.61 | 1.54 | 4.89 | 4.21 | 0.86 | 0.58 | 0.14 | 0.11 |
| percentage | 64.02 | 3.62 | 79.34[a] | 67.81[b] | 3.18 | 71.78[c] | 81.87[d] | 3.18 | 79.64 | 79.47 | 3.18 | 80.40[e] | 74.70[f] | 2.75 | 77.8 | 75.96 | 1.51 | 0.40 | 0.33 | 0.02 |
| Propionate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.02 | 0.01 | 1.13 | 2.96 | 0.87 | 2.78 | 0.22 | 0.87 | 0.31 | 0.22 | 0.87 | 0.38 | 0.50 | 0.75 | 11.48 | 0.97 | 0.42 | 0.77 | 0.15 | 0.13 |
| percentage | 2.75 | 0.67 | 10.75[g] | 18.51[h] | 3.27 | 20.37 | 7.35 | 3.27 | 7.61[i] | 6.40[j] | 3.27 | 6.49 | 10.95 | 2.89 | 11.3 | 10.8 | 1.91 | 0.81 | 0.05 | 0.01 |
| Isobutyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.01 | 0.01 | 0.14[k] | 0.49[l] | 0.10 | 0.26[m] | 0.06[n] | 0.10 | 0.07 | 0.07 | 0.10 | 0.08 | 0.16 | 0.08 | 0.14 | 0.19 | 0.05 | 0.42 | 0.09 | 0.07 |
| percentage | 2.46 | 1.15 | 2.87 | 3.75 | 0.56 | 1.9 | 2.06 | 0.56 | 1.95 | 1.97 | 0.56 | 2.39 | 2.10 | 0.49 | 2.27 | 2.47 | 0.31 | 0.59 | 0.06 | 0.69 |
| Butyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.21 | 0.1 | 0.15 | 0.63 | 0.16 | 0.30 | 0.06 | 0.16 | 0.07 | 0.08 | 0.16 | 0.10 | 0.18 | 0.14 | 0.15 | 0.239 | 0.08 | 0.46 | 0.27 | 0.22 |
| percentage | 25.4 | 4.09 | 2.44 | 4.00 | 0.67 | 2.14 | 2.07 | 0.67 | 2.12 | 2.37 | 0.67 | 2.12 | 3.9 | 0.58 | 2.2 | 3.08 | 0.32 | 0.07 | 0.27 | 0.38 |
| Isovalerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.01 | 0.01 | 0.07 | 0.52 | 0.13 | 0.27 | 0.03 | 0.13 | 0.03 | 0.07 | 0.13 | 0.06 | 0.08 | 0.11 | 0.11 | 0.18 | 0.06 | 0.46 | 0.25 | 0.11 |
| percentage | 2.08 | 1.69 | 1.31 | 3.46 | 0.53 | 1.79 | 1.16 | 0.53 | 1.04 | 1.92 | 0.53 | 1.07 | 1.94 | 0.46 | 1.30 | 2.12 | 0.26 | 0.03 | 0.25 | 0.11 |
| Valerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.03 | 0.01 | 0.14 | 0.17 | 0.07 | 0.27 | 0.15 | 0.07 | 0.22 | 0.26 | 0.07 | 0.25 | 0.30 | 0.07 | 0.22 | 0.02 | 0.05 | 0.92 | 0.17 | 0.35 |
| percentage | 4.16 | 0.78 | 3.12 | 2.32 | 1.75 | 1.89 | 5.36 | 1.75 | 7.51 | 7.73 | 1.75 | 7.53 | 5.08 | 1.64 | 5.01 | 5.12 | 1.43 | 0.90 | 0.00 | 0.12 |
| Total VFA Concentration | 0.74 | 0.21 | 6.81[o] | 12.81[p] | 2.93 | 12.41[q] | 2.89[r] | 2.93 | 3.49 | 3.57 | 2.93 | 3.99 | 4.82 | 2.54 | 6.67 | 6.02 | 1.42 | 0.75 | 0.14 | 0.10 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b,c,d,k,l,m,n,o,p,q,r] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

[e,f] Subcolumn means within row and week category with different superscripts differ *(* $P < 0.17$).

[g,h] Subcolumn means within row and week category with different superscripts differ ( $P < 0.10$).

[i,j] Subcolumn means within row and week category with different superscripts differ ( $P < 0.001$).

**Table 3.3.** Least squares means and associated standard errors for weekly concentrations (mmol/L) and molar percentages of VFA in jejunal digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Acetate | | | | | | | | | | | | | | | | | | | | |
| concentration | 2.85 | 1.50 | 1.13 | 1.95 | 0.26 | 0.94 | 1.09 | 0.26 | 1.22 | 1.80 | 0.26 | 0.94 | 0.79 | 0.22 | 1.06 | 1.41 | 0.12 | 0.06 | 0.02 | 0.21 |
| percentage | 53.61 | 1.56 | 69.05 | 65.26 | 3.53 | 61.68 | 56.47 | 3.53 | 63.25 | 65.24 | 3.53 | 59.64 | 64.07 | 3.15 | 63.4 | 62.76 | 2.22 | 0.76 | 0.10 | 0.32 |
| Propionate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.34 | 0.21 | 0.07 | 0.20 | 0.05 | 0.13 | 0.14 | 0.05 | 0.10 | 0.14 | 0.05 | 0.10 | 0.06 | 0.04 | 0.10 | 0.13 | 0.03 | 0.28 | 0.39 | 0.26 |
| percentage | 5.58 | 0.34 | 4.66 | 6.77 | 1.29 | 6.92 | 6.41 | 1.29 | 5.37 | 4.96 | 1.29 | 5.58 | 5.52 | 1.16 | 5.63 | 5.92 | 0.83 | 0.72 | 0.60 | 0.62 |
| Isobutyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.09 | 0.02 | 0.04 | 0.05 | 0.03 | 0.12 | 0.11 | 0.03 | 0.10 | 0.14 | 0.03 | 0.08 | 0.05 | 0.03 | 0.08 | 0.09 | 0.02 | 0.85 | 0.04 | 0.58 |
| percentage | 3.09 | 0.98 | 3.00 | 2.02 | 0.85 | 6.71 | 5.41 | 0.85 | 5.20 | 5.40 | 0.85 | 4.48 | 4.09 | 0.74 | 4.85 | 4.23 | 0.41 | 0.30 | 0.00 | 0.82 |
| Butyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.28 | 0.19 | 0.07 | 0.11 | 0.04 | 0.13 | 0.15 | 0.04 | 0.10 | 0.11 | 0.04 | 0.04 | 0.02 | 0.04 | 0.09 | 0.10 | 0.02 | 0.69 | 0.06 | 0.89 |
| percentage | 4.52 | 0.40 | 4.44 | 3.73 | 1.19 | 6.80 | 6.67 | 1.19 | 5.07 | 4.15 | 1.19 | 2.67 | 1.77 | 1.06 | 4.75 | 4.08 | 0.73 | 0.38 | 0.00 | 0.98 |
| Isovalerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.06 | 0.02 | 0.01 | 0.03 | 0.05 | 0.01 | 0.09 | 0.05 | 0.05 | 0.07 | 0.05 | 0.28 | 0.21 | 0.04 | 0.09 | 0.09 | 0.03 | 0.77 | <0.001 | 0.15 |
| percentage | 1.32 | 0.46 | 1.27 | 1.05 | 0.95 | 1.12 | 4.18 | 0.95 | 2.60 | 2.63 | 0.95 | 17.12 | 17.11 | 0.85 | 5.53 | 6.24 | 0.59 | 0.23 | <0.001 | 0.19 |
| Valerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 1.89 | 1.14 | 0.28[a] | 0.61[b] | 0.07 | 0.25[c] | 0.41[d] | 0.07 | 0.36 | 0.48 | 0.07 | 0.18 | 0.09 | 0.06 | 0.27 | 0.40 | 0.03 | 0.01 | 0.00 | 0.02 |
| percentage | 32.07 | 2.21 | 17.61[e] | 21.20[f] | 1.60 | 16.80[g] | 20.89[h] | 1.60 | 18.53 | 17.66 | 1.60 | 10.50[i] | 7.198[j] | 1.39 | 15.86 | 16.74 | 0.80 | 0.44 | <0.001 | 0.09 |
| Total VFA | 5.52 | 3.07 | 1.62 | 2.96 | 0.42 | 1.60 | 2.01 | 0.42 | 1.96 | 2.77 | 0.42 | 1.61 | 1.23 | 0.36 | 1.70 | 2.24 | 0.20 | 0.07 | 0.08 | 0.17 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction
[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.002$).

[c,d,g,h] Subcolumn means within row and week category with different superscripts differ *(* $P < 0.10$).

[e,f,i,j] Subcolumn means within row and week category with different superscripts differ *(* $P < 0.15$).

**Table 3.4.** Least squares means and associated standard errors for weekly concentrations (mMol/L) and molar percentages of VFA in the proximal colon digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Acetate | | | | | | | | | | | | | | | | | | | | |
| concentration | 7.42 | 1.20 | 12.38 | 14.23 | 3.02 | 12.46 | 25.85 | 3.02 | 11.39 | 22.62 | 3.31 | 14.93 | 28.91 | 2.62 | 12.79 | 22.90 | 1.50 | <.001 | 0.03 | 0.15 |
| percentage | 75.41 | 6.21 | 72.61 | 64.72 | 3.80 | 57.13 | 64.45 | 3.80 | 55.95 | 63.85 | 4.16 | 65.07 | 60.67 | 3.29 | 62.69 | 63.84 | 1.89 | 0.78 | 0.11 | 0.09 |
| Propionate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.38 | 0.28 | 1.97 | 3.14 | 0.89 | 3.72 | 8.11 | 0.89 | 3.36 | 6.12 | 0.98 | 3.14 | 6.54 | 0.77 | 3.05 | 5.98 | 0.44 | <.001 | 0.004 | 0.33 |
| percentage | 3.91 | 2.81 | 9.80 | 13.77 | 2.15 | 16.25 | 20.42 | 2.15 | 17.59 | 18.00 | 2.32 | 13.59 | 13.98 | 1.91 | 14.25 | 16.24 | 1.16 | 0.10 | 0.003 | 0.59 |
| Isobutyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.19 | 0.10 | 0.10 | 0.21 | 0.09 | 0.17 | 0.28 | 0.09 | 0.20 | 0.18 | 0.10 | 0.25 | 0.60 | 0.08 | 0.18 | 0.29 | 0.03 | 0.03 | 0.01 | 0.15 |
| percentage | 3.08 | 1.77 | 0.50 | 0.89 | 0.25 | 0.70 | 0.68 | 0.25 | 1.19 | 0.64 | 0.31 | 1.12 | 1.45 | 0.22 | 0.84 | 0.91 | 0.13 | 0.67 | 0.03 | 0.37 |
| Butyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 1.50 | 0.52 | 2.30 | 3.68 | 1.66 | 5.15 | 4.75 | 1.66 | 3.97 | 5.23 | 1.73 | 3.64[a] | 8.78[b] | 1.56 | 3.77 | 5.61 | 1.35 | 0.02 | 0.03 | 0.05 |
| percentage | 13.70 | 3.81 | 13.23 | 16.92 | 3.61 | 22.16[c] | 11.56[d] | 3.61 | 20.85 | 15.55 | 3.79 | 16.02 | 18.73 | 3.35 | 18.07 | 15.69 | 2.78 | 0.20 | 0.69 | 0.02 |
| Isovalerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.23 | 0.05 | 0.25 | 0.28 | 0.14 | 0.37 | 0.28 | 0.14 | 0.50[e] | 0.17[f] | 0.15 | 0.35[g] | 0.83[h] | 0.12 | 0.36 | 0.39 | 0.07 | 0.79 | 0.05 | 0.02 |
| percentage | 2.92 | 0.65 | 1.68 | 1.13 | 0.58 | 1.57 | 0.73 | 0.58 | 2.72 | 0.67 | 0.63 | 1.61 | 1.96 | 0.51 | 1.87 | 1.06 | 0.28 | 0.05 | 0.62 | 0.16 |
| Valerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.13 | 0.03 | 0.16 | 0.46 | 0.27 | 0.32 | 0.63 | 0.27 | 0.33 | 0.24 | 0.29 | 0.59 | 1.34 | 0.24 | 0.36 | 0.62 | 0.13 | 0.06 | 0.01 | 0.32 |
| percentage | 1.51 | 0.32 | 1.69 | 2.08 | 0.72 | 1.71 | 1.68 | 0.72 | 1.56 | 0.82 | 0.79 | 2.60 | 2.93 | 0.62 | 1.89 | 1.88 | 0.36 | 0.98 | 0.13 | 0.85 |
| Total VFA | 9.93 | 1.27 | 17.46 | 22.32 | 4.36 | 22.51 | 40.20 | 4.36 | 19.92 | 34.87 | 4.76 | 22.90 | 47.21 | 3.79 | 20.77 | 35.85 | 2.11 | <.0001 | 0.003 | 0.12 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b,c,d] Subcolumn means within row and week category with different superscripts differ ($P < 0.01$).

[e,f] Subcolumn means within row and week category with different superscripts differ ($P < 0.13$).

[g,h] Subcolumn means within row and week category with different superscripts differ ($P < 0.005$).

**Table 3.5.** Least squares means and associated standard errors for weekly concentrations (mmol/L) and molar percentages of VFA in distal colon digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overal diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Acetate | | | | | | | | | | | | | | | | | | | | |
| concentration | 9.76 | 3.60 | 15.71 | 14.46 | 3.07 | 13.01 | 27.24 | 3.07 | 9.24 | 17.59 | 3.73 | 14.5 | 21.2 | 2.67 | 13.12 | 20.12 | 1.65 | <0.01 | 0.18 | 0.12 |
| percentage | 70.25 | 8.57 | 67.66 | 61.65 | 5.15 | 52.11 | 59.87 | 5.15 | 47.2 | 53.96 | 6.07 | 59.84 | 56.29 | 4.59 | 56.7 | 57.94 | 3.31 | 0.71 | 0.07 | 0.36 |
| Propionate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.54 | 0.40 | 3.08 | 3.77 | 1.13 | 4.64 | 9.99 | 1.13 | 3.73 | 6.15 | 1.38 | 3.97 | 5.87 | 0.99 | 3.86 | 6.44 | 0.62 | <0.01 | 0.02 | 0.22 |
| percentage | 5.20 | 4.18 | 67.66 | 61.65 | 5.15 | 52.11 | 59.87 | 5.15 | 47.20 | 53.96 | 6.07 | 59.84 | 56.29 | 4.59 | 16.56 | 17.56 | 1.61 | 0.71 | 0.07 | 0.36 |
| Isobutyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.10 | 0.07 | 0.17 | 0.23 | 0.09 | 0.25 | 0.38 | 0.09 | 0.24 | 0.33 | 0.11 | 0.35 | 0.58 | 0.07 | 0.25 | 0.38 | 0.04 | 0.05 | 0.03 | 0.75 |
| percentage | 0.74 | 0.42 | 0.65 | 0.87 | 0.37 | 0.82 | 0.84 | 0.37 | 1.35 | 1.16 | 0.46 | 1.59 | 1.95 | 0.32 | 1.10 | 1.21 | 0.19 | 0.70 | 0.03 | 0.89 |
| Butyrate | | | | | | | | | | | | | | | | | | | | |
| concentration | 2.53 | 0.86 | 3.44 | 4.25 | 2.52 | 7.24 | 6.22 | 2.52 | 5.22 | 7.25 | 2.73 | 4.19 | 8.35 | 2.39 | 5.02 | 6.52 | 2.15 | 0.18 | 0.25 | 0.33 |
| percentage | 21.92 | 5.86 | 17.16 | 18.73 | 4.49 | 27.30[a] | 14.43[b] | 4.49 | 25.92 | 22.47 | 5.00 | 17.58 | 20.68 | 4.19 | 21.99 | 19.08 | 3.57 | 0.21 | 0.30 | 0.07 |
| Isovalerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.14 | 0.04 | 0.30 | 0.45 | 0.24 | 0.47 | 0.34 | 0.24 | 0.69 | 0.30 | 0.29 | 0.50 | 1.05 | 0.21 | 0.49 | 0.53 | 0.12 | 0.80 | 0.25 | 0.22 |
| percentage | 1.35 | 0.47 | 1.34 | 1.57 | 0.86 | 1.57 | 0.75 | 0.86 | 3.99 | 1.12 | 1.05 | 2.26 | 2.9 | 0.74 | 2.29 | 1.58 | 0.44 | 0.27 | 0.27 | 0.26 |
| Valerate | | | | | | | | | | | | | | | | | | | | |
| concentration | 0.09 | 0.03 | 0.01 | 0.54 | 0.33 | 0.18 | 0.74 | 0.33 | 0.27 | 0.28 | 0.4 | 0.62 | 1.06 | 0.29 | 0.27 | 0.65 | 0.2 | 0.1 | 0.21 | 0.83 |
| percentage | 0.68 | 0.16 | 0.09 | 1.9 | 1.01 | 0.62 | 1.63 | 1.01 | 1.34 | 0.82 | 1.2 | 2.45 | 3.82 | 0.89 | 1.13 | 2.04 | 0.61 | 0.18 | 0.05 | 0.67 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total VFA | 13.00 | 2.93 | 22.63 | 23.62 | 4.53 | 25.7 | 44.84 | 4.53 | 19.4 | 31.8 | 5.13 | 24.13 | 33.63 | 4.17 | 22.96 | 33.47 | 3.4 | <0.001 | 0.02 | 0.11 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W= treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

**Table 3.6.** Least squares means and associated standard errors of bacteria concentrations[1] in neonatal calf digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[3] | SE | CON[4] | PSY[5] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | | | | | | | | | | | | | | | | | | | | |
| *Bifidobacteria* | 9.70 | 0.73 | 9.67 | 10.12 | 0.47 | 9.27 | 10.01 | 0.54 | 9.96 | 10.21 | 0.47 | 9.89 | 10.90 | 0.42 | 9.698 | 10.31 | 0.329 | 0.05 | 0.35 | 0.77 |
| *E. coli* | 9.63 | 0.28 | 10.36 | 9.70 | 0.60 | 9.85 | 9.25 | 0.60 | 10.34 | 9.57 | 0.60 | 10.33 | 9.65 | 0.52 | 10.22 | 9.542 | 0.289 | 0.11 | 0.83 | 1.00 |
| *Clostridium perfringens* | 9.14 | 0.35 | 8.93 | 9.62 | 0.31 | 9.01 | 8.36 | 0.31 | 9.08 | 8.91 | 0.31 | 8.68 | 8.96 | 0.27 | 8.925 | 8.961 | 0.152 | 0.87 | 0.29 | 0.21 |
| *Lactobacilli* | 6.76 | 0.78 | 5.46 | 6.39 | 0.48 | 9.85 | 11.26 | 0.83 | 9.44 | 10.71 | 0.83 | 7.94 | 9.10 | 0.42 | 8.173 | 9.365 | 0.35 | 0.02 | <0.001 | 0.98 |
| Jejunum | | | | | | | | | | | | | | | | | | | | |
| *Bifidobacteria* | 8.47 | 0.36 | 5.91 | 8.18 | 0.88 | 5.75 | 4.94 | 0.99 | 6.48 | 7.23 | 0.88 | 5.84 | 5.68 | 0.81 | 5.996 | 6.507 | 0.674 | 0.30 | 0.07 | 0.16 |
| *E. coli* | 7.70 | 0.77 | 5.59 | 5.96 | 0.92 | 6.29 | 5.13 | 0.75 | 6.34 | 5.25 | 0.92 | 4.96 | 4.79 | 0.65 | 5.795 | 5.283 | 0.407 | 0.38 | 0.51 | 0.75 |
| *Clostridium perfringens* | 5.54 | 0.35 | 4.72[a] | 6.77[b] | 0.46 | 5.67 | 5.69 | 0.56 | 5.73[c] | 4.68[d] | 0.46 | 5.72 | 5.35 | 0.40 | 5.462 | 5.622 | 0.257 | 0.62 | 0.64 | 0.02 |
| *Lactobacilli* | 8.12 | 0.43 | 4.20 | 4.41 | 0.40 | 9.07 | 3.58 | 0.54 | . | 10.26 | 0.54 | 4.73 | 4.74 | 0.35 | . | 5.747 | 0.297 | 0.19 | <0.001 | 0.39 |
| Colon | | | | | | | | | | | | | | | | | | | | |
| *Bifidobacteria* | 10.38 | 0.41 | 10.34 | 10.89 | 0.46 | 10.10 | 10.63 | 0.56 | 11.26 | 11.35 | 0.46 | 10.66 | 10.44 | 0.40 | 10.59 | 10.83 | 0.238 | 0.49 | 0.26 | 0.80 |
| *E. coli* | 9.90 | 0.25 | 9.19 | 9.20 | 0.63 | 8.79 | 8.57 | 0.63 | 8.71 | 9.21 | 0.63 | 8.34 | 8.92 | 0.55 | 8.756 | 8.978 | 0.334 | 0.62 | 0.78 | 0.90 |
| *Clostridium perfringens* | 8.55 | 0.33 | 7.62 | 8.10 | 0.61 | 7.93 | 7.75 | 0.61 | 9.49 | 9.68 | 0.61 | 9.19 | 9.48 | 0.55 | 8.557 | 8.754 | 0.414 | 0.58 | 0.003 | 0.92 |
| *Lactobacilli* | 8.66 | 0.51 | 7.79 | 7.71 | 0.95 | 10.18 | 11.37 | 1.41 | 10.92 | 11.80 | 1.41 | 9.12 | 9.69 | 0.95 | 9.503 | 10.14 | 0.804 | 0.39 | 0.03 | 0.91 |

[1] Data expressed as $\log_{10}$ CFU/g sample on a DM basis

[2] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[3] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[4] CON=Control diet

[5] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.005$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.12$).

**Table 3.7.** Least squares means and associated standard errors for weekly measurements of reticulo-rumen (RR).

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 24.67 | 1.15 | 26.50 | 31.84 | 2.18 | 29.84 | 30.84 | 2.18 | 32.00 | 33.84 | 2.18 | 33.75 | 33.10 | 1.97 | 30.52 | 32.40 | 1.47 | 0.15 | 0.07 | 0.39 |
| Weight, g | 182.6 | 6.4 | 183.5 | 238.5 | 25.3 | 248.9 | 210.8 | 25.3 | 242.8 | 312.7 | 25.3 | 255.2 | 280.2 | 22.4 | 232.6 | 260.6 | 15.0 | 0.09 | 0.03 | 0.13 |
| G RR per kg BW | 3.73 | 0.10 | 4.14 | 5.17 | 0.40 | 5.39 | 4.82 | 0.40 | 4.87 | 5.80 | 0.40 | 4.74 | 5.12 | 0.35 | 4.79 | 5.23 | 0.20 | 0.12 | 0.39 | 0.20 |
| Density (g/cm) | 7.46 | 0.32 | 6.92 | 7.48 | 0.47 | 8.28[c] | 6.81[d] | 0.47 | 7.61[e] | 9.18[f] | 0.47 | 7.61[g] | 8.46[h] | 0.41 | 7.61 | 7.98 | 0.23 | 0.25 | 0.08 | 0.03 |
| cm RR per kg BW | 0.49 | 0.04 | 0.60 | 0.69 | 0.04 | 0.65 | 0.70 | 0.04 | 0.64 | 0.63 | 0.04 | 0.63 | 0.61 | 0.04 | 0.63 | 0.66 | 0.02 | 0.31 | 0.49 | 0.50 |
| (g/cm)/ kg BW | 0.15 | 0.01 | 0.16 | 0.16 | 0.01 | 0.18 | 0.16 | 0.01 | 0.15 | 0.17 | 0.01 | 0.14 | 0.16 | 0.01 | 0.16 | 0.16 | 0.00 | 0.59 | 0.18 | 0.15 |

* Baseline values are shown for informational purposes and are not included in the statistical analyses

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[c,d,e,f] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

[g,h] Subcolumn means within row and week category with different superscripts differ *(* $P = 0.14$).

**Table 3.8.** Least squares means and associated standard errors of weekly omasal (OM) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 6.83 | 0.21 | 6.58 | 6.67 | 0.59 | 9.17 | 6.83 | 0.59 | 7.33 | 7.33 | 0.59 | 7.13 | 7.15 | 0.51 | 7.552 | 6.996 | 0.283 | 0.18 | 0.16 | 0.13 |
| Weight, g | 68.47 | 3.85 | 59.47 | 63.6 | 8.64 | 83.27 | 66.93 | 8.64 | 80.83 | 71.1 | 8.64 | 78.48 | 75.52 | 7.48 | 75.51 | 69.29 | 4.182 | 0.3 | 0.25 | 0.67 |
| g OM per kg BW | 1.4 | 0.07 | 1.37 | 1.38 | 0.14 | 1.81 | 1.53 | 0.14 | 1.64 | 1.35 | 0.14 | 1.44 | 1.37 | 0.12 | 1.568 | 1.407 | 0.068 | 0.11 | 0.16 | 0.61 |
| Density (g/cm) | 10.03 | 0.52 | 9.05 | 9.42 | 1.11 | 9.47 | 9.74 | 1.11 | 11.08 | 9.74 | 1.11 | 10.96 | 10.62 | 0.96 | 10.14 | 9.881 | 0.538 | 0.74 | 0.43 | 0.86 |
| Cm OM per kg BW | 0.14 | 0 | 0.15 | 0.15 | 0.01 | 0.2 | 0.16 | 0.01 | 0.15 | 0.14 | 0.01 | 0.13 | 0.13 | 0.01 | 0.158 | 0.144 | 0.006 | 0.11 | 0.01 | 0.28 |
| (g/cm)/ kg BW | 0.21 | 0.01 | 0.21 | 0.21 | 0.02 | 0.21 | 0.22 | 0.02 | 0.23 | 0.19 | 0.02 | 0.2 | 0.19 | 0.02 | 0.211 | 0.202 | 0.011 | 0.53 | 0.86 | 0.64 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Table 3.9.** Least squares means and associated standard errors of weekly abomasal (AB) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 35.3 | 4.2 | 31.2 | 36.7 | 1.7 | 28.8 | 29.7 | 1.7 | 35.7 | 33.2 | 1.7 | 32.9 | 34.6 | 1.4 | 32.1 | 33.5 | 0.8 | 0.23 | 0.02 | 0.15 |
| Weight, g | 277.6 | 11.6 | 224.1 | 257.2 | 21.4 | 254.7 | 231.0 | 21.4 | 239.8 | 254.7 | 21.4 | 240.5 | 263.6 | 18.9 | 239.8 | 251.6 | 12.5 | 0.39 | 0.92 | 0.50 |
| g AB per kg BW | 5.58 | 0.25 | 5.07 | 5.55 | 0.36 | 5.53 | 5.26 | 0.36 | 4.81 | 4.74 | 0.36 | 4.46 | 4.86 | 0.31 | 4.97 | 5.10 | 0.17 | 0.59 | 0.09 | 0.65 |
| Density (g/cm) | 7.28 | 0.39 | 7.21 | 7.05 | 0.69 | 8.83 | 7.93 | 0.69 | 7.65 | 6.76 | 0.69 | 7.36 | 7.75 | 0.61 | 7.54 | 7.60 | 0.40 | 0.89 | 0.23 | 0.54 |
| Cm AB per kg BW | 0.72 | 0.12 | 0.72 | 0.81 | 0.05 | 0.63 | 0.69 | 0.05 | 0.72 | 0.63 | 0.05 | 0.61 | 0.63 | 0.04 | 0.67 | 0.69 | 0.02 | 0.65 | 0.05 | 0.35 |
| (g/cm)/ kg BW | 0.16 | 0.01 | 0.16 | 0.15 | 0.01 | 0.19 | 0.18 | 0.01 | 0.14 | 0.14 | 0.01 | 0.14 | 0.15 | 0.01 | 0.16 | 0.15 | 0.01 | 0.94 | 0.004 | 0.49 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Table 3.10.** Least squares means and associated standard errors of duodenal (DU) growth parameters.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | T | W | T*W |
| Length, cm | 30.2 | 30.6 | 2.1 | 0.89 | 0.15 | 0.45 |
| Weight, g | 50.6 | 67.9 | 8.2 | 0.03 | 0.83 | 0.83 |
| g DU per kg BW | 1.05 | 1.38 | 0.16 | 0.02 | 0.73 | 0.78 |
| Density (g/cm) | 1.80 | 2.22 | 0.28 | 0.06 | 0.25 | 0.94 |
| Cm DU per kg BW | 0.64 | 0.63 | 0.05 | 0.86 | 0.03 | 0.64 |
| (g/cm)/ kg BW | 0.04 | 0.05 | 0.01 | 0.06 | 0.27 | 0.74 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week Interaction

[2] CON=Control diet.

[3] PSY= Psyllium-supplemented diet

**Table 3.11.** Least squares means and associated standard errors of weekly jejunal (JE) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 1633.6 | 65.4 | 1573.8 | 1756.5 | 126.0 | 1683.5 | 1399.0 | 126.0 | 1796.8 | 1448.1 | 126.0 | 1731.5 | 1573.8 | 109.1 | 1686.4 | 1542.1 | 61.0 | 0.09 | 0.76 | 0.19 |
| Weight, g | 1028.9 | 99.1 | 893.3 | 955.1 | 102.3 | 936.0 | 968.4 | 102.3 | 900.8 | 1128.4 | 102.3 | 953.8 | 1081.6 | 88.6 | 921.0 | 1033.4 | 49.5 | 0.12 | 0.72 | 0.78 |
| g JE per kg BW | 19.65 | 0.42 | 20.28 | 20.75 | 1.39 | 20.36 | 22.30 | 1.39 | 18.18 | 21.23 | 1.39 | 17.55 | 19.77 | 1.20 | 19.09 | 21.01 | 0.67 | 0.06 | 0.22 | 0.82 |
| Density (g/cm) | 0.65 | 0.09 | 0.57 | 0.54 | 0.04 | 0.55[a] | 0.69[b] | 0.04 | 0.49[c] | 0.78[d] | 0.04 | 0.55[e] | 0.69[f] | 0.03 | 0.54 | 0.68 | 0.02 | <0.001 | 0.10 | 0.004 |
| Cm JE per kg BW | 32.40 | 3.02 | 36.02 | 38.45 | 1.66 | 36.70[g] | 32.24[h] | 1.66 | 36.24[i] | 27.35[j] | 1.66 | 31.85[k] | 28.66[j] | 1.44 | 35.20 | 31.67 | 0.81 | 0.01 | 0.001 | 0.03 |
| (g/cm)/kg BW | 0.01 | 0.00 | 0.01 | 0.01 | 0.00 | 0.01[m] | 0.02[n] | 0.00 | 0.01[o] | 0.02[p] | 0.00 | 0.01[q] | 0.01[r] | 0.00 | 0.01 | 0.01 | 0.00 | <0.001 | 0.02 | 0.01 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a, b, c , d ,l,j,m,n,o,p,q,r,e,f] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

[g,h] Subcolumn means within row and week category with different superscripts differ ( $P < 0.10$).

[k,l] Subcolumn means within row and week category with different superscripts differ *(* $P < 0.15$).

**Table 3.12.** Least squares means and associated standard errors of ileum (IE) growth parameters.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | T | W | T*W |
| Length, cm | 58.9 | 70.1 | 11.9 | 0.51 | 0.45 | 0.99 |
| Weight, g | 52.5 | 67.5 | 9.4 | 0.27 | 0.46 | 0.48 |
| g IE per kg BW | 1.12 | 1.39 | 0.21 | 0.37 | 0.70 | 0.43 |
| Density (g/cm) | 0.92 | 1.12 | 0.12 | 0.08 | 0.09 | 0.40 |
| Cm IE per kg BW | 1.26 | 1.47 | 0.28 | 0.59 | 0.38 | 0.99 |
| (g/cm)/ kg BW | 0.02 | 0.02 | 0.00 | 0.15 | 0.23 | 0.30 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W= treatment x week interaction

[2] CON=Control diet

[3] PSY= Psyllium-supplemented diet

**Table 3.13.** Least squares means and associated standard errors of colon (COL) growth parameters.

| | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | T | W | T*W |
| Length, cm | 191.3 | 184.1 | 12.6 | 0.51 | 0.16 | 0.67 |
| Weight, g | 407.9 | 467.3 | 18.6 | 0.04 | 0.84 | 0.49 |
| g COL per kg BW | 8.43 | 9.45 | 0.45 | 0.01 | 0.008 | 0.21 |
| Density (g/cm) | 2.19 | 2.58 | 0.21 | 0.06 | 0.21 | 0.93 |
| Cm COL per kg BW | 3.98 | 3.74 | 0.22 | 0.41 | 0.08 | 0.77 |
| (g/cm)/ kg BW | 0.05 | 0.05 | 0.00 | 0.04 | 0.01 | 0.61 |

[1] *P*-value. T= effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON=Control diet

[3] PSY= Psyllium-supplemented diet

# CHAPTER 4

## Effects of inclusion of psyllium in milk replacer on ion transport and histomorphological measures in the gastrointestinal tract of neonatal dairy calves

### Introduction

Optimal health and growth of dairy calves is an important determinant of dairy farm profitability. The majority (56.5%) of unweaned heifer deaths can be attributed to scours (diarrhea) or other digestive challenges (National Animal Health Monitoring System, 2007). Therefore, efforts to improve gastrointestinal tract function and disease resistance should lead to improved welfare and growth performance. Psyllium has been studied in many species for its potential effects on digestive function, rate of passage, nutrient absorption, and intestinal morphology. A predominant role of dietary fiber, particularly viscous fermentable fibers, in the stomach and small intestine is to limit the rate of release of nutrients by increasing the viscosity of the digesta, thereby reducing the rate of nutrient transport to the epithelium (Morris, 2001). As a viscous fiber, psyllium would be expected to slow the rate of glucose absorption. For example, glucose absorption decreased in type 2 diabetic patients when psyllium was consumed (Sierra *et al.*2002). Improvement in glucose tolerance from consumption of viscous fiber is thought to be more likely due to slower absorption of carbohydrate rather than decreased absorption (Sierra *et al.*, 2002). It could be postulated, therefore, that changes in release of absorbable nutrients in the intestine might alter expression or function of nutrient transporters.

As a fermentable fiber, psyllium would be expected to favor intestinal anaerobic bacteria with the capacity to ferment it. Increased fermentation in the lower tract results in greater concentrations of VFA, which in turn increase mass of the gastrointestinal tract in other species (Leng-Peschlow, 1991; Edwards *et al.* 1992; Schneeman and Richter, 1993; Edwards *et al.* 2003). The VFA also have been shown to stimulate intestinal villus length and crypt depth (Kuzmuk et al., 2005) and increase rates of nutrient transport in the small intestine (Tappenden *et al.,* 2003).

Butyrate is the VFA most responsible for stimulation of rumen epithelial development and differentiation, with propionate being the second most stimulatory (Heinrichs, 2005). Therefore, an increase in production of VFA as a result of inclusion of psyllium in milk replacer could potentially stimulate ruminal papillae development, as well as villi development throughout the lower gastrointestinal tract. Minimal changes in ruminal papillae growth and development would be expected when psyllium is supplemented to milk replacer, however, because of the esophageal groove closure that allows most milk replacer to bypass the rumen. However, if there is greater fermentation and production of VFA in the lower gastrointestinal tract, VFA that enter the bloodstream could impact rumen papillae growth and development via systemic effects.

In newborn calves less than 3 d old, the papillae of the rumen are partially developed. However, if the calf receives a diet of only milk or milk replacer, the papillae will show regressive changes, both in length and shape (Tamate *et al.*, 1962). This regression of growth is theorized to result from the lack of VFA reaching the rumen from the milk diet, because of esophageal groove closure. Rumen development present in newborn calves can be attributed to VFA in the maternal blood, which supplies the fetal

rumen tissue with VFA that stimulate papillae growth and development (Tamate *et al.*, 1962). In a similar manner, therefore, VFA present in the calf's blood from lower gastrointestinal fermentation and absorption could impact ruminal papillae development.

The potential impact of psyllium on nutrient transport and gastrointestinal histomorphology in young dairy calves is not known. My hypothesis was that psyllium would slow the rate of glucose and amino acid availability in the intestine, and as a result there would be alterations in rates of nutrient transport that could be measured in vitro. Furthermore, VFA from fermentation of psyllium in the lower gut would lead to enhanced villus size and crypt depth and to greater rates of nutrient transport throughout the digestive tract, which in turn might improve digestive function and gut health. The purpose of this portion of the study, therefore, was to determine the effects of inclusion of psyllium in milk replacers on ion transport measures and histomorphological development in the gastrointestinal tract of neonatal dairy calves.

## Materials and Methods

### Animal Care

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol number 04083).

The study was conducted during two periods, the first (May 2004) using 12 calves and the second (June 2004) using 22 calves. Male Holstein calves were purchased from Stone Ridge Dairy (Belleflower, IL) at less than 36 h of age.

At the beginning of the trial period, daily late-afternoon trips were made to Stone Ridge Dairy to select and purchase calves. At Stone Ridge Dairy, all male Holstein

calves less than 36 h of age in the calf pack were evaluated for potential purchase by trial personnel. The evaluation included body temperature, heart and lung auscultation, hydration status, mobility, navel status, and overall thriftiness. Also, the calves must have received at least one feeding of colostrum by Stone Ridge personnel. Calves deemed satisfactory were then transported to the University of Illinois Dairy Nutrition Field Laboratory.

Upon arrival, each calf received an eartag in the left ear for identification purposes. Calves were then measured for withers height, body length, heart girth circumference, and body weight. Ear notches were taken from each calf and placed in formalin for determination of persistently infected bovine viral diarrhea (PI-BVD) status. Each calf was vaccinated against infectious bovine rhinotracheitis virus and parainfluenza-3 intranasally (2 ml of TSV-2; Pfizer Animal Health, Exton, PA). In addition, calves received 15 mL of Quatracon-2X™ Antiserum (Boehringer Ingleheim Vetmedica, Inc.; St. Joseph, MO) subcutaneously in the neck. Each calf also received 2 mL of Excenel (Pfizer Animal Health; Exton, PA) subcutaneously in the neck.for the first 3 d of the study. After entry injections, the calves were moved to southward-facing individual calf hutches (Calf-Tel; Hampel Corp., Germantown, WI) on 15 to 20 cm of crushed limestone located on the south side of the Dairy Nutrition Field Laboratory. No bedding was used, to minimize ingestion of bedding material that might confound treatment inferences.

**Assignment to Treatments**

Calves were blocked by pairs based upon birth date, body weight, and total protein score in pairs and then randomly assigned within pair to each of the dietary

treatments. These pairs of calves were then randomly assigned to harvest week. Of the 34 calves assigned to treatments, 1 calf died at 2 d of age. Necropsy results (University of Illinois College of Veterinary Medicine, Urbana, IL) indicated that the cause of mortality was likely related to birth trauma. All other calves completed the trial in good health.

**Feeding**

On the night of arrival, each calf was offered colostrum for ad libitum intake. On the morning following arrival, each calf again was offered colostrum. Treatment feedings were administered at 0600 and 1800 h. Calves were fed milk replacers reconstituted to 12.5% dry matter at a rate of 12% of BW daily, adjusted weekly as calves grew. Milk replacers (Land O'Lakes Animal Milk Products Co.; Arden Hills, MN) with or without a 1.1% inclusion of psyllium were formulated to contain 22% protein and 20% fat, and contained only milk proteins. Neither milk replacer contained growth-promoting antibiotics. Water was available to calves for ad libitum consumption, with fresh warm water provided twice daily after each milk replacer feeding. No other feeds were offered.

**Health Monitoring**

Calves were observed at least twice daily for general health, including appearance (alertness), appetite (ability to consume feed), and fecal scores. Fecal scores were recorded daily, using the following guidelines: 1 = dry, hard; 2 = soft, formed; 3 = pudding-like; 4 = mix of liquids with some solids; and 5 = liquid. If the calf's hydration status was low (skin tenting evident), 500 mL Lactated Ringers Solution (Abbott Laboratories; Abbott Park, IL) was administered subcutaneously. If a calf appeared unhealthy, 3 mL of Excenel (Pfizer, Exton, PA) was administered subcutaneously for a

period of 3 d. If body temperature was over 39° C, 1 mL of Banamine (Schering-Plough Animal Health; Union, NJ) was administered subcutaneously. Calves received a booster of TSV-2 at 2 wk of age.

**Calf Harvest Procedure**

Pairs of calves were harvested for analysis weekly. For period 1, 2 calves were euthanized after arrival before experimental treatments began (week zero) for baseline measurements. For the remaining weeks, one pair of calves was euthanized weekly, with the exception of week 4. In week 4 of period 1, 4 calves were euthanized. For period 2, 4 baseline calves were euthanized at week zero, and 2 pairs were euthanized during weeks 1, 2, and 3. For week 4, 5 calves were harvested due to the 1 mortality.

Calves were transported to the University of Illinois College of Veterinary Medicine at 0630 h. Calves were not given their morning milk replacer prior to euthanasia. The harvest procedure consisted of the calf being administered Xylazine HCl intramuscularly (50 mg/mL; Fort Dodge Animal Health; Fort Dodge, IA). Once the calf was sedated, it was euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus; Veterinary Laboratories Inc., Lenexa, KS) and then exsanguinated.

When the calf was declared dead, the veterinarian opened the body cavity. The gastrointestinal tract was ligated at the caudal esophagus and rectum and removed as rapidly as possible without damaging the tract. To prevent movement of digesta between compartments, the different segments of the gastrointestinal tract were identified and ligated. The gastrointestinal tract was then divided into three portions: the stomach portion consisting of rumen, reticulum, omasum, and abomasum; the small intestine,

consisting of duodenum, jejunum, and ileum; and the colon. Each portion was processed by the respective team of personnel for tissue and digesta collection.

**Tissue Collection**

Tissues for ion transport analysis were collected as soon as possible after euthanizing calves. Samples of dorsal rumen, medial jejunum, medial ileum, and medial colon were placed in oxygenated (95% $O_2$:5% $CO_2$) Krebs' solution (120 m*M* $MgCl_2$, 120 m*M* $CaCl_2$, 40 m*M* $KH_2PO_4$, 240m*M* $K_2HPO_4$ , 1.15 *M* $NaCl_2$, and 260 m*M* $NaHCO_3$) on ice. These samples were then transported to the laboratory for immediate analysis (within 30 min after euthanasia) of electrophysiological and ion transport measures.

For histomorphology, samples of dorsal rumen, caudal ventral blind sac of rumen, cranial ventral sac of rumen, medial jejunum, medial ileum, and medial colon were placed in glass scintillation vials containing 10% buffered formalin. The locations for rumen sampling were those determined to give greatest accuracy in rumen development according to the methodology of Lesmeister *et al*. (2004). Samples were transferred to 70% ethanol solutions at a later date, as per procedures from Albin (2004).

**Ion Transport and Electrophysiological Response Studies**

Ion transport was evaluated in segments of the rumen, jejunum, ileum, and colon. Modified Ussing chambers were used according to methods described by Kles and Tappenden (2002). Briefly, tissue segments were mounted in modified Ussing chambers (Physiologic Instruments; San Diego, CA), exposing 0.5 $cm^2$ of mucosa and serosa. The tissue segments were bathed in oxygenated modified Krebs' solution that was maintained at 37°C with a circulating water bath (Fisher Scientific). Basal short-circuit current ($I_{sc}$;

indicator of non-specific active ion transport), resistance (R; intestinal barrier function), and potential difference (Pdo; total ion transport) were established and measured during an initial stabilization period of less than 30 min. The $I_{sc}$ was calculated according to Ohm's law, using I = V/R, where I is the current in amperes, V is the electromotive force in volts, and R is resistance in ohms.

Sodium-dependent glucose transport was measured by changes in short-circuit current induced by addition of 10 m*M* D-glucose to the mucosal medium. The modified Ussing chambers were connected to dual-channel voltage clamps (VCC MC2, Physiologic Instruments) connected with data acquisition software (Acquire & Analyze; Physiological Instruments, San Diego, CA) to allow real-time data collection. Peak $I_{sc}$ values (point of maximal change) were measured after each nutrient was added, and the difference between basal levels (measured prior to each nutrient addition) and peak measure was calculated.

Transport of glycylsarcosine, a synthetic dipeptide with methylated peptide bond for increased stability, was measured by addition of glycylsarcosine (to a final dilution of 10 m*M*) to the mucosal Ussing chamber, in the same manner as described for measurement of glucose transport. Amino acid transport (L-glutamine, L-proline, L-arginine, and L-threonine) was measured in the same way as described for glucose transport. Each amino acid was added to the mucosal side of the Ussing chambers to a final concentration of 10 m*M*.

**Histomorphology**

The formalin-fixed rumen and intestinal samples were embedded in paraffin, sliced to approximately 5-μm thickness and stained with hematoxylin and eosin

(University of Illinois Clinical Pathology Laboratory, Urbana, IL). Measurements of papillae length, mid-papillae width, and papillae density were made for the dorsal rumen, caudal ventral blind sac of rumen, and cranial ventral sac of rumen. Villus width, mid-villus height, and crypt depth were measured in samples from the medial jejunum and medial ileum. Crypt depths were measured in the medial colon. Papillae density was recorded as the number of papillae per millimeter of serosal length. All histomorphological measurements were made by using a Nikon Optiphot-2 microscope (Nikon; Melville, NY) and Image-Pro Express software (Version 4.5; Media Cybernetics, Inc., Silver Springs, MD). To determine average measurements, 8 to 10 papillae, villi, or crypts were measured. Mucosal surface area was estimated as (villus height × mid-villus width × villus density). The technician was blinded to nutritional treatment and age of the calf.

**Statistical Analysis**

Statistical analyses were conducted utilizing the Proc Mixed procedure of SAS (SAS version 9.1; SAS Institute Inc.; Cary, NC). The data were analyzed as a randomized complete block design, with period as a random effect. Effects of diet, week, and the interaction of diet and week were included in the model as fixed effects. Baseline calves were not included in the analysis, but means and standard errors are shown for comparison. Significant differences were declared at $P < 0.05$, and trends toward significant effects were noted at $P < 0.15$.

## Results and Discussion

### Electrophysiological and Transport Measurements

No significant differences were observed for $I_{sc}$ values between diets in any of the gastrointestinal tract locations (Table 4.1). However, a significant treatment × week interaction ($P < 0.03$) was noted in the rumen with PSY being higher than CON at wk 1. The $I_{sc}$ values in the ileum tended ($P = 0.12$) to be lower for PSY calves. Higher $I_{sc}$ values (increased short circuit current) would indicate greater ion secretion and active transport. Thus, the possibility exists that psyllium supplementation altered ion secretion and active transport levels at least early in life, although this should be confirmed with additional research.

No significant differences between treatments were observed for resistance values (R) in the gastrointestinal tract locations measured (Table 4.2). Increased R values generally reflect greater barrier function of the gastrointestinal tissue. The lack of differences indicates that psyllium supplementation had no major effect on gut barrier function. Means were greater during the treatment period than in baseline calves indicating an improvement in barrier function as calves grew, although the effect of week within the treatment period was not significant.

No significant differences between treatments were observed for Pdo values in any of the gastrointestinal tract locations (Table 4.3). However, a significant interaction of treatment and week was detected for rumen tissue, in which the PSY treatment was significantly greater at wk 1 ($P < 0.01$). Greater Pdo values indicate greater total ion

transport, which means that psyllium may have increased total ion transport in the rumen during early life but had little difference thereafter.

Diet significantly affected the change in short circuit current induced by addition of 10 mM glucose (∆ glucose) observed with CON calves exhibiting greater ($P < 0.01$) ∆ glucose transport than PSY calves in rumen, ileum, and colon (Table 4.4). In addition, the treatment × week interaction was significant for rumen and colon, in which differences between diets were largest for calves measured at wk 1. These data may be indicative of psyllium slowing the rate of glucose absorption, but not necessarily the quantity of glucose absorption. These data may support the blood glucose data reported in Chapter 2, in which higher blood glucose concentrations were observed pre-prandially for calves in the PSY treatment. Specifically, a slower rate of glucose absorption into the bloodstream could lead to less marked changes in insulin secretion to dispose of the glucose. However, the reduced rates of glucose transport for calves in the PSY treatment contradict research in other monogastric species. For example, in rats undergoing total parenteral nutrition, mucosal glucose uptake was increased when VFA were supplemented (Tappenden *et al.*, 2003).

There were no significant differences between CON and PSY treatments for change in short circuit current induced by addition of 10 mM glutamine (∆ glutamine; Table 4.5). However, a significant treatment × week interaction was noted in the ileum; at wk 2 values for the CON treatment were significantly higher ($P < 0.05$), whereas a tendency was noted at wk 3 for the PSY treatment to be higher ($P < 0.10$).

No effect of treatment was detected for change in short circuit current induced by addition of 10 mM glycylsarcosine (∆ glycylsarcosine) among gastrointestinal tract

locations (Table 4.6). The effect of week was significant for rumen (data not shown) in which means were higher for wk 2.

No effect of treatment was noted for change in short circuit current induced by addition of 10 mM proline (Δ proline) in any of the gastrointestinal tract locations (Table 4.7). A tendency for an effect of week was noted in the rumen ($P < 0.09$) with means being elevated at wk 2 and 3 compared to wk 1 and 4.

No significant effects of treatment were noted for change in short circuit current induced by addition of 10 mM arginine (Δ arginine) transport in any gastrointestinal tract location (Table 4.8). However, a significant ($P < 0.003$) treatment × week interaction was noted in the rumen, with PSY being higher at wk 1 ($P < 0.05$) and CON being higher at wk 2 ($P < 0.001$). Furthermore, a significant effect of week ($P < 0.004$) was noted in the rumen, with wk 4 means being lower than wk 1, 2, and 3. The biological significance and implications are unclear.

Although no significant effects of treatment were observed for change in short circuit current induced by addition of 10 mM threonine (Δ threonine transport; Table 4.9), a significant treatment × week interaction was noted in the rumen ($P < 0.001$). At wk 1, the PSY treatment exhibited significantly greater ($P < 0.01$) Δ threonine transport when compared to the CON treatment.

Overall, few significant differences were noted between treatments for electrophysiological and ion transport measures; changes were only noted for $I_{sc}$ and Pdo values in the rumen at wk 1 (with calves fed PSY being higher), in threonine and arginine transport in the rumen at wk 1 (PSY calves higher), and in glucose transport in the rumen, ileum, and colon ( $P < 0.01$), with the CON treatment exhibiting greater rates of glucose

transport. These latter data potentially indicate an adaptation at the cellular level for glucose transport to be slower when psyllium is included in the diet. However, this result does not agree with expectations based on previous research and the mechanism responsible is not known. The VFA, and most specifically butyrate, are responsible for rumen epithelial growth and development (Heinrichs, 2005). Furthermore, physiological concentrations of butyrate have been shown to upregulate expression of key enterocyte-associated nutrient transporters (Tappenden *et al.*, 2003). Therefore, it is logical that the increased production of VFA from inclusion of psyllium in milk replacer (see Chapter 3) would have increased absorptive rates of glucose and possibly other substrates tested as observed in studies with other animal models (Tappenden *et al.,* 2003), but this was not the case. The significantly greater glucose transport observed in the rumen of the CON calves indicates that the effect was mediated systemically, because the milk replacer would have mostly bypassed the rumen. Further research is needed to determine the mechanism for these effects in neonatal dairy calves.

**Histomorphology of Gastrointestinal Tract**

No significant effect of treatment was observed for histomorphological measures in the rumen (Table 4.10). However, a significant effect of week was noted ($P < 0.01$) for papillae length in the cranial ventral sac, being highest at wk 2. Furthermore, a significant ($P < 0.05$) effect of week was noted for papillae length in the caudal ventral blind sac, with calves at wk 3 exhibiting the lowest values. Papillae development in the rumen was minimal over the course of the study, which was expected because calves did not have access to starter (Davis and Drackley, 1998). In fact, papillae length decreased in the cranial ventral sac ($P < 0.01$) and caudal ventral blind sac ($P < 0.05$).

Measurements of villus height, villus width, and crypt depth did not differ in jejunum, regardless of treatment, age, or their interaction (Table 4.11). In the colon (Table 4.12), dietary treatments did not affect crypt depth or width. However, there was a tendency ($P < 0.07$) toward an effect of week for colon crypt depth, with depths decreasing for wk 1, 2, and 3 but increasing at wk 4.

The lack of many significant differences noted for histomorphological measures could be attributed to the lack of ingestion of dry feed, thereby minimizing microbial end products to enhance ruminal papillae growth and development. Perhaps the amount of psyllium included in the milk replacer was not sufficient to elevate VFA concentrations enough to cause general changes in histomorphology in the jenunum and crypt elongation in the colon. However, numbers of calves were small and further research in this area would be useful to validate whether or not psyllium inclusion in milk replacers affects gastrointestinal histomorphology.

## Conclusions

The significant differences in glucose transport observed in this study were opposite to predictions and are intriguing. This effect would be expected to indicate a cellular level adaptation mediated via systemic effects, and is an area that needs further research. Regarding histomorphological measures, inclusion of PSY did not affect papillae development in the rumen, villus or crypt size in the small intestine, or crypt depth in the colon. This result also contradicts previous data in other species. A larger dataset, perhaps with higher rates of psyllium inclusion, would be useful to draw a more complete conclusion.

## References


Albin, D. M. 2004. Short-chain fatty acids and functional adaptations in the neonatal intestine. Ph.D. Diss., Univ. Illinois, Urbana.

Davis, C. L., and J. K. Drackley. 1998. The Development, Nutrition, and Management of the Young Calf. Iowa State University Press, Ames.

Edwards, C. A., J. Bowen, W. G. Brydon, and M. A. Eastwood. 1992. The effects of ispaghula on rat caecal fermentation and stool output. Br. J. Nutr. 68:473-482.

Edwards, S., M. F. Chaplin, A. D. Blackwood, and P. W. Dettmar. 2003. Primary structure of arabinoxylans of ispaghula husk and wheat bran. Proc. Nutr. Soc. 62:217-222.

Heinrichs, A. J. 2005. Rumen development in the dairy calf. Adv. Dairy Tech. 17:179-187.

Kles, K. A. and K. A. Tappenden. 2002. Hypoxia differentially regulates nutrient transport in rat jejunum regardless of luminal nutrient present. Am. J. Physiol. Gastrointest. Liver Physiol. 283:G1336-G1342.

Kuzmuk, K. N., K. S. Swanson, K. A. Tappenden, L. B. Schook, and G. C. Fahey. 2005. Diet and age affect intestinal morphology and large bowel fermentation end-product concentrations in senior and young adult dogs. J. Nutr. 135: 1940-1945.

Leng-Peschlow, E. 1991. *Plantago ovata* seeds as dietary fibre supplement: physiological and metabolic effects in rats. Br. J. Nutr. 66:331-349.

Lesmeister, K. E., P. R. Tozer, and A. J. Heinrichs. 2004. Development and analysis of a rumen tissue sampling procedure. J. Dairy Sci. 87:1336-1344.

Morris, E. R. 2001. Assembly and Rheology of Non-Starch Polysaccharides. In: B. V. McCleary and L. Prosky (Eds.) Advanced Dietary Fibre Technology. pp. 30-41. Blackwell Science Ltd, Oxford.

National Animal Health Monitoring System. 2007. *Part 1: Reference of Dairy Cattle Health and Management Practices in the United States, 2007*. Ft. Collins, CO: USDA:APHIS:VS:CEAH

Schneeman, B. O., and D. Richter. 1993. Changes in plasma and hepatic lipids, small intestinal histology and pancreatic enzyme activity due to aging and dietary fiber in rats. J. Nutr. 123:1328-1337.

Sierra, M., J. J. Garcia, N. Fernández, M. J. Diez, A. P. Calle, and Farmafibra Group. 2002. Therapeutic effects of psyllium in type 2 diabetic patients. Eur. J. Clin. Nutr. 56:830-842.

Tappenden, K. A., D. M. Albin, A. L. Bartholome, and H. F. Mangian. 2003. Glucagon-like peptide-2 and short-chain fatty acids: a new twist to an old story. J. Nutr. 133:3717-3720.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

**Table 4.1.** Least squares means and associated standard errors for $I_{sc}$ values (mv) among gastrointestinal tract locations.

| | Week 0 | | | Week 1 | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 11.55 | 0.98 | 3.36[a] | 12.82[b] | 3.61 | 7.50 | 7.34 | 3.61 | 13.12 | 9.18 | 3.61 | 9.64 | 4.96 | 3.13 | 6.72 | 8.58 | 1.75 | 0.46 | 0.39 | 0.03 |
| Jejunum | 4.06 | 1.52 | 3.19 | 1.57 | 1.78 | 0.70 | 2.22 | 1.78 | 3.12 | 0.94 | 1.57 | 3.25 | 3.82 | 1.46 | 2.56 | 2.13 | 1.29 | 0.59 | 0.26 | 0.33 |
| Ileum | 1.54 | 0.57 | 1.54 | 0.07 | 0.95 | 1.11 | 0.42 | 0.95 | 1.50 | 0.15 | 0.95 | 1.61 | 1.90 | 0.95 | 1.44 | 0.35 | 0.48 | 0.12 | 0.47 | 0.68 |
| Colon | 2.42 | 0.92 | 0.52 | 2.73 | 2.52 | 5.42 | 3.66 | 2.52 | 1.19 | 1.24 | 2.52 | 5.13 | 3.69 | 2.18 | 3.06 | 2.83 | 1.22 | 0.89 | 0.38 | 0.84 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

**Table 4.2.** Least squares means and associated standard errors for resistance values (R; $\Omega \cdot cm^2$) among gastrointestinal tract locations.

| | Week 0 | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 121.2 | 8.7 | 180.6 | 167.4 | 16.7 | 0.58 | 0.87 | 0.31 |
| Jejunum | 128.7 | 9.0 | 103.9 | 123.5 | 22.4 | 0.30 | 0.84 | 0.32 |
| Ileum | 169.5 | 18.1 | 193.2 | 203.6 | 12.9 | 0.50 | 0.80 | 0.70 |
| Colon | 126.9 | 1.3 | 137.0 | 123.5 | 7.6 | 0.18 | 0.14 | 0.26 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.20$).

**Table 4.3.** Least squares means and associated standard errors of Pdo (mv) values among locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 1.38 | 0.09 | -0.28[a] | 2.90[b] | 0.78 | 1.46 | 0.97 | 0.78 | 2.07 | 1.67 | 0.78 | 1.82 | 0.65 | 0.67 | 1.27 | 1.55 | 0.38 | 0.60 | 0.80 | 0.04 |
| Jejunum | 0.48 | 0.16 | 0.33 | 0.25 | 0.24 | 0.13 | 0.23 | 0.24 | 0.32 | 0.12 | 0.21 | 0.53 | 0.26 | 0.20 | 0.33 | 0.21 | 0.18 | 0.29 | 0.45 | 0.65 |
| Ileum | 0.25 | 0.10 | 0.27 | 0.01 | 0.20 | 0.22 | -0.14 | 0.20 | 0.31 | 0.01 | 0.20 | 0.31 | 0.42 | 0.20 | 0.28 | 0.08 | 0.10 | 0.17 | 0.43 | 0.62 |
| Colon | 0.30 | 0.11 | 0.05 | 0.28 | 0.28 | 0.92 | 0.37 | 0.28 | 0.13 | 0.14 | 0.28 | 0.77 | 0.22 | 0.24 | 0.47 | 0.25 | 0.14 | 0.28 | 0.22 | 0.40 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

**Table 4.4.** Least squares means and associated standard errors for □□glucose transport among gastrointestinal tract locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.24 | 0.15 | 1.55[a] | 0.31[b] | 0.34 | 0.57 | 0.20 | 0.34 | 0.29 | 0.27 | 0.34 | 0.70 | 0.65 | 0.32 | 0.78 | 0.36 | 0.28 | 0.01 | 0.03 | 0.03 |
| Jejunum | 0.84 | 0.41 | 0.06 | 0.24 | 0.32 | 0.32 | 0.05 | 0.32 | 0.17 | 0.67 | 0.26 | 0.10 | 0.31 | 0.22 | 0.16 | 0.32 | 0.14 | 0.42 | 0.73 | 0.59 |
| Ileum | 0.18 | 0.16 | 1.01 | 0.15 | 0.15 | 0.64 | 0.16 | 0.15 | 0.64 | 0.34 | 0.15 | 0.24 | 0.07 | 0.15 | 0.63 | 0.18 | 0.08 | <0.001 | 0.05 | 0.15 |
| Colon | 0.26 | 0.06 | 1.84[c] | -0.01[d] | 0.31 | 0.54 | 0.28 | 0.31 | 0.31 | 0.19 | 0.31 | 0.61[e] | -0.06[f] | 0.27 | 0.82 | 0.11 | 0.16 | 0.003 | 0.13 | 0.04 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b,c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.001$).

[e,f] Subcolumn means within row and week category with different superscripts differ ( $P < 0.10$).

**Table 4.5.** Least squares means and associated standard errors for □□glutamine transport among gastrointestinal tract locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.90 | 0.64 | 0.49 | 0.61 | 0.46 | 1.17 | 0.90 | 0.46 | 0.15 | 1.67 | 0.46 | 0.65 | 0.93 | 0.41 | 0.62 | 1.03 | 0.27 | 0.18 | 0.71 | 0.22 |
| Jejunum | 0.47 | 0.23 | 0.08 | 0.69 | 0.38 | 0.18 | 0.10 | 0.38 | 0.45 | 0.56 | 0.31 | 0.35 | 0.36 | 0.28 | 0.26 | 0.43 | 0.21 | 0.43 | 0.68 | 0.67 |
| Ileum | 0.19 | 0.17 | 0.55[a] | 0.91[b] | 0.18 | 0.64[c] | 0.04[d] | 0.18 | 0.36[e] | 0.83[f] | 0.18 | 0.38 | 0.23 | 0.18 | 0.48 | 0.50 | 0.09 | 0.87 | 0.08 | 0.03 |
| Colon | 0.98 | 0.33 | 0.59 | 0.00 | 0.34 | 0.75 | 0.00 | 0.34 | 0.76 | 0.92 | 0.34 | 0.48 | 0.17 | 0.29 | 0.65 | 0.27 | 0.16 | 0.13 | 0.35 | 0.57 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.20$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

[e,f] Subcolumn means within row and week category with different superscripts differ ( $P < 0.10$).

**Table 4.6.** Least squares means and associated standard errors for Δ glycylsarcosine transport among gastrointestinal tract locations.

| | Week 0 | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.13 | 0.13 | 0.39 | 0.36 | 0.20 | 0.77 | 0.04 | 0.53 |
| Jejunum | 0.04 | 0.02 | 0.16 | 0.15 | 0.05 | 0.89 | 0.23 | 0.57 |
| Ileum | 0.11 | 0.05 | 0.09 | 0.05 | 0.03 | 0.44 | 0.63 | 0.77 |
| Colon | 0.12 | 0.05 | 0.07 | 0.07 | 0.03 | 0.93 | 0.70 | 0.09 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

**Table 4.7.** Least squares means and associated standard errors for Δ proline transport among gastrointestinal tract locations.

| | Week 0 | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | T | W | T*W |
| Rumen | 0.13 | 0.10 | 0.41 | 0.34 | 0.21 | 0.70 | 0.09 | 0.18 |
| Jejunum | 0.00 | 0.00 | 0.05 | 0.04 | 0.02 | 0.93 | 0.12 | 0.18 |
| Ileum | 0.00 | 0.00 | 0.21 | 0.05 | 0.14 | 0.22 | 0.46 | 0.37 |
| Colon | 0.00 | 0.00 | 0.03 | 0.16 | 0.09 | 0.18 | 0.43 | 0.33 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY=Psyllium-supplemented diet

**Table 4.8.** Least squares means and associated standard errors for Δ arginine transport among gastrointestinal tract locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.38 | 0.28 | 0.06[a] | 0.69[b] | 0.23 | 1.46[c] | 0.34[d] | 0.23 | 0.54 | 0.19 | 0.23 | 0.12 | 0.09 | 0.21 | 0.55 | 0.33 | 0.15 | 0.12 | 0.004 | 0.003 |
| Jejunum | 0.00 | 0.00 | 0.18 | 0.18 | 0.07 | 0.02 | 0.03 | 0.07 | 0.09 | 0.04 | 0.06 | 0.00 | 0.09 | 0.05 | 0.07 | 0.08 | 0.04 | 0.78 | 0.08 | 0.50 |
| Ileum | 0.04 | 0.02 | 0.01 | 0.29 | 0.22 | 0.61 | 0.03 | 0.22 | 0.71 | 0.03 | 0.22 | 0.03 | 0.07 | 0.22 | 0.34 | 0.10 | 0.11 | 0.13 | 0.41 | 0.11 |
| Colon | 0.12 | 0.04 | 0.07 | 1.04 | 0.27 | 0.28 | 0.26 | 0.27 | 0.28 | 0.42 | 0.27 | 0.01 | 0.09 | 0.24 | 0.16 | 0.45 | 0.14 | 0.12 | 0.26 | 0.25 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.001$).

**Table 4.9.** Least squares means and associated standard errors for Δ threonine transport among gastrointestinal tract locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.21 | 0.11 | 0.040[a] | 0.72[b] | 0.19 | 1.14[c] | 0.31[d] | 0.19 | 0.20 | 0.36 | 0.19 | 0.37 | 0.27 | 0.18 | 0.44 | 0.41 | 0.15 | 0.82 | 0.02 | <0.001 |
| Jejunum | 0.04 | 0.03 | 0.10 | 0.09 | 0.12 | 0.02 | 0.00 | 0.12 | 0.11 | 0.00 | 0.10 | 0.30 | 0.06 | 0.09 | 0.13 | 0.04 | 0.05 | 0.25 | 0.35 | 0.62 |
| Ileum | 0.05 | 0.02 | 0.16 | 0.01 | 0.13 | 0.38 | 0.01 | 0.13 | 0.03 | 0.05 | 0.13 | 0.04 | 0.05 | 0.13 | 0.03 | 0.15 | 0.08 | 0.15 | 0.49 | 0.33 |
| Colon | 0.03 | 0.01 | 0.00 | 0.18 | 0.08 | 0.02 | 0.00 | 0.08 | 0.08 | -0.01 | 0.08 | 0.06 | 0.01 | 0.07 | 0.04 | 0.04 | 0.04 | 0.93 | 0.73 | 0.29 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b,c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.20$).

**Table 4.10.** Least squares means and associated standard errors for papillae length and width (µm) in the rumen

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Dorsal rumen | | | | | | | | | | | | | | | | | | | | |
| Length | 469.0 | 105.0 | 505.4 | 459.3 | 82.0 | 363.7 | 379.4 | 82.0 | 384.4 | 325.2 | 82.0 | 312.2 | 386.4 | 74.0 | 391.4 | 387.6 | 50.9 | 0.94 | 0.25 | 0.75 |
| Width | 84.0 | 12.2 | 78.0 | 92.2 | 8.4 | 98.6 | 97.2 | 8.4 | 95.1 | 98.3 | 8.4 | 84.4 | 86.2 | 7.7 | 89.0 | 93.5 | 5.9 | 0.36 | 0.12 | 0.69 |
| Cranial ventral sac | | | | | | | | | | | | | | | | | | | | |
| Length | 406.0 | 71.0 | 457.8 | 356.3 | 83.5 | 528.0 | 570.0 | 83.5 | 497.2 | 384.3 | 83.5 | 236.8 | 321.8 | 75.5 | 429.9 | 408.1 | 56.7 | 0.65 | 0.01 | 0.36 |
| Width | 60.0 | 9.1 | 67.5 | 66.8 | 9.4 | 72.0 | 82.9 | 9.4 | 90.6 | 81.0 | 9.4 | 63.6 | 82.3 | 8.3 | 73.4 | 78.3 | 5.6 | 0.42 | 0.21 | 0.34 |
| Caudal ventral blind sac | | | | | | | | | | | | | | | | | | | | |
| Length | 485.0 | 189.0 | 530.7 | 357.9 | 58.4 | 306.4 | 304.4 | 58.4 | 282.3 | 303.4 | 68.9 | 378.0 | 332.5 | 52.1 | 374.3 | 324.6 | 37.4 | 0.21 | 0.05 | 0.32 |
| Width | 66.0 | 5.9 | 80.7 | 74.4 | 5.5 | 80.6 | 74.4 | 5.5 | 69.7 | 83.7 | 6.7 | 85.8 | 90.0 | 4.8 | 79.2 | 80.6 | 2.8 | 0.73 | 0.13 | 0.32 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Table 4.11.** Least squares means and associated standard errors for villi height, villi width, and crypt depth (µm) in the jejunum

| | Week 0 | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|
| Measurement | Baseline[2] | SE | CON[3] | PSY[4] | SE | T | W | T*W |
| Villi height | 560 | 75 | 701.7 | 598.2 | 58.1 | 0.21 | 0.85 | 0.57 |
| Villi width | 99 | 16 | 101.9 | 100.2 | 9.0 | 0.84 | 0.88 | 0.60 |
| Crypt depth[a] | 251 | 52 | 249.2 | 273.7 | 29.8 | 0.61 | 0.21 | 0.16 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY=Psyllium-supplemented diet

**Table 4.12.** Least squares means and associated standard errors for crypt width and depth (μm) in the colon

| | Week 0 | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|
| Measurement | Baseline[2] | SE | CON[2] | PSY[3] | SE | T | W | T*W |
| Crypt depth | 454 | 32 | 346.9 | 312.5 | 22.0 | 0.16 | 0.07 | 0.99 |
| Crypt width | 75 | 3.9 | 58.6 | 56.7 | 3.9 | 0.67 | 0.40 | 0.49 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY=Psyllium-supplemented diet

# CHAPTER 5

## Conclusions and Implications

Overall, this trial yielded many intriguing results. The effects of psyllium inclusion at 1.1% in neonatal dairy calf milk replacers are potentially beneficial to the calf. With psyllium acting as a viscous, fermentable substrate inside the calf, there were several benefits on gastrointestinal growth and development.

The observation of increased digesta viscosity in the PSY treatment is quite logical, as psyllium is a viscous soluble fiber. The increase in digesta viscosity and increase in MRT go hand in hand. An area for further research to be done in this area would be effects of psyllium supplementation when calf starters are available. It could be hypothesized that psyllium inclusion in the milk replacer and calf starter consumption could be synergistic. There is the potential for more complete digestion of dietary components due to a slower rate of passage. Although there were no significant differences in digestibilities in this trial, the trial was not performed in a typical calf rearing system with consumption of calf starter, nor was milk replacer offered at a biologically normal rate to allow for maximum growth and performance.

Fermentation of psyllium within the gastrointestinal tract resulted in significantly higher concentrations of VFA in the proximal and distal colon, which in turn resulted in an increase in size and scale of the gastrointestinal tract. The increased size and scale of the gastrointestinal system of neonatal dairy calves could be of potential benefit to calf health from the standpoint of potentially greater absorptive capacities of the tissue, and greater tissue resistance to disease intrusion into the blood. Furthermore, inclusion of

psyllium as a fermentable substrate could potentially assist in preparing the calf for more rapid gastrointestinal growth and development once calf starter consumption begins.

The lack of significant microbial changes in the gastrointestinal tract despite the increase in VFA could be due to subtle differences or a small sample size with an assay with a larger margin of error compared to other assays utilized in this study. This is an area that merits further research with a larger sample size to determine potential effects of inclusion of psyllium in milk replacers on microbial populations of the gastrointestinal system of the neonatal dairy calf.

One of the more intriguing findings of this study was the significantly higher glucose transport rates for the control (non-supplemented) calves, with the CON calves exhibiting higher rates of glusose transport in the rumen, ileum, and colon. These data agree with the blood glucose data indicating a trend for higher blood glucose concentrations pre-prandially for the PSY calves. Therefore, it can be theorized that the rate of glucose absorption was slowed with inclusion of psyllium (ion transport data), so that glucose was absorbed over a longer period of time (blood glucose data). This theory supports the idea of viscosity slowing the rate of absorption, but contradicts research performed in other monogastric species in which elevated rates of glucose absorption were noted as a result of increased VFA absorption. However, this data set does suggest that there is some sort of systemic effect of psyllium inclusion, as the $\Delta$ glucose rates were lower in the rumens of calves fed PSYdespite the esophageal groove shunting milk replacer past the rumen. Furthermore, there was a tendency for the PSY rumens to be larger; also indicative of the presence of some sort of systemic feedback mechanism.

Overall, this trial yielded strong data in support of potential benefit to psyllium supplementation in milk replacers but also indicates the need for further research in the area. Regarding the microbial results and the histomorphology data, a much larger data set would be advantageous due to the larger margin of error with those two laboratory assays . In order to evaluate psyllium's effectiveness in a "real-world" application, a study must be done with calf starter inclusion and milk replacers fed at a more biologically appropriate level with much larger numbers of calves. Most importantly, in order for psyllium to be used on farm, it must be cost-effective and provide a decent return on the investment for today's modern progressive dairy producers to utilize this technology.

# APPENDIX

**Appendix 1.** Least squares means and associated standard errors for water, dry matter, and nutrient intakes.

| | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Intake | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Morning, L | 1.04 | 1.18 | 0.3 | 2.12 | 2.24 | 0.33 | 3.23 | 3.84 | 0.38 | 2.22 | 1.36 | 0.5 | 2.15 | 2.16 | 0.19 | 0.99 | <.0001 | 0.44 |
| Night, L | 0.9 | 1.14 | 0.29 | 1.2 | 1.06 | 0.32 | 1.48 | 2.56 | 0.36 | 1.26 | 1.59 | 0.5 | 1.21 | 1.58 | 0.21 | 0.12 | 0.01 | 0.3 |
| Daily, L | 1.81 | 1.94 | 0.52 | 3.29 | 3.27 | 0.57 | 4.7 | 6.39 | 0.65 | 3.48 | 2.31 | 0.9 | 3.32 | 3.47 | 0.33 | 0.75 | <.0001 | 0.28 |
| DMI, g/d | 669 | 686 | 26.3 | 685 | 697 | 28.5 | 719 | 752 | 32.3 | 776 | 791 | 40.1 | 712 | 732 | 21.7 | 0.31 | 0.001 | 0.98 |
| Protein, g/d | 146 | 148 | 5.7 | 149 | 151 | 5.7 | 157 | 162 | 7.0 | 169 | 171 | 8.7 | 155 | 158 | 4.7 | 0.50 | 0.013 | 0.98 |
| ME, kcal/d | 2915 | 2978 | 115 | 2988 | 3025 | 124 | 3135 | 3262 | 140 | 3384 | 3434 | 174 | 3175 | 3105 | 95 | 0.39 | 0.013 | 0.98 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON = Control diet

[3] PSY = Psyllium-supplemented diet

**Appendix 2.** Least squares means and associated standard errors for average daily growth.

| | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Body length, cm/d | 0.10 | 0.00 | 0.07 | 0.39[a] | 0.05[b] | 0.08 | 0.15 | 0.27 | 0.10 | 0.16 | 0.14 | 0.12 | 0.20 | 0.12 | 0.05 | 0.17 | 0.08 | 0.04 |
| Heart girth, cm/d | 0.29 | 0.27 | 0.07 | 0.15 | 0.16 | 0.07 | 0.34 | 0.26 | 0.08 | 0.00 | 0.00 | 0.11 | 0.19 | 0.17 | 0.04 | 0.76 | 0.01 | 0.94 |
| Withers height, cm/d | 0.24 | 0.20 | 0.07 | 0.26 | 0.14 | 0.08 | 0.21 | 0.28 | 0.10 | 0.16 | 0.15 | 0.13 | 0.22 | 0.19 | 0.05 | 0.71 | 0.85 | 0.69 |
| Body weight, kg/d | 0.10 | 0.12 | 0.05 | 0.29 | 0.24 | 0.06 | 0.45 | 0.47 | 0.07 | 0.25 | 0.33 | 0.09 | 0.29 | 0.27 | 0.04 | 0.66 | <.001 | 0.82 |
| Predicted BW gain[4], kg/d | 0.51 | 0.52 | | 0.52 | 0.52 | | 0.54 | 0.55 | | 0.59 | 0.60 | | 0.54 | 0.55 | | | | |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON = Control diet

[3] PSY = Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ($P < 0.05$).

[4] Predicted average daily gain according to NRC (2001). Not analyzed statistically, but shown for information purposes.

**Appendix 3.** Least squares means and associated standard errors for viscosity of digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[1] | SE | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 13.28 | 1.45 | 7.78 | 8.28 | 1.02 | 7.3 | 8.28 | 1.02 | 10.37 | 9.21 | 1.02 | 15.22 | 9.44 | 0.88 | 10.17 | 8.80 | 0.98 | 0.33 | 0.08 | 0.25 |
| Abomasum | . | . | 19.6 | 31.01 | 4.46 | 21.86 | 45.54 | 5.17 | 16.9 | 35.01 | 4.46 | 14.02 | 24.89 | 4.46 | 18.10 | 34.11 | 6.88 | 0.004 | 0.28 | 0.77 |
| Jejunum[4] | 477.29 | 197.98 | 19.37 | 16.34 | 3.39 | 21.03 | 24.69 | 3.39 | 15.58 | 18.38 | 3.39 | 29.79 | 20.21 | 3.12 | 21.44 | 19.91 | 4.81 | 0.69 | 0.36 | 0.54 |
| Colon[4] | 4.49 | 0.2 | 3.93 | 4.99 | 0.14 | 4.16 | 4.6 | 0.14 | 3.27 | 4.66 | 0.14 | 3.31 | 4.7 | 0.12 | 3.67 | 4.74 | 0.14 | <0.001 | 0.16 | 0.24 |

[1] Baseline values are shown for comparison purposes and are not included in the statistical analyses.

[2] CON=Control Treatment.

[3] PSY= Psyllium Treatment

[4] Data were $Log^{10}$ transformed for statistical analysis, but back-transformed means are shown for ease of interpretation.

**Appendix 4.** Least squares means and associated standard errors for viscosity of digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[1] | SE | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 13.28 | 1.45 | 7.78 | 8.28 | 1.02 | 7.3 | 8.28 | 1.02 | 10.37 | 9.21 | 1.02 | 15.22 | 9.44 | 0.88 | 10.17 | 8.80 | 0.98 | 0.33 | 0.08 | 0.25 |
| Abomasum | . | . | 19.6 | 31.01 | 4.46 | 21.86 | 45.54 | 5.17 | 16.9 | 35.01 | 4.46 | 14.02 | 24.89 | 4.46 | 18.10 | 34.11 | 6.88 | 0.004 | 0.28 | 0.77 |
| Jejunum[4] | 477.29 | 197.98 | 19.37 | 16.34 | 3.39 | 21.03 | 24.69 | 3.39 | 15.58 | 18.38 | 3.39 | 29.79 | 20.21 | 3.12 | 21.44 | 19.91 | 4.81 | 0.69 | 0.36 | 0.54 |
| Colon[4] | 4.49 | 0.2 | 3.93 | 4.99 | 0.14 | 4.16 | 4.6 | 0.14 | 3.27 | 4.66 | 0.14 | 3.31 | 4.7 | 0.12 | 3.67 | 4.74 | 0.14 | <0.001 | 0.16 | 0.24 |

[1] Baseline values are shown for comparison purposes and are not included in the statistical analyses.

[2] CON=Control Treatment.

[3] PSY= Psyllium Treatment

[4] Data were $Log^{10}$ transformed for statistical analysis, but back-transformed means are shown for ease of interpretation.

**Appendix 5.** Least squares means and associated standard errors for mean digesta retention time in the total tract.

| | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | CON[2] | PSY[3] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Mean retention time, h | 8.35 | 9.46 | 0.47 | 8.26 | 10.24 | 0.47 | 8.73 | 9.42 | 0.47 | 8.45 | 9.71 | 0.59 | 0.106 | 0.93 | 0.76 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] CON=Control Treatment.

[3] PSY= Psyllium Treatment

**Appendix 6.** Least squares means and associated standard errors for weekly dry matter contents (%) of gastrointestinal tract digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 2.4 | 0 | 1.7 | 1.3 | 0.00 | 1.2 | 2.2 | 0.00 | 1.4 | 1.2 | 0.00 | 2 | 2.3 | 0.00 | 1.5 | 1.7 | 0.00 | 0.52 | 0.18 | 0.32 |
| Abomasum | 38.4 | 0.06 | 2.9 | 2.2 | 0.02 | 2.5 | 2.6 | 0.02 | 3.3 | 2.9 | 0.02 | 6.3 | 3 | 0.01 | 3.8 | 2.7 | 0.01 | 0.28 | 0.3 | 0.52 |
| Jejunum | 5 | 0 | 9.5 | 7.3 | 0.01 | 8.3 | 8.1 | 0.01 | 6.9 | 6.2 | 0.01 | 8 | 8.9 | 0.01 | 8.2 | 7.6 | 0.01 | 0.55 | 0.38 | 0.62 |
| Proximal colon | 22.2 | 0.04 | 16.1 | 14.7 | 0.02 | 18 | 13.4 | 0.02 | 18.2 | 14.9 | 0.02 | 16.7 | 12.2 | 0.02 | 17.3 | 13.8 | 0.01 | 0.017 | 0.74 | 0.84 |
| Distal colon | 29.1 | 0.06 | 20.1 | 19.5 | 0.02 | 21.5 | 15.9 | 0.02 | 23.4 | 21.1 | 0.03 | 18.8 | 14.8 | 0.02 | 20.9 | 17.8 | 0.01 | 0.05 | 0.1 | 0.65 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for comparison purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 7.** Least squares means and associated standard errors for weekly dry matter contents (%) of gastrointestinal tract digesta.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 2.4 | 0 | 1.7 | 1.3 | 0.00 | 1.2 | 2.2 | 0.00 | 1.4 | 1.2 | 0.00 | 2 | 2.3 | 0.00 | 1.5 | 1.7 | 0.00 | 0.52 | 0.18 | 0.32 |
| Abo-masum | 38.4 | 0.06 | 2.9 | 2.2 | 0.02 | 2.5 | 2.6 | 0.02 | 3.3 | 2.9 | 0.02 | 6.3 | 3 | 0.01 | 3.8 | 2.7 | 0.01 | 0.28 | 0.3 | 0.52 |
| Jejunum | 5 | 0 | 9.5 | 7.3 | 0.01 | 8.3 | 8.1 | 0.01 | 6.9 | 6.2 | 0.01 | 8 | 8.9 | 0.01 | 8.2 | 7.6 | 0.01 | 0.55 | 0.38 | 0.62 |
| Proximal colon | 22.2 | 0.04 | 16.1 | 14.7 | 0.02 | 18 | 13.4 | 0.02 | 18.2 | 14.9 | 0.02 | 16.7 | 12.2 | 0.02 | 17.3 | 13.8 | 0.01 | 0.017 | 0.74 | 0.84 |
| Distal colon | 29.1 | 0.06 | 20.1 | 19.5 | 0.02 | 21.5 | 15.9 | 0.02 | 23.4 | 21.1 | 0.03 | 18.8 | 14.8 | 0.02 | 20.9 | 17.8 | 0.01 | 0.05 | 0.1 | 0.65 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for comparison purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 8.** Least squares means and associated standard errors for weekly pre-prandial blood constituents.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Glucose, mg/dL | 102.6 | 3.2 | 95.9 | 98.0 | 4.6 | 94.5 | 99.3 | 4.8 | 96.5 | 97.8 | 5.2 | 91.2 | 101.6 | 6 | 94.6 | 99.2 | 4.2 | 0.064 | 1.00 | 0.67 |
| NEFA | 395 | 29.5 | 282 | 264 | 26.8 | 263 | 220 | 30.2 | 198 | 175 | 33 | 231 | 265 | 41.2 | 244 | 231 | 22 | 0.52 | 0.011 | 0.66 |
| BHBA, mmol/L | 0.06 | 0.01 | 0.05 | 0.04 | 0.01 | 0.05 | 0.05 | 0.02 | 0.06 | 0.05 | 0.02 | 0.1 | 0.1 | 0.02 | 0.06 | 0.06 | 0.01 | 0.80 | 0.081 | 0.96 |
| Cholest-erol, mg/dL | 27.9 | 1.6 | 59 | 51 | 3.6 | 69.3 | 66.7 | 4.00 | 76.6 | 85.8 | 4.7 | 95.2 | 83.1 | 6.14 | 75.1 | 71.6 | 2.5 | 0.28 | <.001 | 0.123 |
| Urea N, mg/dL | 12.6 | 0.94 | 6.1 | 6.0 | 0.66 | 6.4 | 5.5 | 0.71 | 6.4 | 6.0 | 0.80 | 6.3 | 6.9 | 1.0 | 6.3 | 6.1 | 0.54 | 0.68 | 0.85 | 0.81 |
| Total protein, mg/dL | 5.77 | 0.11 | 5.92 | 5.67 | 0.26 | 5.94 | 5.59 | 0.27 | 5.63 | 5.65 | 0.29 | 5.43 | 5.48 | 0.34 | 5.73 | 5.6 | 0.23 | 0.36 | 0.40 | 0.69 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 9**. Least squares means and associated standard errors for weekly post-prandial blood constituents.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Glucose, mg/dL | 103.59 | 4.23 | 110.9 | 109.5 | 4.1 | 101 | 104.2 | 4.6 | 96.3 | 100.8 | 5.5 | 101.5 | 107.8 | 7.2 | 102.5 | 105.7 | 2.8 | 0.42 | 0.073 | 0.87 |
| NEFA | 261 | 22.4 | 212 | 238 | 17.3 | 179 | 178 | 19.4 | 192 | 168 | 22.3 | 180 | 158 | 29.7 | 191 | 185 | 12.6 | 0.73 | 0.013 | 0.52 |
| BHBA, mmol/L | 0.05 | 0.01 | 0.0 | 0.0 | 0.01 | 0.0 | 0.0 | 0.01 | 0 | 0 | 0.01 | 0.03 | 0.04 | 0.01 | 0.02 | 0.02 | 0.01 | 0.45 | <0.001 | 0.74 |
| Cholesterol, mg/dL | 26.4 | 1.3 | 53.9 | 51.3 | 4.5 | 66 | 66.9 | 4.9 | 67 | 79.2 | 5.6 | 84 | 73 | 7.1 | 67.9 | 68.1 | 3 | 0.96 | <0.001 | 0.18 |
| Urea N, mg/dL | 12 | 0.98 | 5.9 | 6.1 | 0.72 | 6.5 | 5.7 | 0.74 | 5.9 | 5.6 | 0.79 | 6.2 | 5.9 | 0.91 | 6.2 | 5.8 | 0.73 | 0.44 | 0.89 | 0.64 |
| Total protein, mg/dL | 5.1 | 0.43 | 5.3 | 5.2 | 0.6 | 5.44 | 5.43 | 0.61 | 4.9 | 5.17 | 0.62 | 4.8 | 4.64 | 0.65 | 5.13 | 5.1 | 0.61 | 0.99 | 0.018 | 0.76 |

[1] *P*-value. T = effect of treatment, W = effect of week, T*W = treatment x week interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 10.** Least squares means and associated standard errors of weekly duodenal (DU) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 27.5 | 2.4 | 25.7 | 28.0 | 4.4 | 40.3 | 32.7 | 4.4 | 29.7 | 31.0 | 4.4 | 25.3 | 30.8 | 3.8 | 30.2 | 30.6 | 2.1 | 0.89 | 0.15 | 0.45 |
| Weight, g | 50.6 | 4.0 | 49.9 | 68.1 | 11.3 | 51.3 | 65.6 | 11.3 | 53.9 | 63.7 | 11.3 | 51.2 | 64.2 | 10.9 | 50.6 | 67.9 | 8.2 | 0.03 | 0.83 | 0.83 |
| g DU per kg BW | 0.98 | 0.04 | 1.12 | 1.49 | 0.23 | 1.09 | 1.47 | 0.23 | 1.06 | 1.17 | 0.23 | 0.94 | 1.41 | 0.21 | 1.05 | 1.38 | 0.16 | 0.02 | 0.73 | 0.78 |
| Density (g/cm) | 1.94 | 0.05 | 1.92 | 2.41 | 0.39 | 1.42 | 1.99 | 0.39 | 1.82 | 2.02 | 0.39 | 2.05 | 2.48 | 0.35 | 1.80 | 2.22 | 0.28 | 0.06 | 0.25 | 0.94 |
| Cm DU per kg BW | 0.56 | 0.04 | 0.60 | 0.61 | 0.10 | 0.89 | 0.75 | 0.10 | 0.60 | 0.59 | 0.10 | 0.47 | 0.57 | 0.09 | 0.64 | 0.63 | 0.05 | 0.86 | 0.03 | 0.64 |
| (g/cm)/ kg BW | 0.04 | 0.00 | 0.04 | 0.05 | 0.01 | 0.03 | 0.05 | 0.01 | 0.04 | 0.04 | 0.01 | 0.04 | 0.05 | 0.01 | 0.04 | 0.05 | 0.01 | 0.06 | 0.27 | 0.74 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet.

[4] PSY= Psyllium-supplemented diet.

**Appendix 11.** Least squares means and associated standard errors of weekly ileum (IE) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 94.9 | 38.5 | 79.7 | 88.3 | 24.6 | 36.7 | 50.3 | 24.6 | 61.7 | 64.7 | 24.6 | 57.8 | 77.0 | 21.3 | 58.9 | 70.1 | 11.9 | 0.51 | 0.45 | 0.99 |
| Weight, g | 124.2 | 68.3 | 66.8 | 45.5 | 19.4 | 28.0 | 61.7 | 19.4 | 60.3 | 91.9 | 19.4 | 54.8 | 71.0 | 16.8 | 52.5 | 67.5 | 9.4 | 0.27 | 0.46 | 0.48 |
| g IE per kg BW | 2.42 | 1.26 | 1.61 | 1.03 | 0.44 | 0.62 | 1.40 | 0.44 | 1.21 | 1.82 | 0.44 | 1.04 | 1.32 | 0.38 | 1.12 | 1.39 | 0.21 | 0.37 | 0.70 | 0.43 |
| Density (g/cm) | 1.22 | 0.21 | 0.80 | 0.80 | 0.19 | 0.80 | 1.26 | 0.19 | 1.09 | 1.40 | 0.19 | 0.99 | 1.02 | 0.17 | 0.92 | 1.12 | 0.12 | 0.08 | 0.09 | 0.40 |
| Cm IE per kg BW | 1.85 | 0.67 | 1.87 | 2.05 | 0.57 | 0.80 | 1.15 | 0.57 | 1.25 | 1.27 | 0.57 | 1.11 | 1.41 | 0.50 | 1.26 | 1.47 | 0.28 | 0.59 | 0.38 | 0.99 |
| (g/cm)/ kg BW | 0.02 | 0.00 | 0.02 | 0.02 | 0.00 | 0.02 | 0.03 | 0.00 | 0.02 | 0.03 | 0.00 | 0.02 | 0.02 | 0.00 | 0.02 | 0.02 | 0.00 | 0.15 | 0.23 | 0.30 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 12.** Least squares means and associated standard errors of weekly colon (COL) growth parameters.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Length, cm | 215.2 | 25.3 | 217.6 | 192.5 | 18.7 | 175.0 | 163.6 | 18.7 | 175.7 | 189.5 | 18.7 | 196.8 | 190.7 | 16.9 | 191.3 | 184.1 | 12.6 | 0.51 | 0.16 | 0.67 |
| Weight, g | 369.0 | 19.3 | 416.5 | 424.8 | 38.5 | 416.5 | 470.0 | 38.5 | 389.9 | 517.5 | 38.5 | 408.6 | 457.0 | 33.3 | 407.9 | 467.3 | 18.6 | 0.04 | 0.84 | 0.49 |
| g COL per kg BW | 7.51 | 0.29 | 9.36 | 9.17 | 0.65 | 8.97 | 10.72 | 0.65 | 7.76 | 9.60 | 0.65 | 7.63 | 8.31 | 0.59 | 8.43 | 9.45 | 0.45 | 0.01 | 0.01 | 0.21 |
| Density (g/cm) | 1.78 | 0.10 | 2.01 | 2.19 | 0.32 | 2.42 | 2.91 | 0.32 | 2.24 | 2.76 | 0.32 | 2.09 | 2.45 | 0.29 | 2.19 | 2.58 | 0.21 | 0.06 | 0.21 | 0.93 |
| Cm COL per kg BW | 4.28 | 0.26 | 4.95 | 4.17 | 0.43 | 3.82 | 3.73 | 0.43 | 3.49 | 3.52 | 0.43 | 3.67 | 3.53 | 0.37 | 3.98 | 3.74 | 0.22 | 0.41 | 0.08 | 0.77 |
| (g/cm)/ kg BW | 0.04 | 0.00 | 0.05 | 0.05 | 0.01 | 0.05 | 0.07 | 0.01 | 0.05 | 0.05 | 0.01 | 0.04 | 0.04 | 0.01 | 0.05 | 0.05 | 0.00 | 0.04 | 0.01 | 0.61 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 13.** Least squares means and associated standard errors for resistance values (R; Ω·cm$^2$) among gastrointestinal tract locations.

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | $P^1$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Base-line[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 121.2 | 8.7 | 170.7 | 212.0 | 34.5 | 208.8 | 131.4 | 34.5 | 156.4 | 178.9 | 34.5 | 186.6 | 147.3 | 29.9 | 180.6 | 167.4 | 16.7 | 0.58 | 0.87 | 0.31 |
| Jejunum | 128.7 | 9.0 | 101.8 | 150.3 | 36.0 | 98.7 | 113.8 | 36.0 | 83.0 | 127.8 | 30.3 | 132.3 | 102.3 | 27.5 | 103.9 | 123.5 | 22.4 | 0.30 | 0.84 | 0.32 |
| Ileum | 169.5 | 18.1 | 185.7 | 204.3 | 22.1 | 207.1 | 215.6 | 22.1 | 197.6 | 181.6 | 22.1 | 182.3 | 213.0 | 22.1 | 193.2 | 203.6 | 12.9 | 0.50 | 0.80 | 0.70 |
| Colon | 126.9 | 1.3 | 117.1 | 125.3 | 14.0 | 161.5 | 116.3 | 14.0 | 116.4 | 115.9 | 14.0 | 153.1 | 136.5 | 12.2 | 137.0 | 123.5 | 7.6 | 0.18 | 0.14 | 0.26 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet.

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.01$).

[c,d] Subcolumn means within row and week category with different superscripts differ ( $P < 0.20$).

**Appendix 14.** Least squares means and associated standard errors for Δ glycylsarcosine transport among gastrointestinal tract locations

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.13 | 0.13 | 0.22 | 0.24 | 0.25 | 0.85 | 0.57 | 0.25 | 0.18 | 0.41 | 0.25 | 0.32 | 0.22 | 0.23 | 0.39 | 0.36 | 0.20 | 0.77 | 0.04 | 0.53 |
| Jejunum | 0.04 | 0.02 | 0.17 | 0.22 | 0.10 | 0.00 | 0.08 | 0.10 | 0.30 | 0.15 | 0.08 | 0.16 | 0.15 | 0.07 | 0.16 | 0.15 | 0.05 | 0.89 | 0.23 | 0.57 |
| Ileum | 0.11 | 0.05 | 0.13 | 0.11 | 0.06 | 0.09 | 0.00 | 0.06 | 0.05 | 0.08 | 0.06 | 0.09 | 0.02 | 0.06 | 0.09 | 0.05 | 0.03 | 0.44 | 0.63 | 0.77 |
| Colon | 0.12 | 0.05 | 0.13 | 0.03 | 0.06 | 0.07 | 0.00 | 0.06 | 0.001[a] | 0.208[b] | 0.06 | 0.06 | 0.04 | 0.05 | 0.07 | 0.07 | 0.03 | 0.93 | 0.70 | 0.09 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

[a,b] Subcolumn means within row and week category with different superscripts differ ( $P < 0.05$).

**Appendix 15.** Least squares means and associated standard errors for Δ proline transport among gastrointestinal tract locations

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Rumen | 0.13 | 0.10 | 0.08 | 0.28 | 0.30 | 1.14 | 0.42 | 0.30 | 0.14 | 0.52 | 0.30 | 0.27 | 0.16 | 0.28 | 0.41 | 0.34 | 0.21 | 0.70 | 0.09 | 0.18 |
| Jejunum | 0.00 | 0.00 | 0.00 | 0.01 | 0.04 | 0.05 | 0.00 | 0.04 | 0.09 | 0.04 | 0.03 | 0.04 | 0.12 | 0.03 | 0.05 | 0.04 | 0.02 | 0.93 | 0.12 | 0.18 |
| Ileum | 0.00 | 0.00 | 0.20 | 0.06 | 0.21 | 0.56 | 0.03 | 0.21 | 0.03 | 0.07 | 0.21 | 0.05 | 0.04 | 0.21 | 0.21 | 0.05 | 0.14 | 0.22 | 0.46 | 0.37 |
| Colon | 0.00 | 0.00 | 0.05 | 0.02 | 0.14 | 0.06 | 0.08 | 0.14 | 0.02 | 0.43 | 0.14 | 0.00 | 0.10 | 0.13 | 0.03 | 0.16 | 0.09 | 0.18 | 0.43 | 0.33 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet

**Appendix 16.** Least squares means and associated standard errors for villi height, villi width, and crypt depth (µm) in the jejunum

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Villi height | 560 | 75 | 695 | 592 | 113 | 567 | 656 | 138 | 734 | 552 | 113 | 811 | 592 | 98 | 702 | 598 | 58 | 0.21 | 0.85 | 0.57 |
| Villi width | 99 | 16 | 112 | 101 | 13 | 92 | 108 | 13 | 107 | 92 | 13 | 97 | 99 | 12 | 102 | 100 | 9 | 0.84 | 0.88 | 0.60 |
| Crypt Depth[a] | 251 | 52 | 253 | 192 | 35 | 253 | 305 | 40 | 307 | 250 | 35 | 307 | 250 | 35 | 249 | 274 | 30 | 0.61 | 0.21 | 0.16 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=treatment x week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY=Psyllium-supplemented diet

**Appendix 17.** Least squares means and associated standard errors for crypt width and depth (µm) in the colon

| | Week 0 | | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | | Overall diet means | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Baseline[2] | SE | CON[3] | PSY[4] | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | CON | PSY | SE | T | W | T*W |
| Crypt depth[a] | 454 | 32 | 396 | 358 | 38 | 360 | 335 | 38 | 305 | 275 | 38 | 326 | 282 | 33 | 347 | 312.5 | 22.0 | 0.16 | 0.07 | 0.99 |
| Crypt width | 75 | 4 | 61 | 65 | 7 | 57 | 64 | 7 | 59 | 50 | 7 | 58 | 49 | 6 | 59 | 56.7 | 3.9 | 0.67 | 0.40 | 0.49 |

[1] *P*-value. T= effect of treatment, W=effect of week, T*W=Treatment x Week Interaction

[2] Baseline values are shown for informational purposes and are not included in the statistical analyses.

[3] CON=Control diet

[4] PSY= Psyllium-supplemented diet