2019

Growth Performance, Metabolic and Rumen Profile, Nutrient Utilization, and Health of Calves Fed Condensed Whey Solubles with Starter Pellets

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GROWTH PERFORMANCE, METABOLIC AND RUMEN PROFILE, NUTRIENT UTILIZATION, AND HEALTH OF CALVES FED CONDENSED WHEY SOLUBLES WITH STARTER PELLETS

BY

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A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Science

Specialization in Dairy Science

South Dakota State University

2019

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This thesis is dedicated to the memory of Dr. Richard Koritansky.

He taught me a great number of things about cows and faith.

ACKNOWLEDGMENTS

I would like to start by thanking my incredible family for their love, encouragement, patience, and relentless support over the years. I am especially thankful for the work ethic and love of learning that my parents instilled in me. Additionally, my extended family has been so generous and so supportive; my love and respect for agriculture stems from them. I want to especially thank my uncle Mick who set in motion the chain of events that not only helped me discover my passion for ruminant nutrition, but my desire to pursue my masters in dairy nutrition. My sincerest appreciation goes out to my wonderful husband. He has grounded me, encouraged me, and motivated me every step of the way. These graduate studies have been possible because of the laughter and patience that he brings to our house.

I want to express my sincere gratitude to my advisor Dr. Jill Anderson for providing me the opportunity to study here at SDSU. I am thankful for her guidance, patience, encouragement, and valuable advice. I have thoroughly enjoyed being a part of her lab as well as being her assistant coach for the dairy cattle judging team. She is an incredible mentor and I have learned so much from her.

I would like to also thank my graduate committee, Dr. Vikram Mistry, Dr. Llyod Metzger, Dr. Johan Osorio, and Dr. Matthew Diersen for the time spent reviewing my thesis. I also want to thank all of the faculty members of the Dairy and Food Science Department for their willing help whenever I needed it. I want to also thank the SDSU DRTF farm crew and employees, especially Pete Linke. My farm trial could not have been possible without their assistance to maintain calf health and the facilities. I would

like to thank Idaho Milk Product for their financially support of my study and creation of this production. I believe it will be a great value-added product for dairy producers.

I would also like to thank my fellow graduate students Chelsea Schossow, Rhea Lawrence, Nirosh Senevirathne, Karla Hernandez-Rodriguez, Lauren Shearer, Lydia Olaqunju, Fernanda Rosa, Rodrigo Grazziotin, Nathaly Carpinelli, and Sonia Rodriquez-Jimenez. Their help and guidance on the farm, in the lab, and in our classes has been invaluable. I have a learned a great deal from my studies and even more from the great minds studying next to me. I also want to thank my undergraduate friends for their assistance during my feeding trial, special thanks to Alex Preszler, Kelli Choudek, Kirby Krogstad, and Jacob Weg.

Last, but not least, I want to express my deepest gratitude to God. These graduate studies would not have been possible without His grace, strength, and perseverance. With eager excitement I more toward my future career in His name.

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LIST OF ABREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADIA	Acid detergent insoluble ash
AA	Amino acids
BCS	Body condition score
BHB	Beta hydroxyl butyrate
BW	Body weight
CON	Control
CWS	Condensed whey solubles
CWSH	Condensed whey solubles high dosage
CWSL	Condensed whey solubles low dosage
CP	Crude Protein
d	Day
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FOS	Fructo-oligosaccharide
GC	Gas chromatography
GI	Gastrointestinal
GOS	Galacto-oligosaccharide
h	Hour(s)

K_2EDTA	Potassium ethylene diamine tetra-acetic acid
Lin	Linear orthogonal effect
Mcal	Mega calories
MOS	Mannan-oligosaccharide
NaFl	Sodium fluoride
NDF	Neutral detergent fiber
NFC	Non-fibrous carbohydrate
OM	Organic matter
PUN	Plasma urea nitrogen
Q	Quadratic orthogonal effect
SCFA	Short chain fatty acid
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SBM	Soybean meal
Trt	Treatment
USDA	United States Department of Agriculture
VFA	Volatile fatty acid
Wk	Week

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ABSTRACT

GROWTH PERFORMANCE, METABOLIC AND RUMEN PROFILE, NUTRIENT UTILIZATION, AND HEALTH OF CALVES FED CONDENSED WHEY SOLUBLES WITH STARTER PELLETS

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2019

A study was conducted for this thesis to evaluate the effectiveness of a new product's ability to improve calf health and growth performance. In addition to the results from the study, this thesis also includes a literature review of immune and rumen maturation in calves and a discussion of ways to protect and encourage those fundamental progressions. For the main research project 48 dairy calves were used in a randomized complete block design study to evaluate the growth performance, rumen fermentation and metabolic profiles, nutrient utilization, and health scores when calves were supplemented Condensed Whey Solubles (CWS) with their starter pellets. The calves were enrolled in the study for twelve weeks. The CWS was offered with an aliquot of the daily starter pellets to encourage consumption of CWS and solid feed. The daily dosage of CWS was consistent, either none for control calves, 40 mL/d for CWSL calves or 80 mL/d for CWSH calves. The remainder of the daily starter pellet was fed after the aliquot was consumed and starter pellets were fed ad libitum. Supplementing CWS improved dry matter intake, increased withers and hip heights in the post weaning stage, and lowered body temperatures post weaning. Moreover, CWS improved fecal consistencies for CWSL calves in the post weaning stage. Supplementing CWS is an

option for producers to improve growth and health while maintaining metabolic and rumen development.

INTRODUCTION

Investigating optimal ways to care for and raise dairy calves is critical for producers. The care and attention calves receive during their first 12 wk of life impact their efficiency and productivity later in life. However, during these critical weeks, calves are developing a functional rumen and maturing their immune system and remain very vulnerable to illness. Researchers have investigated the maturation and developmental processes of calves extensively. Therefore, this thesis aims to catalog the literature and knowledge to date on the topic. The immune system begins its progression at the passive transfer of immunities from colostrum. From that point on the calf's immune defense is shifting and evolving into its own entity. This process is coupled with the calf's transition from milk dependency to consuming solid feed, which matures the rumen. Developing the rumen and the microbial ecosystem within is crucial for the calf's ability to extract energy from fibrous material. However, if the calf's fragile immune system is battling an illness, then metabolized energy will be shunted to fuel the immune defense and not towards rumen maturation and skeletal growth. Because immune status is so important, alternatives have been explored, and products have been developed that can enhance the immune system. One of those promising avenues is the use of oligosaccharides as prebiotics in calf diets. These complex carbohydrates have been shown to improve calf health, which is demonstrated through lower body temperatures and better fecal consistencies. Additionally, several studies have found that feeding oligosaccharides encourage growth. These benefits may be caused by supporting the immune system so

energy can go towards growth or because of the product themselves; regardless, oligosaccharides have proven themselves to be worth investigating. Therefore, the experiment found in this thesis examines a new product that is concentrated with prebiotic compounds derived from milk carbohydrates. Several health and growth benefits with feeding this new product called, Condensed Whey Solubles (CWS), were found. The findings supported the hypothesis that calves fed CWS would have improved growth performance and display a healthier immune status. Therefore, the objective of this thesis are to use previous literature to support the relevance of researching CWS, describe execution and results of the CWS study, and additionally to interpreting results for future investigations.

CHAPTER 1

LITERATURE REVIEW

Introduction

Dairy calves are a significant investment of both time and money for producers. Nutritional and health management in the first few months of life is crucial for a calf's long-term performance. Therefore, producers and researchers are constantly searching for the most efficient and effective management tools and products. Those first few months are critical because the calf is undergoing major digestive tract and immunological changes. Calves receive their first form of immunity through passive transfer from their mothers. The calves are vulnerable for weeks thereafter while they develop their immune defense. Furthermore, born with a gastrointestinal (GI) tract that more closely mimics the monogastric digestive system, calves have to transition into a ruminant. This process has been heavily researched with a focus on making the rumen functional through starter intake and rapidly fermentable carbohydrates. Evolutionarily speaking, no matter how nutritionally rich a calf's diet is, if that calf is sick, then energy will go to maintenance and immune function, not growth or development. But research has also found nutritional avenues where certain nutrients can be used as diet supplements acting primarily as preventative measures against illness.

In this literature review the challenges young calves have to overcome early in life will be defined and discussed. Additionally, previous research investigating ways to improve this developmental stage will be covered. There are several different managerial methods to promote calf health and development, but the research here will be focused on feeding practices and alternatives to feed-grade antibiotics. Then, a developing new

product, Condensed Whey Solubles (CWS), will be introduced. Condensed whey solubles has properties similar to previously studied nutrients that have proven to be beneficial to growth and health. A pilot study initiated the investigation of CWS, but results elicited further research into its mechanisms of benefit to the calf. Therefore, the objective of this literature review is to define the challenges young calves have to overcome early in life, discuss current methods to help calves overcome these obstacles, and introduce the need for a second study on supplementing CWS.

Calf Rearing Importance and Challenges

Raising healthy calves and heifers is the goal of all dairy operations because those calves and heifers will eventually join the lactating herd. Moreover, raising replacement heifers is a significant expenditure for operations, averaging at least two years of input before the first lactation (Gabler et al., 2000). Research on optimal and cost-efficient ways to improve the growth and health of dairy calves is a focus within dairy science. Many researchers have evaluated the impact early-life nutrition and management has on an animal once fully developed. Their findings quantify that a properly cared for calf and heifer leads to better growth performance, increased production, and longevity within the herd (Rincker et al., 2011; Heinrichs et al., 2011; Soberon et al., 2012).

However, calves face two major obstacles at birth. Their first challenge is that they have an undeveloped immune system. Their second struggle is that their rumens, which will supply them energy the majority of their lives, are nonfunctioning and need maturation. The National Animal Health Monitoring System (NAHMS) reported in 2014 the dairy heifer calf morbidity rate of 38.1% and a mortality rate of 5% (Urie et al., 2018a). Of those morbidity cases, 33.4% were due to respiratory illness and 56.0% of the

cases were due to digestive issues. Within the mortality rate digestive, respiratory, or a combination were the primary causes of death. Digestive problems claimed 32.0% of the deaths alone (Urie et al., 2018b). These numbers are better than the 1992 report when the morbidity and mortality rates for preweaned heifer calves was 36.1 and 8.4% respectively, but there is still room for improvement (USDA, 1992). Additionally, the primary form of treatment in the survey data collected by NAHMS was antibiotics, which is both expensive and a continually pressing concern of consumers (Urie et al., 2018a). Therefore, research and calf management techniques should continue to pursue ways to improve calf performance and health.

Immune Development

Calves develop within their dams through a cotyledonary placenta. Therefore, nutrients are not passed directly through blood, calves do not receive prepartum antibodies, and that is why calves are born with undeveloped immune systems (Senger, 2003). In response, dairy cows produce colostrum, a form of milk that is higher in total solids, fat, proteins, and contains immunoglobulins. The colostrum is secreted for about 24 hours after parturition before the secretory cells in the alveoli of the mammary gland transition into making milk within the following 48 hours. This passive transfer of antibodies is critical for establishing the calf's immunity. Colostrum is checked for quality before being administered to the calf. If the quality is insufficient then the farm must use a colostrum replacer or frozen colostrum in accordance to the management protocol. Regardless of protocol variances, delivering the colostrum is time-critical because as time progresses the calf's gut loses the ability to absorb the immunoglobulins (Weaver et al., 2000).

Immunoglobulins from colostrum are the calf's initial building block for their immune development. However, calves remain very susceptible to diseases for the following 12 weeks; with the highest chance of death happening before week 4 of life (Urie et al., 2018b). The industry has developed ways to aid the calf's developing immune system. One of those methods is housing. Preweaned dairy calves are mostly afflicted by diseases that are inhaled or ingested (Wells et al., 1996). A well-bedded, ventilated, individual hutch promotes calf health and it also stops the spread of disease (Cummins et al., 1991; Quigley et al., 1995). The other technique to help the developing system is through nutrition since one of the primary ways young calves get sick is through digestive infections. Their basic nutritional requirements have to be met; but in addition, the industry has investigated dietary additives that promote immunity. In the past, producers used feed-grade antibiotics. There were positive results, but at the end of 2016 this feeding practice was ruled to require a veterinarian prescription by the Veterinary Feed Directive. Therefore, researchers and producers have increased their search for effective alternatives which include: iron binding antimicrobial proteins (Joslin et al., 2002), prebiotics such as oligosaccharides (Heinrichs et al., 2003), and probiotics (Timmerman et al., 2005) to name the most researched. The pursuit continues to find options that will supplement the immune system until it is adequately developed to protect the calf.

Ruminal Development

Dairy calves are born with rumens that are underdeveloped and essentially non-functional (Lyford, 1988). The esophageal grove at the base of their esophagus shunts suckled milk past the rumen and directly into the abomasum for degradation (Van Soest,

1982). However, eventually calves have to transition from being functional monogastrics to plant-based ruminants. At birth, the rumen and reticulum combined hold nearly the same volume as the abomasum, but by 12 weeks old they hold nearly six times as much as the abomasum (Lyford, 1988). Several factors influence the calf's ability to make the transition. In addition to unlimited water availability and solid foods offered at an early age, the immune status influences calf development. The immune system is developing alongside the rumen; therefore, if the immune status is compromised, then the body will channel nutrients to improving health and not growth of body or development of mature GI tract. The functional development of the rumen in a calf is critical for producers because they are establishing the calf's metabolic proficiency for her future production life.

A pivotal stage in this transition is called weaning. A calf's primary source of nutrients has to come from milk or milk replacer for at least four weeks, but then it is up to the producer's discretion when to initiate weaning. Producers tend to wean their calves around 6 to 8 weeks of age or once they start consuming 1kg of starter pellets per day (Hopkins, 1997). The weaning process can be very stressful for calves, with the risk of decreased feed efficiency, intake, and ADG; as well as depressing the immune system (Eckert et al, 2015; Steele et al., 2017). There are different ways to wean calves, but research shows that the step-down method is the most beneficial for the calf. In the step-down model a calf receives one liquid feeding per day for about 10 to 14 days. Khan et al. (2007a) found that the gradual step-down method, versus the conventional abrupt weaning, increased growth and solid feed intake. Later, Steele et al. (2017) found that in

addition to increased ADG postweaning, the step-down calves also had greater rumen volatile fatty acid (VFA) production.

Stress is minimized at weaning when calves have already begun consuming solid feed and their rumens have gained functionality. There is no concrete initiation to rumen maturation. Although born sterile, bacteria can be found in the rumen within a day postpartum. Additionally, the colonies of bacteria and other microbiota found in a typical adult rumen are present within two weeks of age and stabilize around two months old (Meale et al., 2017a). Suspended in an anaerobic, water saturated environment, microbes interact with and break down solid food in the rumen. The by-products of the microbial fermentation process are VFA, the most prevalent being acetic, butyric, and propionic acid. Volatile fatty acids provide 70 to 80% of the energy requirement for a fully developed cow (Owens and Goetsch, 1988). Bergman (1990) ranked these prominent VFA's, butyric > acetic > propionic, for their rumen developmental abilities. In addition to digestion of the microbes themselves, acetic and propionic acid are absorbed through the rumen wall and primarily provide energy to the calf (Fahey and Berger, 1988). Butyric acid, however, is converted to BHB in the rumen mucosa and is used primarily by the rumen (Sander et al., 1959; Hodson et al., 1965).

The rumen's ability to utilize VFA is contingent on its ability to absorb the acids. Papillae are the sites of VFA absorption within the rumen, and their finger-like structure increases surface area for uptake (Lyford, 1988). Butyric acid's primary purpose within the rumen is papillae growth by providing energy for epithelial proliferation (Sander et al., 1959; Baldwin et al., 2004). Tamate et al. (1962) and Hamada et al. (1976) introduced inert objects to the calf diet and saw increases in rumen volume and muscular strength,

but no epithelial improvement. These studies support that physical rumen growth is encouraged by the nature of the solid food, but epithelial development is stimulated by the chemical process of fermentation. The aforementioned is why solid intake is positively correlated with rumen development. Tamate et al. (1962) photographed the visual rumen differences between a 12 week-old calves fed only milk versus a 12 wk old calf fed milk, hay, and grain. The differences were astonishing. The rumen in the milk-fed calf was light in color and smooth with no presence of papillae, but the second rumen of the calf fed milk, grain, and hay was dark in color and the papillae growth was dense. This was further supported by Coverdale et al. (2004) which reported that calves had greater weight gain, feed efficiency, and VFA concentrations with a diet mixture of forage and coarse starter. Additionally, increased solid feed intake at an earlier age results in the rumen becoming functional sooner (Biesheuval et al., 1991; Khan et al., 2016; Meale et al., 2017b).

Furthermore, Heinrichs and Jones (2016) published a series of photos illustrating the ruminal differences between calf diets. One series of pictures captured the rumens of 6 wk old calves fed only milk, milk and grain, or milk and hay. These illustrations supported Tamate et al. (1962) data but they also showed the importance of grain. The hay and milk rumen were darker in color but lacked papillae development like the milk only rumen (Heinrichs and Jones, 2016). In an invited review, Khan et al. (2016) summarized the effects of concentrates and forages on rumen development. Their research concluded that forages have a greater impact on rumen expansion, weight, and reduced passage rate for increased degradation. Concentrates, on the other hand, have a

greater impact on papillae differentiation and growth, butyrate production, and total VFA production.

Carbohydrates in Calf Diets

Protein, carbohydrates, and fat are the three energy sources found within a diet. Carbohydrates make up 60 to 70% of a cow's diet (NRC, 2001). A carbohydrate is a molecule containing carbon, hydrogen, oxygen with some amount of water. When carbohydrates are fermented by the microbes and the VFA which are produced are absorbed, the body converts those end products of rumen fermentation into glucose or fatty acids to utilizes them for energy. If the glucose is not absorbed directly, it is synthesized via gluconeogenic pathways from propionate, glycerol, and lactate. Glucose is very important for body function. It is used all across the body for cellular maintenance and proliferation. This is particularly crucial in calves who need it for muscular growth, the beginning stages of mammary development, as well as the rumen epithelial maturation (Fahey and Berger, 1988).

Carbohydrates are divided into classes of saccharides. Monosaccharides and disaccharides have the smallest molecular weights and are classified as sugars; examples include, glucose, fructose, and galactose. Oligosaccharides contain two to ten saccharide units such as lactose and fructo-oligosaccharide. Carbohydrates with more than ten saccharides are polysaccharides and these are commonly known as starch, cellulose, hemicellulose, and lignin. Polysaccharides are further divided into structural and nonstructural carbohydrates. Structural carbohydrates, cellulose and hemicellulose, are primarily found in cell walls of plants, are very fibrous, and not readily available to a

developing rumen (NRC, 2001). Therefore, nonstructural carbohydrates are the primary source of glucose in calf diets.

The initial carbohydrates come from milk: lactose, glucose, and galactose. The nonstructural carbohydrates that make up the bulk of the calf's diet postweaning can be found in sources such as corn, oats, molasses, barley, and soyhulls (Huntington, 1997; Hill et al., 2008). Because it is so rapidly fermented, starch is the primary nonstructural carbohydrate found in calf pellets (NRC, 2001). Herrera- Saldana et al. (1990) ranked starch sources on degradability through *in vitro* and *in situ* trials and the results were: oats > wheat > barley > corn > sorghum. However, it is also well documented that processing the starch sources via grinding, rolling, or cracking improves nutrient availability and therefore increases starch digestibility (Lykos and Varga, 1995, Huntington 1997; Bateman et al., 2009). Corn is the most commonly used source for starch because it is the least expensive feed ingredient in the USA and it is 80% starch (Miller and Hoover, 1998; Hill et al., 2008). Moreover, Kahn et al. (2007b) reported that calves fed corn as their starch source had greater ADG than calves fed oats, barley, or wheat. Oats however are often fed to improve coarseness to the feed and are around 45% starch (Hill et al., 2008). Molasses is a rapidly fermented sugar that provides energy and increased palatability (NRC, 2001). Feeding too much molasses can reduce ADG and increase health issues in calves (Lesmeister and Heinrichs, 2005). Barley averages 57% starch on a DM basis (NRC, 2001). Jarrah et al. (2013) found that processing barley did not improve calf performance. Soybean hulls have less starch compared to other feeds at 19% and they are also low cost, but add bulk to the diet (Hill et al., 2008).

Dietary Oligosaccharides as Prebiotics

The industry has researched antibiotic alternatives for decades. Since 2016, when producers could no longer freely feed antibiotics, there has been a push to find effective replacements to aid the fragile rumens and immune systems of calves. Two of the more researched substitutions are pro- and prebiotics. Probiotics are defined as live cultures of microorganisms that improve the microbial communities within the gastrointestinal tract (Uyeno et al., 2015). Some examples of constituents that have been found to have probiotic effects are yeast (Quigley et al., 1992; Lascano and Heinrichs, 2007), specific strains of bacteria (Abe et al., 1995), multispecies probiotics (Timmerman et al, 2004), and kefir (Fouladgar et al., 2016). Most probiotics are fed as preventative measures, but Renaud et al. (2019) reported that a multispecies probiotic was able to reduce the duration of diarrhea incidences in calves when fed as a treatment. Probiotics have also been found to improve health and increase ADG (Timmerman et al., 2005; Signorini et al., 2012), but probiotic efficiencies do vary because of products being inconsistent (Uyeno et al., 2015). The inconsistency is partially due to probiotics being distinguished as either defined or undefined. In a defined probiotic there are known specific strains of microorganisms. The risk for those defined probiotics is the chance that it will not support the GI microbial communities needed for that farm. An undefined probiotic is a mixture that is not completely characterized or quantified. Undefined probiotics tend to be more efficient, but there is also a chance the impact is muted by the numerous microorganisms (Gaggia et al., 2010). Additionally, since they are live cultures, appropriate storage and handling are critical to ensure the viability of probiotic product.

Prebiotics are defined as selectively fermented or indigestible food that cause specific changes in the GI microbiota that benefit the health of the host (Gibson et al., 2004). The mechanisms through which prebiotic ingredients benefit the host are still being investigated; but in short, prebiotics avoid traditional metabolic pathways through resisting fermentation of microbes, gastric acids, and enzymatic degradation (Gibson, 2004). Van Loo and Vancraeynest (2008) reported that prebiotics potentially displace pathogenic bacteria by competing for nutrients or attaching to absorbency sites in the gut epithelium. They also stated that another mechanism could be encouraging short-chain fatty acid production which lowers intestinal pH and stimulates an immune system response. Geigerova et al. (2017) later suggested that prebiotics can be used to support the benefits of and survival of administered probiotics as alternatives for antibiotics (2017). Furthermore, it is also suggested that prebiotics bind to harmful bacteria such as *Escherichia coli* and *Salmonella sp* and pass through the digestive system (Newman, 1994).

Animals have enzymes that digest most carbohydrates; however, some galactans and oligosaccharides cannot be digested. Researchers have also discovered prebiotic health benefits of these undigested carbohydrates. There are several different types of oligosaccharides found in nature. Some of the more researched ones in livestock species are fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), and galacto-oligosaccharides (GOS). Fructo-oligosaccharides are primarily found in the energy storage organs of plants such as wheat, bananas, barley, and asparagus. Fructo-oligosaccharides appear to encourage the growth of beneficial bacteria (Sghir et al., 1998) while inhibiting the colonization of pathogens (Grizard and Barthomeuf, 1999). Donovan

et al. (2002) concluded that FOS and probiotic bacteria could be used as alternatives to antibiotics. Additionally, Webb et al. (1992) reported increased weight gain when FOS was added to milk replacer for calves. Fructo-oligosaccharides have also been found to impact feed conversion rate and shifted VFA production from acetate to butyrate in veal calves (Grand et al., 2013). Furthermore, Respondek et al. (2008, 2011) observed FOS increasing insulin sensitivity in obese dogs and horses and increased growth performance in pigs and poultry (Flickinger et al., 2003).

Most MOS is derived from the cell walls of *Saccharomyces cerevisiae* (yeast) through enzymatic hydrolysis and centrifuged for isolation (Spring et al., 2000). Spring et al. (2000) study on chickens hypothesized that MOS provides a competitive binding site for harmful bacteria which results in that bacteria exiting the digestive tract without attaching to the epithelium. Savage et al., (1996) suggests that MOS stimulate antibody production to improve health. The benefits of MOS in human intestinal health has also been researched extensively (Jenkins et al., 1999; Gibson et al., 1999; Singh et al., 2018). Dvorak and Jacques (1997, 1998) observed MOS improve performance of pigs and increased grain intake and weight gain in dairy calves. Later, Heinrichs et al., (2003, 2013) reported that MOS improved feed intake and fecal consistency scores in dairy calves.

Lastly, GOS is present in cow milk and is usually derived from the cheese making by-product, whey permeate (Yanahira et al., 1995). Whey permeate is rich with lactose and the GOS can be separated from the lactose through an enzymatic reaction using β-galactosidase (Torres et al., 2010). The resulting syrup is a mixture of glucose, galactose, lactose, and oligosaccharides; collectively the combination is called GOS (Castro et al.,

2016). The mechanisms through which GOS benefits the host is unclear. However, most research supports that GOS encourages the growth of the beneficial bacteria *Lactobacillus* and *Bifidobacteri*a and works synergistically if fed with probiotics (Macfarlane et al., 2008; Castro et al., 2016). Galacto-oligosaccharides are also present in human milk and therefore potential human benefits have been investigated (Macfarlane et al., 2008; Matsumoto et al., 2017; Ashwini et al., 2019). Aly et al. (2016) found that GOS supplemented in infant formula increased iron bioavailability. In livestock, Tsukahara et al. (2009) studied GOS being fed to weaning pigs and concluded that GOS improved growth performance and health status of the small intestine. Castro et al. (2016) reported that dairy calves supplemented with GOS had greater intestinal epithelial development and more lactic acid bacteria in their colon during the first two weeks of life. However, GOS calves also had lower SCFA concentrations and higher fecal consistency scores. They attributed these negative results to the treatment dosage exceeding the colon's absorption abilities and suggest more osmotic balance and concentrated dosage for future research. Additionally, cows supplemented with a mixture of yeast and GOS had a tendency to improve N metabolism (Mwenya et al., 2005).

Of the oligosaccharides previously mentioned, GOS has been researched the least in regard to dairy calf health and growth benefits. This is largely due to the variability found in the GOS compound profile as well as the efficiency of and differences among β-galactosidases used (Angus et al., 2005). Investigating the prebiotic potential of oligosaccharides should continue to be explored.

Condensed Whey Solubles Pilot Study

Senevirathne et al. (2018a) conducted a pilot study beginning in May of 2017. The product, condensed whey solubles (CWS), is made from milk permeate and the composition is around 25% lactose and 20% prebiotic properties containing oligosaccharides. The objectives of the study were to evaluate the effect CWS had on calf growth, health, nutrient utilization, and rumen development, hypothesizing that CWS calves would perform better than control calves. The study was a randomized complete block design where the Holstein dairy calves were blocked by sex and birthdate. The calves were then randomly assigned to either the control group with no CWS supplementation or assigned to 40 mL/d supplementation of CWS. Previous literature supporting oligosaccharides benefits to the GI tract influenced the trial's design. The trial administered the CWS to the calves with the pasteurized waste milk that was fed during the preweaning stage to evaluate CWS impact on the abomasum and lower GI tract since the product would be shunted past the rumen. Then the treatment calves were dosed the CWS on their calf starter pellets postweaning to investigate ruminal digestion benefits. The calves were fed 2.83 L of pasteurized waste milk twice daily during wk 1 through 5. The calves were weaned during wk 6 with only one milk feeding in the morning and completely off milk at the start of wk 7. Intakes and health evaluations were recorded daily. Health parameters included rectal body temperature, ocular and nasal discharge, as well as fecal consistency. Body weight, body condition scores, and frame growth were measured once a week, 3 h after morning feeding. Additionally, blood samples were also taken weekly at the same time as body measurements. Rumen fluid samples were collected via esophageal tube around 4 h after morning feeding at the end of wk 8 and 12.

Fecal grabs samples were also collected at 15 different time points over the course of three days to evaluate apparent total tract digestibility of DM, CP, and fiber. Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$ and tendencies were $0.05 \leq P < 0.10$.

The results from the study concluded that CWS improved ($P = 0.03$) BHB blood concentrations (34.37 and 36.07 mg/dL; SEM = 1.03 for CON and CWS respectively), but there were no treatment differences in frame growth or rumen profile. Some of most intriguing results from the pilot study were the differences found in starter pellet and total DMI postweaning. The DMI was similar between treatments preweaning, but from the transition week on the CWS calves had greater ($P < 0.01$) starter DMI (2.19 and 2.29 kg/d; SEM= 0.25) and total DMI (2.19 and 2.31 kg/d; SEM = 0.25) postweaning. Corresponding increased body weight tended ($P = 0.05$) to be greater in treatment calves postweaning as well (109.29 and 113.27 kg/d; SEM = 0.05). Additionally, fecal scores and rectal temperatures were similar between treatments preweaning, but postweaning the CWS calves had lower ($P < 0.01$) fecal consistency scores (0.45 and 0.28; SEM = 0.03) and lower ($P = 0.01$) rectal temperatures (39.81 and 39.7°C; SEM = 0.02).

Collectively these results provoked reconsidering where in the digestive tract the CWS was having the greatest impact since most of the statistical treatment effects were observed during the postweaning stage. During the postweaning portion of the trial, the CWS administered on the starter were sequestered to rumen microbial fermentation. This resulted in increased DMI intake and greater body weight, potentially if the CWS had been available on the starter pellets preweaning, intake would have been encouraged. Additionally, CWS has concentrated amounts of digestion resistant oligosaccharide, and

the pilot study saw improved health postweaning in treatment calve. Those results call for further investigation into CWS potentially having the most prebiotic effect beginning in the rumen. Further research is required to investigate if CWS would have the greatest impact when applied only to starter pellet.

Conclusion

Specific calf rearing practices differ among operations, but all producers have to overcome the developmental challenges with which calves are born. Addressing these challenges and investigating strategies to improve calf health and growth performance continues to be a focus of dairy research because early life development has life-long impacts on milk production and longevity.

The initial challenge is the vulnerable and underdeveloped immune system. After the critical colostrum dose calves remain the most susceptible to disease those first twelve weeks of life. Traditionally, producers would feed calves antibiotics through milk and milk replacer, but since that is no longer an option, the industry searches for ways to supplement and protect the developing immune system. Research has investigated the potential prebiotic benefits of oligosaccharides as antibiotic alternatives. Although the exact mechanisms through which oligosaccharides work is still unsure, research has found that oligosaccharides improve health scores and increase various aspects of performance in comparison to animals on control diets.

The other challenge calves have to overcome is a nonfunctional rumen that needs to become fully functional before weaning and continue to mature through their first two years of life. This rumen development is crucial, and many factors influence its development including the immune status of the calf and consumption of solid feed.

Dairy scientists have been researching how to increase starter intake for over twenty years (Kertz et al., 2017). Through that research they have discovered that earlier starter intake minimizes stress at weaning and improves overall calf performance because the rumen becomes functional sooner.

A pilot study was conducted on a product containing concentrated milk sugars and galacto-oligosaccharides to investigate its potential health and growth benefits. The results revealed however, that the CWS product might have the greatest impact beginning the dosage in the rumen since most treatment differences were seen postweaning. Therefore, the proposed follow-up study has the potential to support the hypothesis that oligosaccharides have prebiotic effects on the GI tract starting from the rumen as well as encourage earlier starter intake, rumen development, and improve growth performance.

CHAPTER 2

EFFECTS OF SUPPLEMENTING CONDENSED WHEY SOLUBLES WITH STARTER PELLETS ON GROWTH PERFORMANCE, METABOLIC AND RUMEN PROFILE, NUTRIENT UTILIZATION, AND HEALTH.

ABSTRACT

The objective was to evaluate growth, rumen fermentation, metabolic profile, and health of calves supplemented with condensed whey solubles (CWS) on starter pellets. Forty-eight 2 d-old calves in huts were used in a 12-wk randomized complete block design study. Calves were blocked by breed (33 Holstein, 15 Brown Swiss), sex (30 female; 18 male), and birth date. Treatments were: 1) control (CON) with no supplement, 2) 40 mL/d CWS (CWSL), and 3) 80 mL/d CWS (CWSH). Treatments were top-dressed on an aliquot of starter pellet. Amount of starter pellets in aliquot increased with consumption. The remainder of ad libitum-fed starter pellets were offered after morning feeding. Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5, 1×/d in wk 6, and weaned at d 42. Individual intakes of milk and starter pellets were measured daily. Fecal scores (0 = firm, 3 = watery) were observed and recorded daily. Additionally, respiratory scores (healthy ≤ 3, sick ≥ 5) were observed daily and recorded and were calculated from the sum of rectal temperature, cough, ocular and nasal discharge scores. Body weights (BW), frame measures, and jugular blood samples were taken 1 d/wk around 3 h post feeding. Rumen samples were taken wk 8 and 12 via esophageal tubing. Fecal grab samples were collected in wk 12 for total tract digestibility. Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significance was declared at $P < 0.05$ and tendencies were $0.05 \leq P < 0.10$. Total DMI were similar, but

had a treatment by time interaction, tending to increase with CWS post-weaning. Average daily gains were similar, but BW, withers height, and hip height tended to increase post-weaning with CWS. Other frame measures and BCS were similar. Health scores were not different, but post-weaning fecal scores were firmer for CWSL and rectal temperatures were lower for CWS calves. Glucose had a treatment effect due to the increasing inclusion of CWS. Rumen VFA profiles, PUN, cholesterol, triglycerides, and BHB were similar. The wk 12 apparent total tract digestibility on nutrients were similar among treatments. Supplementing CWS improved calf post-weaning intakes, growth, and health with maintained rumen VFA, and metabolic profile.

Keywords: condensed whey solubles, dairy calf, growth performance

INTRODUCTION

Calf health and growth is a focus of producers because those first few months are vital for that animal's efficiency and production life. Early life illness and death is mostly due to pathogens that have been ingested (Urie et al., 2018b). There was a time when producers could feed specific antibiotics to the calves to help eradicate diseases and viruses present on the farm; however, feeding antibiotics without a veterinarian's approval was prohibited in 2016. The industry has since intensified its search for antibiotic alternatives in order to continue to help supplement the calf's developing immune system. Two of the more popularly researched and investigated alternatives are pro- and prebiotics. Probiotics are defined as live cultures that directly impact the host's microbiota, but prebiotics are not as well defined. Prebiotics resist traditional metabolic pathways in order to provide a benefit to the host. An example of a nutrient that has prebiotic characteristics are oligosaccharides. Fructo-oligosaccharides have been found to encourage beneficial bacteria growth (Sghir et al., 1998) and increase weight gain in calves when fed with milk replacer (Webb et al., 1992). Mannan-oligosaccharides have also been reported to increase weight gain in dairy calves (Dvorak and Jacques, 1997) as well as improve fecal consistency scores (Heinrichs et al., 2013). Furthermore, galacto-oligosaccharides are produced through enzymatic treatment of dairy products, mostly whey and milk permeate. In research, galacto-oligosaccharides have been found to encourage the growth of beneficial bacteria and increase intestinal epithelial development (Castro et al., 2016). Galacto-oligosaccharides' profiles are concentrated with milk sugars and complex carbohydrates with ratios dependent on the enzyme used for degradation. Idaho Milk Products developed a Condensed Whey Solubles (CWS) product that is rich

in milk sugars and contains prebiotic compounds. These properties give CWS the potential to positively influence calf health and performance.

The hypothesis of this study was that feeding CWS on calf starter pellet could improve growth performance and encourage starter intake. More specifically, feeding CWS would provide prebiotic benefits by improving the health status of the treatment calves. Additionally, CWS would improve early gastrointestinal rumen development, which would lead to greater nutrient utilization and improved growth. The objective of this study is to determine the effects of supplementing CWS at multiple dosages on growth performance, nutrient utilization, and health of dairy calves.

MATERIALS AND METHODS

Experimental Design

Forty-eight dairy calves at two days old were used in a 12-wk randomized complete block design study. The calves were both Brown Swiss (nine females, six males) and Holsteins (twenty-one females, twelve males). Therefore, the calves were blocked by sex, breed, and birth date with a total of sixteen blocks. The calves were then randomly assigned to one of three treatments within blocks. The treatment diets were: 1) control (CON) with no supplement, 2) 40 mL/d of CWS (CWSL), and 3) 80 mL/d of CWS (CWSH).

Animal Care and Feeding

All animal procedures and uses were approved by the South Dakota State University Intuitional Animal Care and Use Committee under protocol number 17-109E and institutional Animal Welfare Assurance number #A3958-01. The study was

conducted, and all calves were housed at the South Dakota State University Dairy Research and Training Facility (SDSU- DRTF) in Brookings, South Dakota. The farm trial portion of the study began December 8th, 2017 and ended July 7th, 2018. Calves were fed two colostrum feedings by the SDSU-DRTF staff before starting the study. Each calf's blood serum protein concentrations were measured via refractometer (LW Scientific, Lawrenceville, GA) to assess the immune status prior to starting the study.

The calves were housed outside in individual hutches and bedded with straw as needed. The treatments were given to the calves via top-dressing of an aliquot of the daily starter pellets. This top-dressed aliquot was offered roughly one hour before the morning milk feeding. The exception being CON calves which were offered their full daily starter pellet amount roughly one hour before the morning milk feeding. The amount of the aliquoted starter pellets was based on individual starter pellet intake. All calves were initially offered roughly 25g of pellet as fed to their assigned CWS treatment. The amount of starter pellet offered with the CWS treatment would increase in 25g as fed increments until plateauing at 300g as fed of starter pellet for the daily aliquot. The amount of pellets consumed at the time of the morning milk feeding determined if the aliquot would be increased the next day. Orts were weighed once daily, and starter pellets offered was adjusted to ensure 10% feed refusals. Thus, water and starter pellets were fed ad libitum. Calves were also fed 2.83L of pasteurized waste milk two times per day at 0600 and 1800h. one time per day (at 0600) during wk 6, and weaned at d 42.

The starter pellets were custom made by Pipestone Grain Company (Pipestone, MN) to avoid an confounding ingredients often contained in commercial pellets. The calf pellets contained soy hulls, ground corn, soybean meal, wheat middlings, and minerals

(Table 1). The milk was collected from SDSU- DRTF cows two times per day and was pasteurized on site (Platinum DT-30G; Dairy Tech Incorporated, Severance, CO). Calves were observed daily for health concerns and were treated according to SDSU- DRTF management protocols.

Measurements and Sampling

Milk and pellet intake, as well as orts for individual calves were recorded once daily at 0430 h. Weekly samples of the milk were taken after pasteurization, analyzed in the SDSU Manufacturing plant by Dairy Spec FT (Bentley Instruments, Chaska, MN) for composition, and then stored at -20°C. A sample of each new shipment of CWS was taken, dates used recorded, stored at -20°C, and then later analyzed for DM. Weekly samples of the pellets were preserved at -20°C and then composited equally by month at the end of the study for nutrient analysis. Additionally, individual samples of each calf orts were taken at the end of week twelve, stored at -20°C, and then analyzed later for digestibility analysis and calculations.

Health scores were observed and recorded daily. Fecal scores were recorded daily in accordance with University of Wisconsin-Madison School of Veterinary Medicine's Calf Health Scoring Chart. With the scale of 0= normal, firm; 1= semi-formed, pasty, 2= loose, but stays on top of bedding, and 4= watery, shifts through bedding. Respiratory health was also evaluated and recorded daily based on the respiratory score being the sum of rectal temperature, cough, ocular, ear, and nasal discharge scores. Each of the individual scored were also made on a scale of 0 to 3 (University of Wisconsin-Madison School of Veterinary Medicine, Calf Respiratory Scoring Chart).

Fecal grab samples were collected at the end of week 12 over the course of three days at fifteen different time increments. These fecal samples were stored at -20°C until they could be composited in equal amounts at the end of the study for digestibility analysis. In combination with the fecal samples and ort samples, acid detergent insoluble ash (ADIA) was used as the internal digestibility marker to determine total tract digestibility for each calf.

Body weight (BW), body condition scores (BSC), and frame growth measurements were taken weekly, approximately 3 h post morning feeding. The weekly body condition scores were from an average of three different trained individuals who evaluated the calves on the Wildman et al. (1982) scale, where 1 = emaciated and 5 = obese. Frame growth measurements under consideration were hip width, hip height, withers height, heart girth, paunch girth, and body length. These were measured using a store-bought retractable measuring tape with a ruler and level fashioned to it, a store-bought steel framing square, and store-bought soft sewing measuring tape.

At the same time as the growth measurements, blood was sampled weekly approximately 3h after the morning feeding through venipuncture of the jugular vein. The blood was drawn into two 10-mL Vacutainer® tubes containing potassium ethylene diamine tetra-acetic acid (K_2EDTA) for plasma urea nitrogen (PUN), triglyceride, cholesterol, and beta-hydroxybutyrate (BHB) analyses. Blood was also drawn into one 7 ml vacutainer tube containing sodium fluoride for glucose analysis (Becton, Dickson, and Co., Franklin Lakes, NJ). Samples were immediately placed on ice and within 3 hr brought to the lab to be centrifuged at 2,000 rpm for 20 min at 5°C (CR412 centrifuge;

Jouan Inc., Winchester, VA). Once separated, the serum was pipetted into polystyrene tubes and preserved at -20°C for later analyses.

Ruminal fluid samples were taken from each calf at wk 8 and wk 12, approximately 4 hours after feeding via esophageal tubing. The initial 50mL of fluid was discarded due to potential contamination from the bleach-water solution used to rinse the pump and saliva. Once the initial fluid is discarded roughly 20 mL of rumen fluid was collected, immediately measured for pH using a handheld pH meter (Waterproof pH Testr® 30, OAKTON Instruments, Vernon Hills, IL), and then two aliquots of 5 mL were placed in vials for storage at -20°C. The one vial contained 100 μL of 50% sulfuric acid for ammonia N (NH_3-N) analysis. The other vial contained 1 mL of 25% meta-phosphoric acid for volatile fatty acid (VFA) analysis.

Laboratory Analysis

Weekly pellet samples, pellet ingredients, wk 12 starter pellet orts, and wk 12 fecal samples were thawed and composited into representative samples either by calf or month. In duplicate, the composited samples were dried for 48 h at 55°C in a Despatch Oven (style V-23, Despatch Oven Co., Minneapolis, MN), and were ground to a 4 mm particle size using a Wiley Mill (model 3; Arthur H. Thomas Co., Philadelphia, PA). The samples were further ground to a 1mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY) and stored in mason jars until nutrient analysis. To correct analyses to a 100% DM basis, 1g samples of the feed and fecals were run in duplicate and dried for 3 h at 105°C in a muffle furnace in accordance to an abbreviated method from AOAC International's method 935.29 (1998). Ash content was then determined through an abbreviation of AOAC International's., method 942.05

(2002) where the beforementioned 1 g samples were incinerated for 13 h instead of the method's 8 h at 450°C in a muffle furnace. To determine the DM and ash of the CWS 1 g samples, ran in duplicate, were dried for 8 h at 105°C and then incinerated for 13 h at 450°C in muffle furnaces. Organic matter was calculated as OM = (100 - % ash).

The composited monthly pellets, pellet ingredients, wk 12 starter pellet orts, and wk 12 fecal samples were weighted out in 0.5g samples into ANKOM fiber bags in duplicate. Neutral detergent fiber (NDF) (Van Soest et al., 1991) and acid detergent fiber (ADF) (Robertson and Van Soest, 1981; AOAC International, 2002, method 973.18) were analyzed sequentially using the ANKOM 200 fiber analyzer (ANKOM Technology Corp., Fairport, NY). For NDF, heat-stable α- amylase and sodium sulfite were used (AOAC International, 2002, method 2002.04). Ether extract was determined for the monthly pellet composites by weighing out 1 g of the samples in triplicate and running the samples through Ankom XT10 Extraction System (ANKOM Technology Corp., Fairport, NY) with petroleum ether as the solvent (AOAC International's method 920.30, 2002).

The monthly pellets and pellet ingredients were sent to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for analysis of crude protein, starch, and minerals including Ca, P, Mg, K, S, Na, and Cl. Mineral content, excluding Cl, was determined using inductively coupled plasma spectroscopy (AOAC, International 1995). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY). Starch was analyzed using a modified procedure analyzing glucose using YSI Biochemistry Analyzer (YSI Inc., Yellow Springs, OH; Bach Knudsen, 1997). The CP was measured using the combustion method (AOAC

International, 2002; method 990.03). Nonfiber carbohydrates were calculated as % NFC = 100 – (%ash + % CP + %NDF + %EE) according to the NRC (2001).

For apparent total-tract digestibility calculations, the ADIA content was analyzed for the monthly pellet composites, pellet ingredients, wk 12 orts, and wk 12 fecal samples. This was determined by weighing 0.5 g samples into ANKOM fiber bags, analyzing for ADF (Robertson and Soest, 1981) as previously described, and then incinerating the sample bags for 13 h at 450°C in a muffle furnace. The ash percent was determined using a modified procedure of AOAC International's method 935.29 (1998). Calculations for digestibility were done according to Merchen (1988).

Blood metabolites including glucose, BHB, triglyceride, cholesterol, and PUN were analyzed using commercially available enzymatic or colorimetric assay kits on microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA). Serum glucose concentrations were analyzed using glucose oxidase reagent as described by Trinder (1969; catalog no. G7521, Pointe Scientific Inc, Canton, MI). Concentrations of BHB were analyzed by BHB dehydrogenase and diaphorase according to the method described by McMurray et al., (1984; catalog no. H7587-58, Pointe Scientific Inc., Canton, MI). Triglycerides concentration were determined using enzyme glycerol phosphate oxidase after hydrolysis by lipoprotein lipase, as described by Fossati and Lorenzo (1982; catalog no. T7532-500, Pointe Scientific Inc, Canton, MI). Plasma concentrations of cholesterol were analyzed using cholesterol esterase and oxidase (catalog no. C7510, Pointe Scientific Inc.) as described by Allain et al. (1974). The PUN concentrations were determined using diacteylmonoxime in accordance with procedure 0580 of Stanbio Laboratory, Boerne, TX (catalog no. 0580-250, Pointe Scientific Inc, Canton, MI).

Rumen fluid samples were first thawed and vortexed to completely mix contents before pipetting 2mL into a microcentrifuge tube. The samples were then centrifuged at 30,000 x g for 20 min at 4C in a microcentrifuge (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Ammonia N concentrations were analyzed using colorimetric assay performed on a microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) in accordance with the assay described by Chaney and Marbach (1962). The other samples that were preserved for VFA were analyzed for: acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate using an automated gas chromatograph (model 6890, Hewlett-Packard Co., Palo Alto, CA) using a flame ionization detector. Volatile fatty acids were separated on a capillary column (15m x 0.25 mm i.d.; Nukol 17926-01C, Supelco Inc., Bellefonte, PA) using 2-ethylbuturate as an internal standard. The split ratio of 100:1 in the injector port was at a temperature of 250°C and a flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140 and 250°C, respectively.

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Calf intake, growth data, fecal consistency scores, respiratory scores, and metabolic profiles parameters were analyzed as randomized complete block design with repeated measures using MIXED procedures (Littell et al., 2006). Calves were assigned to 16 blocks based on birth date, breed, and sex. The model included treatment, week, sex, stage, breed, and interactions of these terms (stage being defined as preweaning= wk 0-6; postweaning= wk 6-12). Calf nested within block was the random variable. There was no breed by treatment interaction effects, so those results were not reported although these terms were

included in the model. Initial body frame measurements and BW were included as covariates within the model. Repeated measures by week of the feeding period were done on intakes, BW, body frame measures, fecal consistency scores, respiratory scores, VFA, and metabolites using calf (block) as the subject. Akaike's criterion was used to determine the most suitable covariance structure for the repeated measures analysis for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. Least square means were compared using orthogonal contrasts for linear and quadratic effects, and the slice command was used to determine the *P*-values for treatments during individual weeks and stages. As linear and quadratic effects were a key part of the experimental design they were considered a propriety over treatment effects. To determine ADG for body weight and change per day for body frame measurements, the difference was found between each data collection time point and the previous time point and then divided by the number of days in the time period. The MIXED procedures of SAS were used for analysis of data for apparent total-tract digestibility of nutrients. All data are presented as the least square means with the highest standard error of the mean (SEM) among the values. Significant differences were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Nutrient Composition

Tables 2 and 3 show the nutrient composition of the feed ingredients that were in the starter pellets and the nutrient composition of the starter pellets respectively. Starter pellets CP content was 23.11%, which is greater than the recommended 18% by the NRC (2001). The starter pellet was also higher by 3% in EE and 10% NDF, but less than recommended for ADF content by 2% (NRC, 2001). The concentration of calcium and sulfur matched what is recommended in NRC for starter pellets. The other mineral concentrations were greater in the formulated starter pellets compared to NRC recommendation with the exception of sodium which was less than recommended. Primarily the individual pellet ingredients had nutrient compositions that were similar to what is recorded in the Nutrient Composition of Feeds tables in NRC (2001). Table 4 lists the nutrient composition of pasteurized waste milk and CWS. The CWS supplementation product is high in lactose and prebiotic concentrations. The pasteurized waste milk composition of percent fat, protein, lactose, and total solids were within breed averages for Brown Swiss and Holsteins.

Intakes

Starter DMI, total DMI, and intakes of NDF, ADF, and EE are presented in Table 5. Weekly means of the total DMI are found in Figure 1. The mean DMI was 1.53, 1.64, and 1.67 kg/d; with a SEM = 0.08 for CON, CWSL, and CWSH, respectively, which are comparable to total DMI from previous research conducted using CWS (Senevirathne et al., 2018a; Senevirathne et al., 2018b). During the preweaning stage and for the overall means there was no treatment effect for starter DMI and total DMI. However, total DMI

tended ($P = 0.08$) to be greater in treatment calves post weaning. At weaning it can be seen (Figure 1) that treatment calves experienced little to no decrease in DMI, which supports that CWS negates the negative impact weaning can have on calves. This is consistent with Heinrichs et al. (2003) which reported calves on the MOS treatment diet consumed more grain during the weaning week than the other treatment calves. It was observed that CWS calves found the syrup very palatable, but it tended to crystallize or become very thick and hard at cold temperatures which made it less enticing to the calves. Senevirathne et al. (2018a) saw treatment differences for both starter DMI and total DMI overall means, but that research was conducted during warmer months. The DMI gradually increased for all diets, and this can be attributed to the growth and increase in metabolic demand of the calves (Figure 1). There were no differences found in EE, ADF, and NDF intakes. Senevirathne et al. (2018a) study had treatment effects for total CP, starch, ADF, and EE intakes, which is attributed to the treatment differences seen in the pellet DMI.

Growth Performance

Body weights and ADG can be found in Table 6. Body weights were 69.50, 70.81, and 73.24 kg; with SEM 1.59 for CON, CWSL, and CWSH, respectively. There were no differences observed among body weights and ADG. Similarly, Heinrichs et al. (2003) reported no overall treatment effect on body weight, despite MOS calves having greater grain intake during and after weaning. There was a linear tendency ($P = 0.09$) with ADG, and this can be attributed to growth of the calves. In general, BW were slightly less, but comparable to Senevirathne et al. (2018a; 2018b). Additionally, Quigley

et al. (1997) fed galactosyl-lactose, a trisaccharide very similar to GOS, and saw an increase in BW gain and feed efficiency.

Body frame growth measurements and body condition scores are found in Table 7. Body frame growth was not different among treatments overall. However, treatment differences were observed in withers heights ($P = 0.02$) and hip heights ($P = 0.05$) during the post weaning stage. Additionally, there was a treatment effect ($P = 0.05$) on average daily change for withers height. There were no differences among treatments for body length, heart girth, paunch girth, hip width, and body condition scores. The limited differences among treatments in growth performance could be attributed to seasonal variations in growth performance. The majority of the calves were enrolled in the study during the winter which had monthly average temperatures of -6.1, -7.7, -9.4, 1.1ºC for December, January, February, and March, respectively. The results suggest that feeding CWS has some effects on skeletal growth.

Metabolic Profiles

Table 8 records the overall mean and means by stage of plasma concentrations for glucose, cholesterol, triglycerides, plasma urea nitrogen (PUN), and Beta-hydroxybutyrate (BHB). The mean serum glucose concentrations were 96.41, 100.46, and 104.75 mg/dL; with an SEM of 1.63. For glucose there were treatment differences ($P < 0.01$) for the overall mean and both developmental stages. As the CWS was top-dressed on a common starter pellet the diets were slightly imbalanced for glucose and milk carbohydrate intakes. Figure 2 depicts the average weekly glucose concentrations among treatments. These results affirm that treatment calves were digesting sufficient amounts of the CWS previous to weaning. The glucose concentrations decreased with

age. This is due to the primary metabolic energy source shifting from glucose to VFA as the rumen develops (Hammon et al., 2002). There were no treatment differences observed for cholesterol, triglycerides, PUN, and BHB. This is different from the results found by Senevirathne et al (2018a) who saw treatment differences ($P = 0.03$) for BHB, which is associated with rumen functionality and papillae maturation (Baldwin et al., 2004). Senevirathne et al. (2018a) recorded plasma concentrations for glucose, BHB, and PUN that were comparable to the current study. Additionally, concentrations for BHB were consistent with Lesmeister and Heinrichs (2004). Furthermore, cholesterol and triglyceride concentrations in the present study were slightly lower than what was recorded in Ghasemi et al. where calves were observed under cold stress (2017).

Rumen Fermentation

Rumen VFA profiles, ammonia-N, and pH can be found in Table 9. Total VFA, specific VFA proportions, ammonia-N, and pH were not different among treatments. The concentrations of ammonia-N and each VFA proportion are comparable or slightly greater than Senevirathne et al. (2018a). To be specific, the present study found greater proportions of butyrate, acetate, and greater acetate to propionate ratios compared to Senevirathne et al. (2018a). In contrast, the total VFA amount in the current study was significantly greater than what was reported in Castro et al. (2016), but their method of sampling was post calf death rather than esophageal tubing like in the present study. Ghasemi et al. (2017) studied calves under cold stress and reported total VFA, specific VFA proportions, and acetate to propionate ratios are very comparable to the current study. The results of this present study may have been negatively impacted by sampling technique. Samples were taken via esophageal tube and a hand pump. There is no way to

know exactly where in the rumen the sample is being collected from within the forestomach compartments (i.e., rumen vs. reticulum). Additionally, calves have very little rumen fluid and it is difficult to get enough sample and avoid saliva contamination.

Health Score Observations

Fecal scores, rectal temperatures, and respiratory scores are presented in Table 10. Overall fecal scores were not different among treatments. However, during the postweaning stage there was a treatment effect (P <0.01). That treatment effect stems from CWSL whereas CWSH had a negative effect on fecal scores in the post weaning stage which can be seen in Figure 3. Potentially the CWSH dosage made the osmotic ratio too high for the colon comparable to findings in the study conducted by Castro et al. (2016). However, fecal scores among treatments were better in the current study than what was reported in Senevirathne et al. (2018a). Improvement in fecal consistency for treatment calves in the current study is similar to the results from Quigley et al. (1997) who fed galactosyl-lactose to Holstein bull calves. Additionally, Heinrichs et al. (2003) saw improved fecal consistency in calves fed mannose oligosaccharides. There were no treatment differences for overall rectal temperatures. However, CWS calves had lower (P <0.01) rectal temperatures during the post weaning stage. Figure 4 depicts the weekly rectal temperatures by treatment. Temperatures were comparable with Senevirathne et al. (2018a). It has been suggested that prebiotics encourage the growth of beneficial bacteria (Macfarlane et al., 2008), potentially bind with harmful bacteria preventing absorptions (Newman, 1994), or that prebiotics compete for nutrients and absorption sites (Van Loo and Vancraeynest, 2004). These results support that the oligosaccharides in CWS have prebiotic effects on the GI microflora. Respiratory scores were comparable with those

reported in Senevirathne et al. (2018). There was no difference among treatments for respiratory scores. In regard to calves needing to be treated for illnesses, there were no differences among treatments on a percentages of days for diarrhea, fever, or pneumonia.

Apparent Total Tract Digestion of Nutrients

Table 11 presents results for the apparent total tract digestion of nutrients. No differences were observed among treatments for the digestibility of DM, OM, NDF, and ADF. This is similar to Mwenya et al. (2005) who fed an oligosaccharide to cows and observed no treatment digestibility differences. Additionally, Smiricky-Tjardes et al. (2003) observed no treatment differences in apparent total tract digestibility in which they fed oligosaccharides to pigs. However, Senevirathne et al. (2018a) saw a treatment tendency ($P = 0.10$) for treatment calves, having greater NDF digestibility. In regard to percent digestibility, Senevirathne et al. observed 10- 20% greater digestibility for DM, NDF, and ADF in their study versus the current study.

Conclusion

The dairy calves fed CWS with their starter pellets tended to consume more total DMI in the post weaning stage. Corresponding withers and hip heights were also greater in calves post weaning. However, body weights, average daily gains, and most frame growth measurements were not different among treatments. Apparent total tract digestibility of DM, OM, NDF, and ADF were not different among CON and CWS calves. There were no treatment effects on most of the blood metabolites except for glucose. Glucose was greater in CWS calves during both stages and overall from the increasing conclusion of CWS. Rumen profiles were similar among treatments. The CWS improved fecal scores in the CWSL calves during the post weaning stage. Rectal

temperatures overall tended to be lower in treatment calves. Respiratory scores were not different among treatments.

Data were possibly influenced by the climate for that time of year in South Dakota. The majority of calves enrolled on the current study participated from December through April. Therefore, a majority of the calves experienced long periods of cold stress which reduced intake and the ability to put energy towards growth (Young, 1981; NRC, 2001). It was also previously mentioned that product physical attributes were also negatively impacted by the winter temperatures. Senevirathne et al. (2018b) ran a study with similar dosages but with larger calf numbers on a commercial dairy in Idaho. Senevirathne et al. saw treatment effects for BW, ADG, and BHB (2018b). That study also ran March through June 2018; therefore, those calves experienced warmer weather than the calves on the current study conducted in South Dakota. Additionally, most of the calves on the commercial study (Senevirathne et al., 2018b) were Jersey x Holstein F1 crosses so there was a difference in calf body size to supplementation rate compared to the current study.

Though the mechanism of dosage changed during pre-weaning (with pellets versus with milk), most of the statistical results in this current study were during the post weaning stage, similar to the findings from the Senevirathne et al. pilot study (2018a). This suggests that more research is needed to investigate where and how CWS can have the greatest impact on calf growth and maturation. However, CWS administration could have also had a negative impact on results. The significant differences seen in the pilot study may be due to CWS priming the lower intestinal tract during the preweaning stage when the milk and product were shunted past the rumen. More research should be

conducted investigating the potential benefits of simultaneously putting CWS in the milk and on the starter pellets to prime lower GI tract while encouraging starter intake. Results demonstrate that supplementing CWS with starter pellet has the potential to improve calf post-weaning intakes, skeletal growth, and health while maintaining rumen and metabolic profiles.

ACKLNOWLEDGMENTS

This research was funded by Idaho Milk Products, Jerome, ID with additional support from South Dakota State University Agricultural Experiment Station. We also want to thank the farm crew at the SDSU Dairy Research and Training Facilities, Brookings, SD for their assistance with care of the calves.

Table 1. Ingredient composition for calf starter pellets

Ingredients, % DM	Starter Pellets
Corn, Ground	26.96
Soybean meal	21.11
Wheat middlings	40.05
Soybean hulls	6.25
Molasses, dried	3.41
Mineral Mix[1]	1.77
Salt	0.44

[1] 48.2% dicalcium phosphate; 13.7% salt; 28.73% limestone; 3% selenium (0.06%); 5% ruminant trace mineral mix (2.59% calcium; 10.64% magnesium oxide; 1,802 mg/kg cobalt carbonate; 25,022 mg/kg copper sulfate; 340 mg/kg iodine; 100,715 mg/kg iron sulfate; 49,906 mg/kg manganese sulfate, 49,900 mg/kg zinc sulfate; 1.0% mineral oil; rice hulls as carrier); 1.2% liquid molasses; 0.44% vitamin A; 0.13% vitamin D 66 IU/kg; 0.017% vitamin E 275,000 IU/kg

Table 2. Nutrient compositions of corn grain, wheat middlings, soybean hulls, and soybean meal used to make calf starter pellets

Item, % of DM	Corn		Wheat Middlings		Soybean Hulls		Soybean Meal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	85.71	1.99	88.32	1.02	88.04	0.09	89.00	0.35
Ash	0.55	0.17	5.18	1.29	4.88	0.08	7.22	0.40
OM	99.45	0.17	94.82	1.29	95.12	0.08	92.78	0.40
NDF	12.00	1.17	42.58	3.04	61.94	3.17	7.49	0.94
ADF	3.45	0.17	14.49	0.87	45.55	0.77	4.46	1.09
CP	8.21	0.37	18.66	0.88	12.36	0.82	52.60	2.52
EE	5.14	0.68	5.67	0.27	2.79	0.23	2.44	0.12
NFC[1]	74.10	-	27.91	-	18.03	-	30.25	-
Starch	69.24	3.04	15.62	2.02	0.89	0.36	1.82	1.08

[1] % NFC = 100- (% Ash + %CP + %NDF +%EE) (NRC, 2001).

Table 3. Nutrient composition of calf starter pellets

Item[1]	Mean	SD
DM[2], %	87.91	0.86
Ash[2]	6.66	0.16
OM[2]	93.34	0.16
CP[2]	23.11	0.69
EE[2]	6.26	0.41
NDF[2]	22.69	0.90
ADF[2]	8.87	0.39
NFC[2,3]	44.81	-
Starch	25.92	1.21
Ca	0.69	0.04
P	0.94	0.03
Mg	0.38	0.02
K	1.46	0.05
S	0.29	0.01
Na	0.31	0.03
Cl	0.52	0.03

[1] % of DM unless otherwise indicated

[2] Analysis performed on monthly composites (n= 7), each sample was run in duplicate.

[3] % NFC = 100- (% Ash + %CP + %NDF +%EE) (NRC, 2001).

Table 4. Nutrient compositions of pasteurized waste milk and condensed whey solubles (CWS) fed to calves.

	Pasteurized Waste Milk[1]		CWS[2]	
Nutrients, %	Mean	SD	Mean	SD
DM	13.11	0.65	60.01	2.85
Ash	-	-	4.11	0.35
Fat	4.24	0.53	-	-
True Protein	3.03	0.32	-	-
Total Protein	3.22	0.34	-	-
Crude Protein	-	-	1.91	0.24
Solid non fat[3]	8.82	0.41	-	-
Lactose	4.87	0.16	25.33	2.26
Glucose	-	-	7.27	0.55
Galactose	-	-	2.76	0.78
Prebiotics	-	-	20.72	1.98

[1] Milk samples were collected weekly (n= 23), each sample was run in duplicate.
[2] Data were lab analysis provided by IDMP (n= 18), each sample was run in duplicate.
[3] Solid non fat % = Total solid % - Fat %

Table 5. Nutrient intakes for calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]						
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Stage	Trt × Stage	Lin	Q
Starter DMI, g/d											
Mean	1170.5	1253.6	1252.9	71.5	0.55	<0.01	0.06	<0.01	0.02	0.38	0.58
Pre-weaning	181.9	199.1	199.1	74.8	0.98						
Post-weaning	2168.8	2313.3	2315.3	74.8	0.19						
Total DMI, g/d											
Mean	1532.3	1640.2	1669.6	72.8	0.29	<0.01	0.07	<0.01	0.02	0.16	0.61
Pre-weaning	904.9	944.3	985.2	75.9	0.72						
Post-weaning	2168.7	2339.0	2362.6	75.7	0.08						
NDF Intake, g/d											
Mean	302.1	323.6	323.3	18.5	0.55	<0.01	0.06	<0.01	0.02	0.38	0.58
Pre-weaning	46.9	51.4	51.4	19.4	0.98						
Post-weaning	559.7	597.0	597.5	19.4	0.19						
ADF Intake, g/d											
Mean	118.1	126.5	126.4	7.2	0.55	<0.01	0.06	<0.01	0.02	0.38	0.58
Pre-weaning	18.4	20.1	20.1	7.6	0.98						
Post-weaning	218.8	233.4	233.6	7.6	0.19						
Fat Intake, g/d											
Mean	8342.8	8922.7	8918.9	512.1	0.56	<0.01	0.06	<0.01	0.03	0.39	0.29
Pre-weaning	1303.7	1426.4	1422.1	536.8	0.98						
Post-weaning	1453.0	1303.7	1422.1	537.9	0.21						

[1] *P* values for effects of treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt×wk)** and stage (pre-weaning vs post-weaning) and treatment × stage interaction **(Trt × stage)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

[2]Starter DMI incudes only starter DMI.

Table 6. Body weights and average daily gains (ADG) for calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]						
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Stage	Trt × stage	Lin	Q
BW, kg											
Mean	69.50	70.81	73.24	1.59	0.23	<0.01	0.76	<0.01	0.41	0.09	0.74
wk 0	43.28	43.72	42.08	1.80	0.78						
wk 6	65.97	66.64	68.95	2.05	0.54						
wk12	101.50	103.90	107.10	2.06	0.14						
Pre-weaning	55.70	55.54	57.35	1.94	0.75						
Post-weaning	83.44	85.32	88.91	1.95	0.12						
ADG, kg/d											
Mean	0.68	0.72	0.75	0.06	0.38	<0.01	0.08	<0.01	0.99	0.17	0.92
Pre-weaning	0.52	0.55	0.59	0.05	0.64						
Post-weaning	0.84	0.87	0.90	0.05	0.59						

[1]*P* values for effects or treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt × wk)** and stage (pre-weaning vs post-weaning) the treatment x stage interaction **(Trt × stage)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

Table 7. Frame growth measurements and body condition scores for calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]						
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Stage	Trt × stage	Lin	Q
Withers Height, cm											
Mean	82.91	84.24	84.13	0.82	0.35	<0.01	0.30	<0.01	0.79	0.26	0.42
wk 0	74.24	72.25	73.97	1.66	0.56						
wk 6	82.38	84.51	84.39	0.94	0.12						
wk12	89.70	92.56	91.87	0.94	0.04						
Pre-weaning	79.04	80.08	80.06	0.51	0.16						
Post-weaning	86.77	88.39	88.20	0.51	0.02						
ADC, cm/d	0.19	0.24	0.21	0.02	0.05	0.31	0.84	0.41	0.59	0.21	0.05
Hip Height, cm											
Mean	87.57	88.62	88.31	0.44	0.14	<0.01	0.40	<0.01	0.45	0.21	0.16
wk 0	79.42	78.9	79.87	0.09	0.74						
wk 6	87.24	88.88	88.29	0.68	0.13						
wk12	94.72	96.36	96.34	0.68	0.09						
Pre-weaning	83.62	84.67	83.85	0.44	0.11						
Post-weaning	91.49	92.56	92.78	0.44	0.05						
ADC, cm/d	0.17	0.22	0.19	0.01	0.21	0.24	0.56	0.35	0.77	0.18	0.29
Body Length, cm											
Mean	74.50	75.15	74.50	0.73	0.70	<0.01	0.50	<0.01	0.91	0.99	0.41
wk 0	64.93	64.56	65.86	1.06	0.66						
wk 6	73.28	75.87	74.76	1.10	0.15						
wk12	83.42	84.52	83.95	1.10	0.72						
Pre-weaning	69.98	79.89	70.18	0.58	0.39						
Post-weaning	78.92	79.89	78.83	0.58	0.69						
ADC, cm/d	0.22	0.24	0.22	0.16	0.68	0.07	0.14	0.78	0.72	0.89	0.39
Heart Girth, cm											
Mean	92.68	93.62	93.8	0.66	0.35	<0.01	<0.01	<0.01	0.36	0.20	0.58
wk 0	77.87	78.31	78.86	0.90	0.74						
wk 6	91.01	93.8	92.38	0.90	0.03						
wk12	105.87	107.18	107.21	0.90	0.41						
Pre-weaning	85.83	86.88	86.22	0.69	0.43						
Post-weaning	99.46	100.36	101.37	0.69	0.11						
ADC, cm/d	0.33	0.35	0.35	0.01	0.44	<0.01	<0.01	0.98	0.42	0.30	0.53
Paunch Girth, cm											
Mean	100.66	101.22	102.54	0.93	0.31	<0.01	0.77	<0.01	0.54	0.13	0.70
wk 0	79.00	79.56	80.14	0.93	0.69						
wk 6	96.59	99.78	99.04	1.42	0.15						
wk12	121.53	121.16	124.47	1.42	0.17						
Pre-weaning	89.13	112.3	114.66	1.03	0.58						
Post-weaning	112.10	90.14	90.42	1.03	0.12						
ADC, cm/d	0.49	0.50	0.54	0.23	0.33	0.01	0.79	0.05	0.50	0.17	0.47
Hip Width, cm											
Mean	19.50	19.61	19.64	0.22	0.86	<0.01	0.31	<0.01	0.91	0.62	0.85
wk 0	15.78	16.07	15.88	0.25	0.68						

wk 6	19.55	19.17	19.4	0.28	0.51						
wk12	23.17	23.28	23.5	0.28	0.66						
Pre-weaning	17.73	17.79	17.82	0.20	0.94						
Post-weaning	21.25	21.44	24.47	0.20	0.64						
ADC, cm/d	0.09	0.09	0.09	0.004	0.87	<0.01	0.24	0.29	0.51	0.68	0.71
Body Condition Scores											
Mean	2.42	2.42	2.42	0.05	0.99	<0.01	0.85	<0.01	0.45	0.99	0.96
wk 0	1.71	1.8	1.73	0.04	0.18						
wk 6	2.29	2.39	2.42	2.42	0.46						
wk12	2.94	2.90	2.85	2.85	0.71						
Pre-weaning	2.06	2.11	2.08	0.04	0.64						
Post-weaning	2.77	2.73	2.75	0.04	0.71						

[1]*P* values for effects or treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt × wk)** and stage (pre-weaning vs post-weaning) the treatment × stage interaction **(Trt × stage)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

[2] Calves were weaned off pasteurized waste milk at the end of wk 6.

[3] Scale of 1 to 5 with 1 being emaciated and 5 being obese (Wildman et al., 1982).

Table 8. Blood metabolite concentrations for calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]						
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Stage	Trt × Stage	Lin	Q
Glucose[2], mg/dL											
Means	96.41	100.46	104.76	1.63	<0.01	<0.01	0.65	<0.01	0.23	<0.01	0.95
Pre-weaning	107.12	109.61	116.55	2.05	<0.01						
Post-weaning	85.70	91.30	92.85	2.05	<0.01						
Cholesterol[3], mg/dL											
Mean	47.11	49.27	51.50	2.34	0.36	<0.01	0.67	<0.01	0.75	0.16	0.99
Pre-weaning	57.49	61.03	62.51	2.79	0.35						
Post-weaning	36.72	37.52	40.49	2.79	0.57						
Triglycerides,[4] mg/dL											
Mean	15.81	15.75	16.42	1.17	0.90	<0.01	0.26	0.03	0.16	0.70	0.77
Pre-weaning	16.16	18.39	18.05	1.54	0.44						
Post-weaning	15.47	13.11	14.77	1.54	0.42						
PUN[5], mg/dL											
Mean	18.18	17.69	17.56	0.63	0.71	<0.01	0.02	<0.01	0.05	0.45	0.79
Pre-weaning	15.85	15.38	13.94	0.78	0.16						
Post-weaning	20.51	19.97	21.18	0.78	0.50						
BHB[6], mg/dL											
Mean	35.44	35.12	35.95	1.62	0.86	<0.01	0.37	<0.01	0.95	0.73	0.64
Pre-weaning	23.74	23.79	24.55	1.51	0.90						
Post-weaning	47.16	46.55	47.30	1.51	0.91						

[1]*P* values for effects or treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt × wk)** and stage (pre-weaning vs post-weaning) the treatment × stage interaction **(Trt × stage)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

[2] Serum concentrations of glucose

[3] Plasma concentrations of cholesterol

[4] Plasma concentrations of triglycerides

[5] Plasma concentrations of Urea Nitrogen.

[6] Plasma concentrations of Beta-hydroxybutyrate.

Table 9. Rumen fermentation characteristics of calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]				
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Lin	Q
Rumen, pH	5.99	6.27	6.30	0.16	0.18	0.12	0.74	0.13	0.43
Ammonia- N, mg/dL	23.48	20.97	22.71	2.06	0.58	0.94	0.59	0.78	0.34
Total VFA, mM	80.14	72.53	71.46	5.65	0.35	0.36	0.61	0.23	0.56
VFA, mMol/100mMol									
Acetate	47.34	47.03	46.83	0.70	0.83	0.99	0.14	0.57	0.94
Propionate	37.30	37.79	37.67	0.99	0.90	0.93	0.08	0.77	0.76
Butyrate	10.76	10.77	10.86	0.69	0.99	0.77	0.67	0.91	0.96
Isovalerate	1.08	1.09	1.15	0.08	0.78	0.13	0.44	0.50	0.78
Valerate	3.52	3.31	3.44	0.31	0.81	0.32	0.15	0.84	0.58
Acetate:Propionate	1.29	1.26	1.27	0.05	0.90	0.69	0.06	0.74	0.79

[1]*P* values for effects or treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt x wk)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

Table 10. Fecal scores, temperature, respiratory scores, and incidences for calves fed starter pellets (CON) or starter pellets with condensed whey solubles (CWSL, CWSH)

	Treatment				*P-values*[1]						
Item	CON	CWSL	CWSH	SEM	Trt	wk	Trt × wk	Stage	Trt × stage	Lin	Q
Fecal Score											
Mean	0.16	0.18	0.18	0.04	0.85	<0.01	0.02	<0.01	0.09	0.74	0.67
Pre-weaning	0.21	0.28	0.23	0.03	0.24						
Post-weaning	0.11	0.09	0.13	0.03	<0.01						
Temperature, °C											
Mean	39.38	39.37	39.29	0.06	0.11	<0.01	0.03	<0.01	<0.01	0.05	0.33
Pre-weaning	39.37	39.39	39.34	0.03	0.24						
Post-weaning	39.39	39.35	39.24	0.03	<0.01						
Respiratory Score											
Mean	2.50	2.46	2.43	0.06	0.68	0.28	0.88	0.16	0.78	0.39	0.96
Pre-weaning	2.48	2.48	2.38	0.06	0.49						
Post-weaning	2.53	2.44	2.49	0.06	0.75						
Incidences[8], % of d											
Diarrhea	0.22	0.24	0.17	-	0.87						
Fever	0.05	0.07	0.05	-	0.91						
Pneumonia	0.00	0.00	0.02	-	0.70						

[1]*P* values for effects or treatment **(Trt)**, week **(wk)** and the treatment × week interaction **(Trt × wk)** and stage (pre-weaning vs post-weaning) the treatment × stage interaction **(Trt × stage)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

[2] Scale of 0 to 3 with 0 being firm (Normal) and 3 being watery

[3] Respiratory score = (Temperature Score[4] + Nasal Score[5] + Eye Score[6] + Cough Score[7]).

[4] Scale of 0 to 3 with 0 being 37.77- 38.27°C and 3 being ≥ 39.44°C.

[5] Scale of 0 to 3 with 0 being normal and 3 being heavy ocular discharge.

[6] Scale of 0 to 3 being normal and 3 being heavy ocular discharge.

[7] Scale of 0 to 3 with 0 being none and 3 being repeated spontaneous coughs.

[8] Requiring administration of appropriate treatment

Table 11. Week 12 apparent total tract digestibility of nutrients for calves fed starter pellets (CON) and starter pellets with condensed whey solubles (CWSL, CWSH)

Digestibility, %	Treatment				*P-values*[1]		
	CON	CWSL	CWSH	SEM	Trt	Lin	Q
DM	88.70	86.64	88.89	1.18	0.20	0.90	0.07
OM	87.97	85.72	87.68	1.15	0.19	0.83	0.07
NDF	27.70	27.37	25.60	2.22	0.67	0.40	0.56
ADF	34.36	33.73	31.54	2.80	0.66	0.39	0.77

[1] P values for effects of treatment **(Trt)** and orthogonal contrasts linear **(Lin)** and quadratic **(Q).**

Figure 1. Weekly starter dry matter intake (DMI) for calves fed starter pellets (CON) or supplemented with condensed whey solubles (CWSL, CWSH).

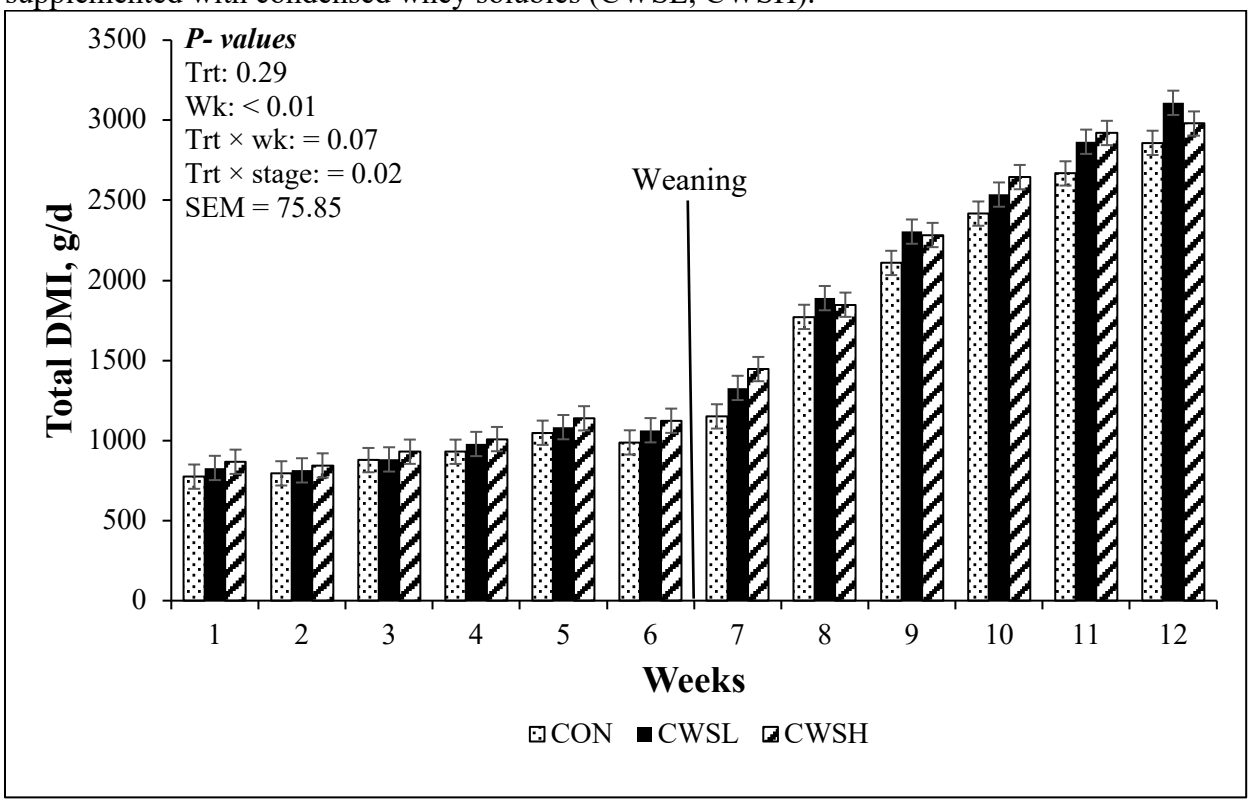


Figure 2. Weekly plasma glucose concentration for calves fed starter pellets (CON) or supplemented with condensed whey solubles (CWSL, CWSH).

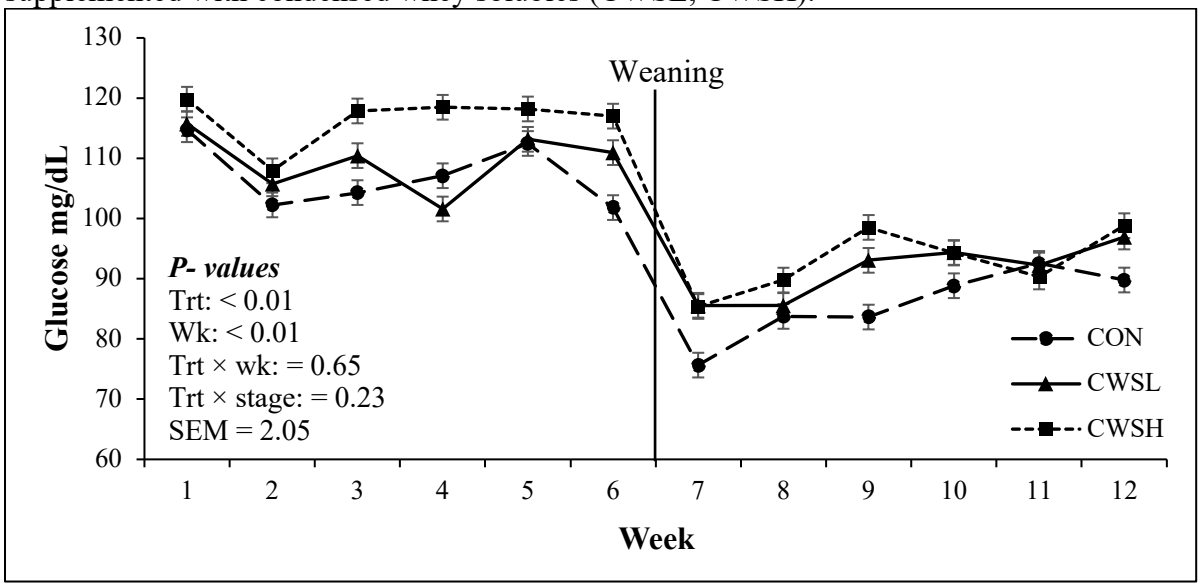


Figure 3. Weekly fecal scores for calves fed starter pellets (CON) or supplemented with condensed whey solubles (CWSL, CWSH).

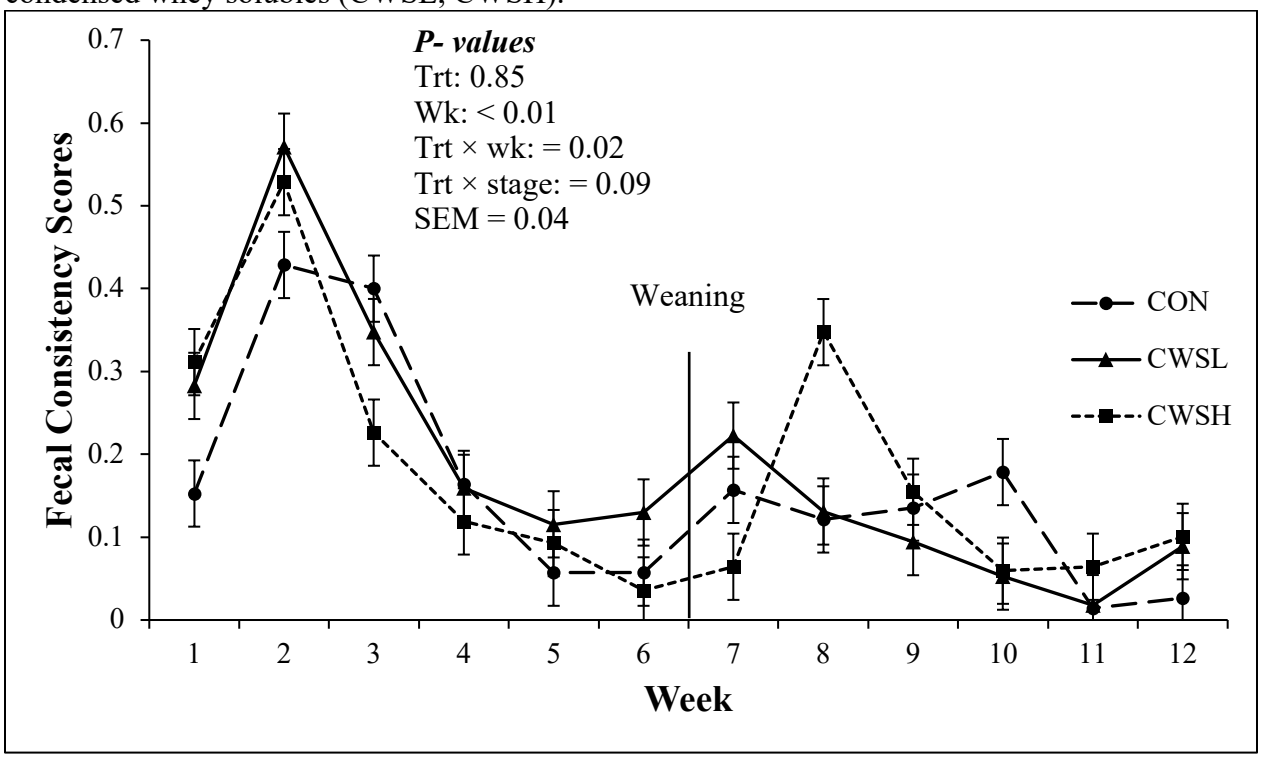
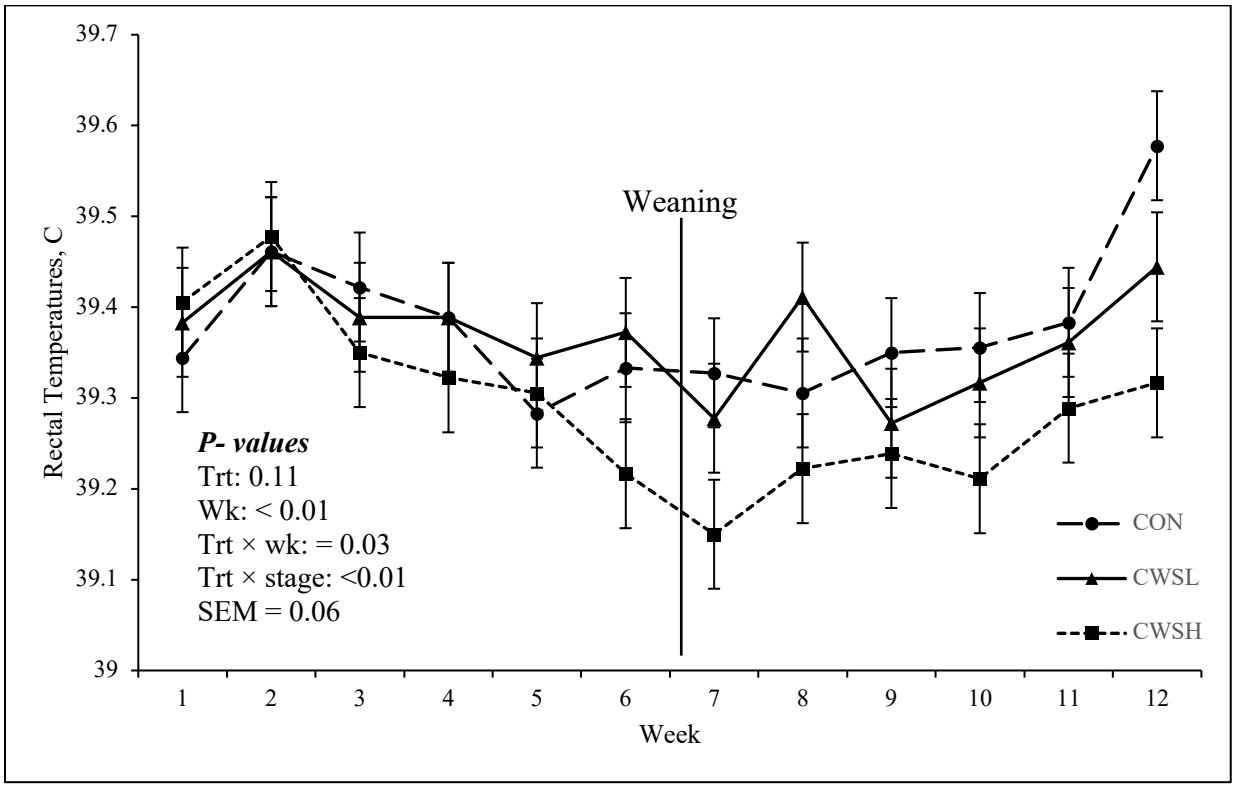


Figure 4. Weekly rectal temperatures for calves fed starter pellets (CON) or supplemented with condensed whey solubles (CWSL, CWSH).



OVERALL CONCLUSIONS

Overall, feeding CWS can benefit the development of dairy calves. It has been previously discussed the mechanism through which the rumen matures. Essentially, the rumen gains functionality as the calf transitions from a strictly liquid diet to gaining nutrients from readily fermented carbohydrates and other solid feed. This process is catalyzed by weaning. One of the first things to be impacted by the stress of weaning calves off milk is intakes, but from the DMI intake graph (Figure 1) one can see that treatment calves did not drop in intake during the weaning period. Therefore, feeding CWS may limit the negative impacts associated with weaning. Moreover, some skeletal growth was observed among treatments for CWS calves post weaning.

Additionally, the development of the immune system was discussed along with sources of supplementation that help the calf establish its immune system. This process is happening at the same time as rumen maturation and has a major impact on the efficiency of the maturation process. Since feed grade antibiotics can no longer be freely fed to calves, alternative supplements, such as compounds within CWS, have been explored. From the results in this thesis, CWS may provide health benefits to calves. Treatment calves saw improved fecal consistencies and lower body temperatures with CWS post weaning.

Research has investigated the development of calves extensively and the literature review within this thesis records their relevant conclusions. However, a full understanding of the benefits found in feeding carbohydrates and prebiotics to calves is inconclusive. There are still debates surrounding the mechanisms through which prebiotics work. Furthermore, it is understood that pre weaning starter intake eases stress

at weaning, but effective ways to encourage that earlier intake is still under-investigated. Condensed Whey Solubles is effective, but further investigation is required to look into the modes through which it works and the ideal way(s) to feed it to growing calves.

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