South Dakota State University

Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

Electronic Theses and Dissertations

2019

Evaluation of Flax Oil Supplementation on Performance of Growing Dairy Heifer Calves and Lactating Cows

Chelsea Rose Schossow
South Dakota State University

Follow this and additional works at: https://openprairie.sdstate.edu/etd

 Part of the Comparative Nutrition Commons, and the Dairy Science Commons

Recommended Citation

Schossow, Chelsea Rose, "Evaluation of Flax Oil Supplementation on Performance of Growing Dairy Heifer Calves and Lactating Cows" (2019). *Electronic Theses and Dissertations*. 3140.
https://openprairie.sdstate.edu/etd/3140

This Thesis - Open Access is brought to you for free and open access by Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact michael.biondo@sdstate.edu.

EVALUATION OF FLAX OIL SUPPLEMENTATION ON PERFORMANCE OF GROWING DAIRY HEIFER CALVES AND LACTATING COWS

BY

CHELSEA ROSE SCHOSSOW

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Sciences

Specialization in Dairy Science

South Dakota State University

2019

EVALUATION OF FLAX OIL SUPPLEMENTATION ON PERFORMANCE OF GROWING DAIRY HEIFER CALVES AND LACTATING COWS

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 thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Jill L. Anderson, Ph.D Date

Thesis Advisor

Vikram Mistry, Ph.D. Date

Head, Dairy and Food Science Department

Kinchel C. Doerner, Ph.D. Date

Dean, Graduate School

I dedicate this thesis to my mother, the strongest, most loving and supportive person I know. Thank you for all the meals, letting me chase my crazy dreams and always only being a phone call away. And to my father, who made me proud to call myself a farmer's daughter, taught me the value of hard work, to be passionate and most importantly that life is unexpected so make the most of it. Although, 23 years was not nearly enough time I'm thankful for the memories we made and miss you every day. Love always, your runt.

ACKNOWLEDGEMENTS

To begin, I would like to thank my advisor Dr. Jill Anderson for her diligent work and continuous support of me during my time at South Dakota State University. I found my love for dairy cattle nutrition in 2013 in her Dairy Science introduction course as a freshman. Thank you for seeing my potential and helping me explore my options. Through your guidance I found myself applying for an undergraduate research grant, receiving the grant, performing a heifer feeding study and presenting my findings at a national conference. I'm very thankful for the experience as it led me to graduate school.

In addition, I would like to thank my graduate committee Dr. Vikram Mistry, Dr. Johan Osorio and Dr. Reinaldo Tonkoski. Thank you for the support and advice as well as reviewing this thesis.

To the students and full time employees at the SDSU DRTF, Davis Dairy plant and the University Feed Mill thank you. Your assistance during the farm trial portion of my work here is greatly appreciated. I would like to personally thank the SDSU DRTF manager Pete Linke for creating a positive teaching environment for all employees and students. I would like to also extend my gratitude to Dr. George Perry and his students for assisting in estradiol blood analysis.

I'm also extremely grateful to my fellow graduate students for your help and companionship during my years here. I would especially like to thank those we helped extensively at the farm to collect data on both the calves and heifers. I know some of those cold winter samples were hard, but we did it! Thank you to Karla Rodriguez-Hernandez, Nirosh Senevirathne, Rhea Lawrence, Michaela Della, Lauren Shearer, and

Lydia Olagunju. In addition, thank you to the undergraduate research assistants, Jacob Weg, Kelli Choudek. and Alex Preszler.

Finally, I would like to thank my family. To my sisters, Breann and Amber, thank you for always listening to my interesting cow stories. My mom for her continued support of my crazy dreams and to my dad who is watching me every day. Lane, thank you for your never-ending support and love as I chase my dreams. Lastly, to our soon to be baby, I'm sorry that you've listened to this thesis over and over again. All I can promise you is a better bedtime story upon your arrival.

TABLE OF CONTENTS

Item **Page**

ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADIA	Acid detergent insoluble ash
AA	Amino acid
BCS	Body condition score
BHB	Beta hydroxyl butyrate
BW	Body weight
CON	Control
CLA	Conjugated linolenic acid
CP	Crude protein
d	Day
DM	Dry matter
DMI	Dry matter intake
DIM	Days in milk
EE	Ether extract
FLAX	Flax oil treatment
GC	Gas chromatography

G:F	Gain to feed ratio
h	Hour
K_2EDTA	Potassium ethylene diamine tetra-acetic acid
LCFA	Long chain fatty Acid
Mcal	Mega calories
MP	Metabolizable protein
MUFA	Mono unsaturated fatty acid
NaFl	Sodium fluoride
NDF	Neutral detergent fiber
NFC	Non-fibrous carbohydrate
NH_3-N	Ammonia nitrogen
OM	Organic matter
PUFA	Poly unsaturated fatty acid
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RIA	Radioimmunoassay
RUP	Rumen undegradable protein
SCFA	Short-chain fatty acid

SE	Standard error
SEM	Standard error of the mean
SFA	Saturated fatty acid
SOY	Soy oil treatment
TMR	Total Mixed Ration
Trt	Treatment
VFA	Volatile fatty acid
wk	Week

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

ABSTRACT

EVALUATION OF FLAX OIL SUPPLEMENTATION ON PERFORMANCE OF GROWING DAIRY HEIFER CALVES AND LACTATING COWS

CHELSEA ROSE SCHOSSOW

2019

Utilization of flax oil is new to the livestock industry, and limited research has been done to see the benefits of feeding the highly unsaturated oil to both dairy calves and lactating cows. Two studies were conducted to evaluate the potential benefits of flax oil as a lipid supplement. The first study compared the supplementation of two plant based oils to growing dairy calves. Thirty-six female Holstein calves in individual hutches were used in a 12-wk randomized complete block design study. Treatments were: 1) control (**CON**) with no oil, 2) 80 g/d of flax oil (**FLAX**), and 3) and 80 g/d of soy oil (**SOY**). Pre-weaning the oils were fed with the milk and post-weaning the oils were top-dressed on starter pellets. Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5 and 1×/d during wk 6. Pellets and water were fed ad libitum. The calves were evaluated on body frame growth, health conditions, dry matter intake, nutrient digestibility, blood metabolite profiles, rumen fermentation, and plasma fatty acid concentrations. Dry matter intakes were greater in CON than FLAX with SOY similar to both. Calf BW and gain: feed were similar but had treatment × wk interactions. The ADG, body condition scores, withers heights and other frame measurements including hip height, heart girth, paunch girth, body length and hip width were similar among treatments. Fecal scores were similar overall but had an interaction of treatment × wk with SOY having greater fecal

scores during weaning and the last two weeks. Body temperatures and respiratory scores were similar. Plasma urea nitrogen concentrations were not different among treatments. Concentrations of plasma beta-hydroxyl butyrate were greater for the CON calves compared to the two oil treatments. Plasma cholesterol was greater in the oil treatments compared to CON. Plasma triglycerides were greater in SOY. A tendency was observed for plasma glucose to be less in SOY. Total concentration of plasma fatty acids was increased with the oil supplements and proportion of individual fatty acids were altered. Supplementing flax and soy oil maintained growth performance compared to CON in the pre-weaning period when fed with milk, but decreased intake and BW during the last two weeks of the post-weaning period when fed with starter pellets. In addition, supplementing flax oil and soy oil maintained PUN concentrations, but altered circulating lipid and energy metabolism. This study demonstrates that the supplementation of plant-based oils to young calves affects their metabolic profile, does not affect body growth, and decreases intake when compared to a diet with no oil supplementation. The second study investigated the potential benefits of supplementing encapsulated flax oil to high producing Holstein dairy cows. Eight multiparous and 4 primiparous Holstein cows (73 ± 40 d in milk, 694 ± 71 kg of body weight) were used in a 3-wk randomized complete block design study. Cows were fed a basal diet containing 52% forages and 48% concentrates on a dry matter basis. Treatments were: 1) control (**CON**) with no oil, and 2) 250 g/d of encapsulated flax oil (**FLAX**). The DMI was not affected by inclusion of the encapsulated flax oil. Milk production, milk fat and milk protein had no differences between treatments. Plasma concentrations of glucose, triglyceride and cholesterol were similar among both treatments. Rumen ammonia tended to be less in the FLAX cows. All

major VFA were similar between treatments. During the short 3 wk study minimal milk fatty acids differences were observed between treatments. However, since the cows on the FLAX treatment produced an average of 0.12 kg/d more of fat in their milk the total amount of the fatty acids was numerically greater than the CON treatment. Supplementing encapsulated flax oil, maintained lactation performance when compared to CON and increased milk fat percentage. Further research would be beneficial in both the calves and cows to validate the results of these preliminary evaluations on flax oil supplementation.

INTRODUCTION

Using byproducts and supplements in the dairy industry is gaining popularity as the price of feedstuffs continues to increase or fluctuate. Feed supplements being considered throughout all ages of dairy cattle are lipids from plant based sources. Advantages of adding lipids into dairy diets include potential increased energy intake for high milk production (Ostergaard et al., 1981; Ruesegger et al., 1985); energy utilization efficiency (Brumby et al., 1978); and aiding in the optimized starch to fiber ratio which improves rumen fermentation (Palmquist and Conrad, 1978). Feeding lipids to young calves is not only beneficial but also crucial to development. The absence of lipids in the diet can affect growth and development according to Lambert et al., (1955) and further suggested that feeding a lipid-free diet may limit growth. Lipids are broken down into fatty acids and glycerol to later be used through intestinal absorption and used as energy. Machmuller et al. (2000), reported that the inclusion of fat into ruminant diets can help improve energy efficiency as it used the fatty acids directly in the metabolic pathway of fat synthesis instead of the need for glucose and acetate as substrates.

One plant based lipid that has gained research interest is flax oil, a byproduct of linseed or flax meal. Flax is known to be one of the oldest cultivated crops and is native to the region that spans from the Mediterranean to India. The main producers of flax are Canada, the United States, and China and production used to be extensively for production of petroleum after World War II (Morris et al., 2007). The fatty acid profile of flax is highly favorable over other lipids as it contains approximately 20 % α-linolenic acid, which is an essential omega-3 fatty acid and a precursor for eicosapentaenoic acid (Conners et al., 2000). This eicosapentaenoic acid is a precursor for the formation of

eicosanoids, which are hormonelike compounds that play a significant role in the immune response of livestock. In terms of milk production, prior data indicate that the milk fatty acid profile and milk α-linolenic acid concentrations have been altered and are greater when cows are fed flax. Kennelly and Khoransani (1994), fed whole flax seed to mid-lactation Holstein cows and reported linear increases in milk long-chain fatty acids and polyunsaturated fatty acids as the amount of flax in the diet increased.

Although there are many benefits to flax oil, there are some challenges associated with lipid supplementation with flax oil. The number one contributor being that the oil is difficult to supplement to ruminants as it is roughly 89 percent unsaturated fat (Jenkins et al.,1997). This makes the oil highly susceptible to oxidation resulting in rancidity and negative effects on nutritional quality. In addition to this, since the oil is high subjectable to rumen biohydrogenation, and unsaturated fatty acids can be toxic to many rumen bacteria at high quantities. Biohydrogenation can be reduced by the same factors that affect hydrolysis. Rates of rumen biohydrogenation of the fatty acids can differ, and increased unsaturation typically leads to a quicker rate (Lock et al., 2005, 2006).

The first study that was conducted evaluated if dairy calves as, pre-ruminants, could utilize the flax oil to benefit body frame growth, average daily gain, starter intake, health parameters, rumen fermentation, blood metabolites, and plasma fatty acid concentrations. The main objective was to a conduct preliminary study to determine if flax oil has potential as a feed supplement for dairy calves. It is hypothesized that feeding flax oil will improve feeding efficiency and energy metabolism resulting in encouragement of lean growth. Additionally, it is hypothesized that due to the high

essential fatty acid profile and antioxidant properties that the flax oil would improve the health of the calves.

Knowing the intensity of biohydrogenation of the unsaturated fatty acids by fully functioning ruminants the second study utilized an encapsulated flax oil product to hopefully escape the rumen for absorption in the small intestines and provide the benefits flax oil has to offer to the cow. The objective of this study was to evaluate the potential benefits of supplementing high producing lactating cows with Nutri-flax coated™ (Nutriad Inc., Schietstandlann, Belgium) and determine how it was utilized. The hypothesis was the supplementing 250 g/d of encapsulated flax oil would improve feed efficiency, milk production and milk fatty acid composition.

CHAPTER 1

LITERATURE REVIEW

Research and evaluation of new feed ingredients are gaining popularity as the dairy industry looks to cut costs yet still produce a wholesome, delicious and nutritious product. Byproducts of currently available feed ingredients are making a greater appearance on ration sheets and in feed bins on farms. Even though the cost of these new ingredients may be lower than the ever-popular corn, soybean meal, and cottonseed, there is potential that these products are not replicating the same results when applied to animal nutrition. From newborn calf to lactating cow, dairy farmers are always looking for a new alternative. As the total world fluid milk production overflows capacity and consumption, one area of increased interest has been how to alter fat percentage and fatty acid profile of fat in milk. Increasing fat percentage in the fluid product, milk, increases the market value to cheese production. In calves, producers are looking to raise replacement heifers at a cheap cost yet still maintain growth and performance. Although lipids tend to have a higher input cost in rations, the output on calf development and future production will typically outweigh the initial input. In addition, lipids are not fully understood in their role in the ruminant animal and how they are utilized.

Calf Rearing

Current Challenges

Raising a healthy heifer calf presents many challenges. From the minute the calf is born their life is in danger as they adapt to the new environment to which they are exposed to. Early stressors include environmental stress including fluctuations in

temperature, as well as immune function play a critical role in the calf's chance at survival. Early colostrum intake is the key to survival. Calves are born with little to no defense or immunity to the vast diseases they will be exposed to throughout life. Getting high-quality colostrum into the calf within the first hour of life is essential for adequate passive transfer and future health. An estimated 31% of preweaning mortality within the first 3 weeks of life can be attributed to failure of passive transfer (Wells et al., 1996). Even with a successful passive transfer from colostrum to calf there is still room for bacteria and viruses to invade the immune system of a young calf. The most common diseases that affect calves are septicemia, diarrhea, and pneumonia (McGuirk et al., 2008). Septicemia is most common in very young calves when their body is adjusting to life outside of the womb (Virtala et al., 1996). Diarrhea or scours is typically observed in calves under 30 d of age (Svensson et al., 2006) and typically goes hand in hand with a gastrointestinal infection which can lead to dehydration as well. Pneumonia is typically observed in calves over the age of 30 d but is still a concern in young calves (Agerholm et al.,1993). Most disease can be avoided with safe bio-secure measures and proper sanitation practices.

Having passed the first hurdle, next comes the commitment to raising the calf to be a replacement heifer and enter into the lactating herd in the next two years. Throughout these two years, there will be no income from the animal, therefore survival and low costs are key. The cost of raising the replacement is influenced by two main concepts, management tactics, and the economy. From birth to weaning the average cost is $87.71 and from weaning to 6 months of age the total cost is $210.75 on average (Heinrich et al., 2016a). Sixty percent of the cost to raise a calf comes from feed and it is

important to keep this input low. Even though raising a calf on a cheap low energy pellet and poor-quality milk replacer may save a few dollars up-front the long-term income will not be as significant as the calf probably did not have enough nutrients to meet the demands of their growth and development. To many producers, it has become a balancing act on how to feed a low-cost feed yet provide the necessary nutrients for the replacement to grow and develop to their fullest potential. One way to reduce input herd costs is with the strategic selection of replacement heifers as they account for approximately 20% of the total dairy farm expenses (Heinrichs et al., 1993). The most important tool for selecting these replacements is understanding the relationship between the growth of the calf and potential future productivity (Wathes et. al., 2008).

Calf Growth and Development

From the day the calf is born until two years of age their body is constantly developing. A calf's digestive system starts very similar to that of a monogastric and will develop into a fully functioning ruminant. Newly born calves are commonly called pre-ruminants as they have the same four chambers in the stomach, yet the abomasum makes up a far larger percentage of the digestive tract than the rumen, reticulum and omasum. At birth, the omasum and abomasum take up a greater amount of space and weight than the reticulum and rumen, yet in 120 days after birth, the rumen will be about one and a half times larger than the abomasum alone (Becker et al., 1951). In addition to size, the rumen papillae also develop over time. Butyrate is the main fatty acid responsible for rumen development, as it provides the energy needed to thicken the developing rumen wall and well form papillae (Govil et al., 2017). Other than butyrate other essential volatile fatty acids in rumen development are propionate and acetate. The main microbial

groups the inhabitant the rumen are the archaea, fungi, anaerobic bacteria, and protozoa. Each of these contributes directly or indirectly to dietary organic matter degradation (Wang et al., 2017). The changes observed in the rumen, both structural and physiological are linked directly with the development of the microorganisms (Klein et al., 1987). To maximize development in the digestive system of the calf, it is beneficial to follow a high plain of nutrition.

Studies have suggested that using calf measurements for growth could be used to predict productivity later in life (Van De Stroet et. al., 2016). In addition, monitoring growth is important to gauge how well the heifer program is performing. One measurement easily used by dairy producers is body weight. Either through the use of a scale or weight tape which measures the heart girth and converts to average weight. Knowing the weight of calves will help identify how well calves are utilizing their feed. Swali and Wathes (2006) reported in their studies that heifer calves with lower body weights continued to have lower BW into first lactation.

It is possible to achieve a large range of average daily gains by changing the plan of nutrition fed to developing calves. It has been found that both feeding calves at a low ADG, or growing too slow, and feeding at a high ADG, or growing too fast, reduced milk production during lactation (Heinrichs et al., 1998). The optimal rate of gain is still highly under investigation. There are many variables that go into the optimum growth rate including genetics, breed, sex for example. Heinrichs (1987), reported that the proper rate of gain is approximately 0.8 kg per day. It is easier to continually grow a calf than try to catch up later with compensatory growth later in life. Raising a healthy calf from the beginning will provide the foundation of a profitable lactating dairy cow later in life.

In addition to body frame growth of calves, it is important to monitor the body condition of calves. Body condition scores can be used to help evaluate the nutrition and management of the heifer feeding program. Heinrichs (1987) reported that ideally for Holstein dairy calves under four months of age the goal should be 2.25. This is on a scale of 1 to 5, with 1 being emaciated and 5 being obese (Wildman et al., 1982).

Feeding Strategies for Young Calves

Every calf born on a dairy farm represents an opportunity to improve herd genetics, increase herd size, and improve the economic returns on the farm. Over the past decade how dairy replacement calves are managed and raised has changed substantially. One major area of change has been the approach to feeding. One thing that has stayed somewhat stable, however, is the demand and need for adequate colostrum consumption and management. Newborn calves are born agammaglobulinemic meaning that they depend majorly on the absorption of immunoglobulins (Ig) from their dam. This delivery is a mechanism called passive transfer as the maternal Ig's are absorbed across the small intestine, typically within the first twenty-four hours of life (Godden et al., 2008). This transfer cannot happen in utero since the placenta separated the maternal and fetal blood supplies.

Typically, within two hours after birth the dam is milked, and her colostrum is tested for quality with a brix refractometer. Generally, if the total solids concentration is greater than 23% the colostrum is deemed high enough quality to feed. This easy and inexpensive quality check can save producers money in the long run as it ensures that the calf is getting high-quality colostrum which in turn could lead to a higher passive transfer and overall better prepared calf for the life ahead.

The technique to administer this nutrient dense colostrum is dependent on producer preference. In Winder's (2018) research, he reported that 51% of respondents claimed that calves were never allowed to nurse directly from their dam after birth, however only 17% of the respondents said they always remove the calf within the first 30 minutes after birth. Direct suckling from the dam is once technique to administer colostrum, but it also sets the calf up for potential problems including early onset infection, or poor quality of the colostrum. Other techniques to deliver the colostrum are through bottle feeding or esophageal tubing. Since the calf is a pre-ruminant and acts more as a monogastric in the early stages of life, the esophageal groove acts as a bypass for the milk to be directed into the abomasum and away from the rumen. In the abomasum a majority of the nutrients are absorbed early on as openings are larger within the first 24 hours. Overall, colostrum management and feeding has not been altered too drastically over the years and still is deemed one of the most important aspects in calf rearing.

Traditionally, dairy calves are fed limited amounts of milk, either as pasteurized or replacer with an ad libitum starter for the first portion of their lives. Hafez and Lineweaver (1968) reported that feeding the limited liquid amounts, typically 8 to 10% of the birth body weight (BW) is much lower than if the calf were to be fed ad libitum milk which is at 16 to 20% of BW. Using this traditional conventional approach, the original theory was that by restricting the liquid fraction of the diet it would stimulate intake of starter feed of pellets earlier, as well, as reduce input costs. In addition, conventional feeding allows only for the maintenance requirements to be met with the addition of

about 0.23 kg/d ADG (Kertz et al.,1979). This conventional approach is very common across dairies as it keeps input cost low and still produces a healthy dairy replacement.

In contrast to the conventional feeding system that has been observed for years, some producers are making the switch to an accelerated feeding system. This system allows calves to consume a greater liquid intake early in life. Traditional milk replacers contain about 20% protein while accelerated products have 26-28 % protein available. Feeding rates in accelerated feeding systems are approximately twice as much as the limited intakes in conventional systems. Stamey et al. (2005), reported that during the first week of life 1.5% of BW is consumed as milk solids, 2% of BW from the second week until weaning. Hill et al. (2006, 2007), found that on the accelerated system calves starter intake lags behind those on the conventional system, however, once they are weaned, they increase and are at approximately the same rate. If managed properly the accelerated program can be effective as calves typically have higher growth rates during the first few weeks of life. One area of concern is at weaning. Since calves are consuming a greater liquid diet than solid pellet diet weaning can be a very stressful period. Khan et al. (2007 a,b), recommends using a step-down method where Jasper and Weary (2002) say a gradual weaning program is desirable. Both programs are successful and are up to the producer's discretion. One major component. however, is ensuring that calves are consistently eating at least 0.90 kg f starter daily to avoid a growth slump (Kehoeet al.,2007). This feeding system has a greater cost than the conventional system, but the benefit observed later in the calf's life can outweigh the initial cost. Benefits of feeding a higher liquid fraction can include reaching breeding age earlier, improved immunity and

ability to withstand infections and increased subsequent milk production (Drackley et al., 2005).

Pasteurized waste milk has gained popularity over the last few decades as it allows producers to use a readily available product that is extremely energy dense, matching the nutrient profile demanded by the calf while keeping costs low. On a dry matter basis, whole milk has greater concentrations of protein and fat (25.4, and 30.8%) when compared to commercial milk replacers where the protein ranges from 18 to 20% and the fat concentrations range from 15 to 20 % (NRC, 2001). This protein and fat nutrient intake difference reported to impact calf growth in Godden et al. (2005) when comparing a commercial 20:20 milk replacer and pasteurized milk fed to dairy calves. In addition to improved growth, Nonnecke et al. (2003) found that the immunoglobulins and non-specific immune factors that are found in whole with promote calf health. Moreover, Scott et al. (2006) reported that on average, dairy operations produce between 2.27 to 9.98 kgs of non-saleable milk each day. By utilizing this milk for calves, it allows producers to avoid potential economic losses. Overall feeding pasteurized milk has numerous benefits to calves growth, development, and health, as well as the economics of the dairy.

Choosing between the strategies in colostrum management and feeding management is at the discretion of the producer. A solid plan of nutrition will help set a young dairy calf up to be successful once she enters the milking herd.

Lipid Digestion in Calves

Feeding lipids to young calves is not only beneficial but also crucial to development. The absence of lipids in the diet can affect growth and development according to Lambert et al., (1955) suggesting that feeding a diet free of lipids lead to limited growth. Lipids are broken down into fatty acids and glycerol to be later used through intestinal absorption and use as energy. Machmuller et al. (2000), reported that the inclusion of fat into ruminant diets could help improve energy efficiency as it used the fatty acids directly in the metabolic pathway of fat synthesis instead of the need for glucose and acetate. There is a variety of lipids commonly available for use in milk replacer and supplementation to calves. Most commonly used are tallow and lard as they are commercially available and a cheaper fat product, which helps decrease total input costs. Tallows and lards contain a variety of different lipids, unsaturated oils, free fatty acids as well as rancid products (Jenkins et al., 1986). To an extent, calves can withstand high concentrations of fat in milk and replacers, but the unsaturated vegetable oils and high dietary free fatty acids can reduce calf gains and feed utilization (Jenkins et al., 1985). Corn oil, which contains high amounts of unsaturated fats, caused scours and poor performance in dairy calves (Jenkins et al., 1996). Young pre-ruminant calves have an increase of 50% to 160% in enzyme activities in the pancreas during the first month of their life (Guilloteau et al., 2009). Previous research demonstrated that pancreatic lipase activity increases with age, yet the enzyme cannot be fully expressed since colipase acts as a limiting factor (Le Huerou-Luron et al., 1992). This can explain why the pre-ruminant can utilize high lipid diets whereas the older calves cannot as their rumen begins to develop. Ruminants are considered to generally well equipped with digestive abomasal and pancreatic enzymes (Guilloteau et al., 1980). The pancreatic enzyme lipase

is important to digest lipids that are bypassing the rumen. As the rumen further develops after weaning colipase production increases and becomes a limiting factor if high amounts of lipids enter into the proximal intestine (Guilloteau et al., 2009). Lipids can be readily digested by calves throughout development yet as the enzymes in the digestive tract develop there are some limitations to the amount that will bypass the rumen and be absorbed in the abomasum and intestines.

Nearly 60% of United States dairy farms use milk replacers according to Heinrichs et al. (1995). Raven et al. (1970), reported that milk lipids would by far be the first choice to use as a lipid source in milk replacers as it is nutritionally unique and has a relatively high proportion of highly digestible medium-chain saturated fatty acids. However, in the United States, most commercial milk replacers contain lipids from animal sources, including lard, tallow and white grease. There has been speculation if there is potential to change the animal lipid content in milk replacers to alternative lipid sources such as vegetable oils. Previous studies have shown variable calf performance due to the inclusion of vegetable oils into the milk replacer. Jenkins et al. (1985), reported similar performance between calves fed milk replacers with tallow as the lipid and milk replacers with coconut oil as the lipid source. He also reported, however, that using corn oil as a substitute lipid source resulted in overall lower performance and growth of the calves. The use of vegetables oils as a lipid source is still under consideration as animal fat sources offer the cheapest source of fat and have greater results than milk replacers with cottonseed, corn and soybean oils (Raven et al., 1970).

Inclusion of lipids into the starter pellets has been commonly disregarded due to potential negative effects and influence on rumen development (Berends et al., 2018).

Additionally, the lipid provides energy to the young calf but not to the rumen bacteria. In addition, Berends et al. (2018), reported that when fat is combined with the fermentable products in a starter pellet, the fat will coat the fermentable components and limit bacteria access, which in turn decreases the potential microbial growth and rumen development. Miller et al. (1959), and Kiehn et al. (1994) both reported that increasing the fat content of a starter will reduce starter intake in growing calves, whereas Johnson et al. (1956), and Araujo et al. (2014), observed no change on starter intakes. The differences in outcomes from these studies can be attributed to the different lipid sources used. Calves prefer the taste of soybean oil compared to that of tallow or lard (Montoro and Bach, 2012).

Hill et al. (2009), reported that there is limited information available on the effects and requirements of specific fatty acids in the dairy calf. In addition, they begin their life consuming high amounts of fat through either pasteurized milk or milk replacer diets, then once weaned their diet is almost completely free of fat or at least very low (NRC, 2001). Typically, calf starter contains little to no sources of C20:4, C20:5 and C22:6, all of which serve as functional fatty acids important for neural development and production of hormones (Klein et al., 2002). However, C18:2 and C18:3 have been shown to elongate into these other functional fatty acids as previously described by Klein et al. (2002). The fatty acids C18:2 and C18:3 are commonly found in plant-based oils, especially flax. Hill et al. (2007), previously reported that a diet with increased C18:2 and C18:3 has benefits to a calf's immune system as well as increased feed efficiency and average daily gain. In relation to the immune system, Drouillard et al. (2002), fed weaned beef heifer calves no flax, 4 % tallow, 4 % flax oil, 10 % flax oil or linseed meal with 4

% tallow. He reported that the incidence of bovine respiratory disease was greater in the control fed heifers over that of the oil treated heifers.

Lactating Dairy Cattle

Current Challenges

Producing a wholesome, delicious and nutritious product, lactating dairy cattle play a large role in the dairy industry. Coined as natures perfect product, milk has been a food staple for centuries. However, with the increased research and development of plant-based milks made from nuts and grains the demand for cows milk has seen a decrease. With decrease in demand and consumption, the price of bulk milk is not ideal for any dairy cattle producer. The United States Department of Agriculture reported that the average Class I base price was $14.84 cwt for 2018. And, future milk prices are likely not to improve in the near future producers are forced to either leave the business or make budget cuts. Each year a crippling number of producers are leaving the industry due to financial struggles and making ends meet. Feed cost is the largest expense on an operation. Between 45 to 66 percent of milk income is accounted for in feed cost (Ishler et al., 2017). Since this is the largest sector it is the first area producers look to when making budget cuts to the business. This typically means removing additives and expensive energy products. Making these cuts to feed costs will temporarily help, but one must look at the long term effects on milk production, cow health, and reproduction. This is why byproducts and alternative feed ingredients have great potential to reduce feed cost.

It is challenging to understand and manage metabolic changes in dairy cows at different stages in lactation. The demand for greater milk production per cow does challenge the health and metabolism (Gross and Bruckmaier, 2019). Dairy cows begin lactation in a negative energy balance causing many to cope with the energetic requirements, which are primarily associated with the metabolic demands by the mammary gland for milk production (Drackley et al., 1999; Ingvartsen et al., 2006). This metabolic stress leads to many effects on reproductive performance, milk quality, and cow health (Bradford et al., 2015: Drackley et al., 2005). The classic approach to account for the metabolic changes was to feed the lactating cow according to her energy and nutrient requirements throughout all stages of lactation and gestation (Overton and Waldron, 2004). However, the cow can only consume a certain feed intake which in turn limits the capacity for glucose and lipid absorption and rumen performance. Requirements for metabolism are rarely met solely from feeding. This leads to a majority of the requirements to be covered by the mobilization of body reserves (Bruckmaier and Gross, 2017; Han van der Kolk et al., 2017). Despite the advancements made in veterinarian care and animal nutrition, dairy cows are still continually at high risk of developing metabolic problems. The greatest incident rates coincide with the energy deficient phase following parturition (Mulligan and Doherty, 2008). Relating back to the early challenge of limited funds and economics in the dairy industry it is hard for producers to meet these demands. Most of which can be commonly solved through high quality feed sources and concentrate supplementation, both of which are not cheap.

Feeding Strategies for High Lactating Cows

Early lactation, known as days 14 to 100, is the stage when milk production rapidly increases, and peak milk is achieved. Reaching peak milk sets up the potential milk yield for the entire lactation. During early lactation fat mobilization to meet energy demands is inevitable due to insufficient intake (Weber et al., 2013). To cover this energy demand, the mobilization of body reserves typically fat tissue, occurs (Ingvartsen and Andersen, 2000). Research has shown that improved overall milk production occurred when CP is increased in the rations of early lactating cows (Cressman et al., 1980; Forster et al., 1983; and Kung et al., 1983). In addition, early lactation cows fed diets with higher percentages of RUP have shown to have higher milk production (Cunningham et al., 1996). Cows during early lactation have a greater demand for MP in order to meet the demands of high milk production (Kalscheur et al., 1999).

The main objective during mid-lactation, between days 100 to 200 days in milk, is to maintain the peak production and maximize DMI. During this time cows should be eating about four percent of their body weight on a DM basis (Heinrichs et al., 2016c). The CP of the ration decreases from the early lactation and cows are no longer utilizing high quantities of stored reserves as they were during the early lactation. After peak production in early lactation is met the requirement for MP can be met by maximizing DMI with a lower concentration of CP (Kalscheur et al., 1999). In conjunction with this, studies in which RUP was increased in the ration during mid-lactation resulted in no dietary effects (Arieli et al., 1996; Robinson et al., 1988).

Feeds Commonly Used in Lactating Dairy Cow Diets

Dairy cows are typically fed a diet composed mostly of forages and concentrates. Forages make up the largest proportion of the dairy cows ration since the cow require a

minimum amount of fiber in the ration. This fiber is important for rumen function, fermentation, increase chewing activity all which help minimize the disappearance of ruminal and total tract digestibility nutrients. According to Staples (1992), a ration with forage of 25% corn silage, 25% alfalfa, and 50% concentrate will lead to the production of 9071.85 kgs of milk per year. Since forages contain cellulose and lignin, they are less digestible than concentrates, and contribute to rumen fill at a greater quantity.

The quality of forages fed also plays a key role in the output of milk production and metabolic activity. Early bloom alfalfa is much more desirable than full bloom. Cows consuming diets of 63% early bloom alfalfa and 37% concentrate produced equal amounts of milk than when compared to cows that were fed a lower amount of full bloom alfalfa and twice as much concentrate (Staples, 1992). Since fiber intake varies amoung cows of different ages and stages in lactation, it is important to match the fiber quantity of the ration to the demand needed by the cow. Van Soest (1963) researched how feeding restricted forage diets affected rumen VFA and showed that as milk yield increased the amount of feed fed as forages decreases as well. In comparison to this Tessman et al. (1988) demonstrated that total milk and protein production declined as the forage in the ration was increased.

In terms of utilizing lipids in the ration, forages must be present. Feeding high forage diets with increased use of lipids helps maintain the rumen function as well as give the lipids a surface to which they can adhere (Palmquist, 1987). Digestion of lipids and fermentation is typically difficult to achieve. Jenkins (1993) found that feeding a high basal diet of hay with lipid allowed for easier digestion and fermentation. In addition, Grant and Weidner (1992) recommended that in order to feed lipid successfully adequate

fiber is necessary. This was observed by Ohajuruja et al. (1991) when he fed animal and vegetable lipids, or calcium soaps up to concentrations of 5% dietary DM in high forage diets without adverse effects on ruminal digestion or metabolism.

Sometimes high forage rations are not efficiently digested due to the low fiber quality; in this scenario concentrates and highly digestible corn silage can be supplemented to increase DM digestibility and nutrient utilization (Moody et al., 2007). Concentrates commonly used in lactating dairy cow rations are corn and soybean products. In contrast, wheat and oats are less common in dairy cow rations. The use of commodity feeds has gained popularity in the past decade as producers cut costs on feed. Commodity feeds are the by-products of commonly used concentrate feeds, for example, corn gluten meal, soybean hulls, brewers' grains. Most commodities are limited in some nutrient as they are a byproduct from the production of another feed (Looper, 2014).

In addition to feed commodities, the use of alternative fed sources from the ethanol, biodiesel, or vegetable oil industry are on the rise. These alternative feed sources include distillers dried grains, distillers wet grain, canola meal, and linseed meal. Distillers grains and canola meal were researched in lactating dairy cow diets, and it was observed that they maintained performance (Schingoethe et al., 2009; Christen et al., 2010). Schingoethe et al. (1999), observed that when distillers wet grains were feed in place of soybean meal and corn it improved the efficiency of converting feed to milk which was illustrated by similar milk production between treatments with decrease intakes. Linseed meal resulted in lower value for milk production in cows when compared to soybean meal and corn distillers grains (Loosli et al., 1960). The research shows that alternative feed sources have potential for use in dairy diets aside from the

linseed meal. Reasons the linseed meal potentially did not maintain or increase milk production could be that it has a lower digestibility due to the higher oil content. Oils fed with high forage diets can coat and inhibit rumen microbe ability to penetrate and break down the forages needed for fermentation and digestibility.

Lipid Sources Used in Dairy Diets

Feeding lipids to lactating dairy cows has gained interest in the last few decades for its potential to increase the energy density in diets. The advantages of adding lipids into dairy diets include potential increased energy intake for high milk production (Ostergaard et al., 1981; Ruesegger et al., 1985), energy utilization efficiency (Brumby et al., 1978), and aiding in the optimized starch to fiber ratio which improves rumen fermentation (Palmquist and Conrad, 1978). In addition to advantages within the cow, feeding fat during warmer environmental temperatures and increased humidity may help with heat stress. Madison et al. (1994), found that feeding supplemental fat increased milk production during the summer more than it did in the winter. In addition, Skaar et al. (1989) reported that when lactating cows were fed diets supplemented with fat they had improved lactation performance. Reasons behind this are that lipids result in less metabolic heat to be dissipated and generated during digestion and metabolism than proteins and carbohydrates.

There are a variety of lipid sources that can be used including plant, animal and specialty fats. Plant-sourced fats, or oilseeds are commonly used for a dietary fat source since they provide both fiber, protein, and fat with a relatively low cost. Oilseeds can be fed whole, but in order to increase the utilization in the rumen for protein, extruding or roasting is very beneficial (Stern et al., 1985). However, after the milling process

typically there is a meal and an oil fraction left. Feeding pure vegetable oils as reported by Mohamed et al. (1988) likely reduced fiber digestion and milk fat percentage. Further research is being conducted to see if encapsulating the vegetable oil will help by-pass the rumen and be more readily available to the mammary gland and milk fat synthesis.

Animal fats, including tallow, and lard are solid at room temperature and typically have greater amounts of oleic acid (Jenkins and Jenny, 1989), as well as increased saturated fatty acids (SFA) than plant-based oilseeds (Bisphlinghoff et al.,1990). Including animal fat to high fiber diet increased the milk fat and decreased concentrations of short and medium chain fatty acids (Lu et al., 1993).

Specialty fats are those that are manufactured and developed to decrease the detrimental effects on rumen fermentation and fiber digestion (Palmquist and Jenkins, 1982). These specialty fats are commonly sold as rumen bypass fats or inert fats. The most commonly used inert fats are Energy Booster 100, containing 98 % total fatty acids and comprised mainly of stearic acid (Milk Specialty Co., Dundee, IL) and Megalac, made of free fatty acids and calcium (Church and Dwight Co. Inc., Princeton, NJ). The difference among animal fats, plant source fats, and these specialty fats is that specialty fats contain mostly saturated fats compared the unsaturated fats that are found in animal and plant source fats. Using these specialty fats Jenkins and Jenny (1989) reported that the saturated fats led to fewer negative effects on production of milk fat, rumen fermentation, and feed intakes.

Milk Fat Synthesis

Milk fat is considered one of the most palatable food components (Luick et al., 1960). The demand for milk fat has led to breeding and selecting cows for higher milk fat production. Carlo Foa is considered to be the first individual to use perfused udders to study the synthesis of milk fat. His original hypothesis was that the udder absorbed certain triglycerides from the blood and SCFA originate in the udder and were synthesized from the non-carbohydrate sources (Foa et al., 1912). This was thought to be where milk synthesis took place until 1940 when Asimoff and Kashevaroff, reported that SCFA went through a completely different pathway (Nikitin et al., 1949). They said that the mammary gland absorbs large amounts of *beta*-hydroxy butyric acid from the blood. Later this was proved by Shaw and Knodt (1941) when they observed that the *beta*-hydroxybutyric absorbed was enough to account for synthesis of milk fatty acids from C_4 through C_{14}. Milk fat is the most variable milk component and can be affected by environmental and physiological factors (Bauman et al., 2006). Synthesis of fatty acids occurs in the cytoplasm of the cell. Each cycle through the malonyl-CoA pathway results in the addition of two carbons being added to the fatty acids chain, forming and synthesizing milk fat and the fatty acids. Acetyl CoA is known as the carrier protein for these fatty acids and elongation of the fatty acids stops at C16 however the enzyme called acyl thioesterases is responsible for cleaving the formed fatty acid from the acetyl CoA carrier protein (Bauman and Grinari, 2000). Acetyl CoA carboxylase is the key milk fat synthesis enzyme in the mammary gland which is increased during lactation. Nutrition is the primary factor that affects milk fat and acts as a tool to help alter the fatty acid profile. Milk fat composed of fatty acids which vary in chain length and carbons come

from the uptake of preformed fatty acids and synthesis of new fatty acids inside the mammary gland.

An increase in trans 18:1 in milk fat content is associated with milk fat depression (Davis and Brown, 1970). Further research by Bauman and Griinari (2003) proposed that as the diet changes the rumen biohydrogenation will alter to produce unique fatty acid intermediates which can act as inhibitors to milk fat synthesis. Another potential inhibitor of milk fat synthesis is the CLA isomer *trans*-10, *cis*-12 and provides an interrelationship between the digestive process in the rumen with the metabolism in the mammary gland which in turn regulates the synthesis of milk fat (Bauman, 2003)

Rumen Lipid Metabolism

As nutritionists continue to increase energy density in diets to meet requirements of dairy cows producing high volumes of milk, there is the recognition that fatty acids both dietary and of rumen origin have significant effects on rumen metabolism. In addition, specific fatty acids that are synthesized in the rumen act as potent regulators from milk fat synthesis in the mammary gland. A large portion of lipid metabolism occurs in the rumen through hydrolysis of the esters in lipids and biohydrogenation of the unsaturated fatty acids (Bauman and Lock, 2006). Hydrolysis of the lipids in the diet occurs based on the bacteria present in the rumen. The extent to which the lipids hydrolysis can be reduced as the amount of fat in the diet increases, and when low pH and ionophores are present, they inhibit the growth and activity of bacteria (Harfoot and Hazelewood, 1997). Since unsaturated fatty acids are toxic to many rumen bacteria the next transformation of lipids in the diet is biohydrogenation of the PUFA. Biohydrogenation can be reduced by the same factors that affect hydrolysis. Rates of

rumen biohydrogenation of the fatty acids can differ, and increased unsaturation typically leads to a quicker rate (Lock et al., 2005: 2006). Lactating dairy cows can easily hydrogenate linoleic and linolenic to the extent of 70 to 95% and 85 to 100%, respectively (Doreau and Fearlay, 1994). Linoleic acid is very common in the rations of dairy cows, in contrast, little amounts of stearic acid are fed, yet we see a reciprocal increase in the steric acid that flows to the duodenum with the increase in dietary linoleic and oleic acids (Bauman and Lock, 2006).

The use of dietary lipid can suppress methane emissions in ruminants, however the role of bacteria, archaea, fungi, and protozoa in this process of mediating lipids effects is still unclear (Wang, et al., 2017). Methane is a natural by-product of rumen fermentation and the formation is reliant on the proper function of the rumen and is caused by the accumulation of hydrogen (Kumar et al., 2014). Since methane emissions are a concerning topic in regard to global warming, changes in diets, livestock management, and genetic selection are currently a large focus in research and the public. Nutritional study strategies have shown dietary lipids and used of poly-unsaturated fatty acids (PUFA) to decrease the hydrogen availability in the rumen (Beauchemin et al., 2008; and Hristov et al., 2013). The PUFA would increase biohydrogenation which would limit the growth of new archaea which could potentially slow down the release of methane.

When calcium soaps from palm fatty acids or canola are fed, they are termed as rumen-protected. This protected term does not mean that they are protected from rumen biohydrogenation, but simply they have minimal effects on the microbial population of the rumen (Palmquist, 2006). Basically, the feeding of protected unsaturated fatty acids

will protect against the adverse effect on microbial fermentation, but the bypass of the fatty acids to the duodenum will not increase (Lundy et al., 2004). Overall dietary lipids once in the rumen go through extensive hydrolysis and biohydrogenation which leaves the rumen free of highly unsaturated fatty acids as they rapidly exit. Milk fat is dependent on many factors but having an understanding of the interactions of rumen fermentation in regard to dietary fat will help troubleshoot some commercial problems.

Lipid Absorption in the Small Intestine

A vast majority of lipids are digested in the small intestine and 5 % occurs in the duodenum, 20% in the upper jejunum, 25% in the mid and lower jejunum, and the last 50% are digested in the ileum (Leat and Harrision, 1975). There are four parts in the absorption and transport of lipids in the small intestine including, effects of bile and pancreatic secretions on the lipid, absorption of lipid into intestinal epithelial cells, metabolism and transport of the absorbed lipid, and the cellular exit and transport to the lacteals of the lymphatic system (Mattoson et al., 1952). Free fatty acids that pass out of the rumen are usually attached to feed particles. The formation of micelles is important in fat absorption as this is the form that fatty acids can be absorbed through the gut wall. Once passed through the gut wall they are converted in triglycerides where they develop into chylomicrons and lipoproteins. Once developed they can enter the lymphatic system which delivers them to the body tissues to be use as either an energy source, milk fat, or for adipose fat deposition (Bauchart et al.1993).

Bioactivities of Fatty Acids

Largely, it has been determined that the lipid components of flax have beneficial health effects. In addition, since flax oil is primarily made up of PUFA, roughly 89 percent (Jenkins, et al. 1997), there is a chance that it could affect the production of inflammatory mediators including eicosanoids and cytokines, both of which stimulate epithelial cell proliferation (Ruthig et al., 1999; Hankenson et al., 2000). Other than immune response dietary fatty acids can also impact the oocyte quality and development in lactating cattle. Since fatty acids are high sources of energy, they have a large impact on the structure and function of biological membranes (Cribier et al., 1993). Lucy et al., 1993 and Mattos et al., 2000 found that supplementation of the dairy ration with lipids increased the total number of follicles as well as stimulated the growth and size of the preovulatory follicle. Moreover, the increased fatty acid precursor availability in the diet is paired with increased steroid and eicosanoid secretion (Wang et al., 2005). Increasing dietary fat has shown positive influence in the concentration of prostaglandins, and steroid hormones (Staples et al., 1998). However, feeding of the fatty acids should be monitored as diets enhanced with linoleic acid during the prepartum period have been reported to increase retained placenta incidences (Barnouin et al., 1991). In contrast to this, alpha-linolenic acid fed in the postpartum period improved pregnancy rate (Kassa et al., 2002).

Lipid Research Potential

Many research studies have been conducted over the years on the lipids generally supplemented into dairy diets, including corn oil, tallow, lard, and soybean oil. A peak in interest has begun to look at other plant-based oils that might have greater effects on lowering methane production, increasing immunity, and improving reproduction. In

addition, with humans watching their health now more than ever, the increase for omega-3 enriched foods are on the rise. Flax oil, a byproduct of linseed meal is gaining popularity. However, since this product is highly unsaturated this has led to confusion on how to include this oil appropriately in rations for dairy calves and cows. New attempts to encapsulate the product or to feed the product to calves prior to development of the rumen to avoid biohydrogenation and hydrolysis of the butyl esters should be made and research conducted

Use of Flax oil

Flax

Flax is known to be one of the oldest cultivated crops and is native to the region that spans from the Mediterranean to India. The main producers of flax are, Canada, the United States and China and production used to be extensively for production of petroleum after World War II (Morris et al., 2007). The crop is a spring annual with a 90 to 100 day growing season consisting of a 45 to 60-day vegetative period, then a 15 to 25-day flowering period and a 30 to 40 maturation period (Oplinger et al., 1989). Ensuring that flax is harvested at the correct time is very important as an early harvest can result in reduced yields. A late harvest will alter the chemical make-up of the oil resulting in a decrease in quality and value. The plant itself is considered an excellent companion crop to grasses and legumes because it has short stature allowing light to reach forage seedings and a less extensive root system which allows for limited competition for soil moisture (Oplinger et al., 1989). Once in the flowering stage, the flax flower, blue in color, has five petals and five-celled capsules that contain the flax seeds, and up to 10 seeds by maturity (Oelke et al., 1987).

Benefits of Flax

Flax contains about 20 percent α-linolenic acid, which is an essential omega-3 fatty acid as well as a precursor for eicosapentaenoic acid (Conners et al., 2000). This eicosapentaenoic acid is a precursor for the formation of eicosanoids, hormonelike compounds that play a significant role in the immune response of livestock. Addition research by Conners et al. (2000), suggested that the eicosapentaenoic acid can elongate into docosahexaenoic acid, which is another omega-3 fatty acid essential for brain and eye health as well as cell membrane integrity. Thompson et al. (2004), reported that flax is the richest plant source of the lignan precursor secoisolariciresionol diglycoside. This is converted to mammalian phytoestrogens by the rumen microorganisms which are thought to have potential use in hormone replacement and possibly prevent cancer (Harris and Haggerty, 1993). Another benefit is the potential to enhance the fluid milk by increasing the content of alpha-linolenic acid, which not only is in important to human nutrition but is commonly found in high quantities in flax (Institute of Medicine, 2002). The conjugation of linolenic acid has been shown to inhibit the promotion, and progression of malignant tumors in livestock (Kelley et al., 2007).

Feeding Flax to Livestock

Compared to most grains and oilseeds, the composition of flax is extremely variable based on variety and environmental factors (Daun et al., 2003). Protein values range from 18.8 to 24.4 % (Daun and Pryzbylski, 2000). Flax is 94% DM with 35% lipid and CP of 22.8% (Lardy and Anderson, 1999). Compared to soybean meal the DM is comparable, lipid content is significantly higher, and the CP content is about half. Since flax is high in oil, it can be considered as a fat source for livestock. As mentioned

previously, fat is traditionally used to increase the energy density of the diet without using expensive protein sources. In addition, flax with such a high oil content can help reduce dust, eliminate fines as well as aid in the processing of pellets acting as a binder (Byers and Schelling, 1988).

As for affects in milk, on multiple accounts changes in the milk fatty acid profile and milk α-linolenic acid concentration have been altered including that of total fatty acid profile and concentrations of fatty acids that lead to the most health benefits. Kennelly and Khoransani (1994), fed whole flax seed to mid-lactation Holstein cows and reported that linear increases in milk long-chain fatty acids and polyunsaturated fatty acids were increased as the level of flax in the diet increased. In comparison, Goodridge et al. (2001) reported that in the milk alpha-linolenic acid linearly increased with the inclusion of dietary flax.

In feeding calves for health effects Drouillard et al. (2002) reported that when weaned heifer calves were fed either 10 percent flax meal, 4 percent flax oil, 4 percent tallow or no fat after 40 days the incidences of bovine respiratory disease was greatest in the control group. Additionally, looking into reproduction Petit et al. (2001) reported that dairy cows fed 17 percent flax compared with dairy cows fed other sources of fat had greater conception rates. They attributed this increased response to the better maintained energy balance for the cows on the flax-fed diet. Hill et al. (2009), reported that when feeding flax oil to calves and increase in ADG and starter intake was observed. Feeding flax to preruminants may be an option as Karcher et al. (2014) observed that calves fed flax oil had improved feed efficiency in the first 28 days, however, post weaning this improvement was not sustained.

Overall, feeding flax to dairy cattle can be a useful source of nutrients as it is high in protein and fat and is a great source of essential fatty acids.

Oil Extraction

Since the flax seed contains about 40 percent oil and 60 percent meal, the extraction of oil is beneficial in maximizing the production as well as improving the oil quality. Improving the quality enhances the contents of fatty acids that are present. The main extraction process is by mechanical cold press. In this method, the meal is separated from the oil with low initial operation costs as well as eliminating contaminants (Cakaloglu et al., 2018). Advantages of the cold press include a quick process which can use small quantities of raw oilseeds and the by-product is a protein-rich meal (Singh et al., 2000). The disadvantage to this system is that the oil yield is not as high as the solvent extraction method leaving approximately 7 percent of the oil in the seed (Savoire et al., 2013). The cold press can be classified as expellers which were first given their name in 1902 by Anderson. This is the most common type of cold press used as the oil is separated from the meal through the rotating cold metal bars which are moving at a constant speed (Uitterhaegen et al., 2017). By using cold the press extraction method, the oils are suitable for direct consumption and do not need to be refined.

Encapsulation of Flax Oil

Vegetable oils, in general, are being used as a vehicle for encapsulation of lipids. Since flax oil is roughly 89 percent unsaturated fat (Jenkins et al.,1997) the lipid is highly susceptible to oxidation resulting in rancidity and negative effects on nutritional quality. In order to prevent negative effects from the lipid and decrease biohydrogenation, it is

important to encapsulate the highly unsaturated flax oil with a nonessential oil including that of corn oil, soybean oil, and other popular vegetable oils. Encapsulated flax meal goes through a process in which the end-product is a bead. One study compared an unencapsulated oil to an encapsulated flax oil in which the encapsulated fat did not undergo oxidation (Can Karaca et al., 2013).

Conclusions

Raising a successful replacement from calf to lactating cow comes with many challenges and there are numerous strategies to consider. In consideration of calves, frame growth, mammary gland development, and rumen development are top areas to consider when designing a calf management practice. Average daily gain is primarily used as a good indication whether calves are growing at a consistent rate. Careful consideration should be taken though as it has been found that both feeding calves at a low ADG or growing too slow, and feeding at a high ADG or growing too fast, reduced milk production during lactation (Heinrichs et al., 1998). The optimal rate of gain is still highly under investigation as there are still many unknowns in the world of calf nutrition.

At birth, the calves omasum and abomasum take up a greater amount of space and weight than the reticulum and rumen. Over the first 120 days after birth, the rumen develop into a fully functioning organ and will be about one and a half times larger than the abomasum alone (Becker et al., 1951). It is crucial during this time to develop not only the rumen size but also the papillae and microbe ability. The changes observed in the rumen, both structural and physiological are linked directly with the development of the microorganisms (Klein et al., 1987). Development of these microorganisms depends on their ability to thrive in a healthy environment. The production of acetate, butyrate and

propionate is crucial in feeding the archaea, fungi, anaerobic bacteria and protozoa which aid in the digestion of fibers and concentrates that will be eventually fed to the developing heifer.

Calves begin their life on a high milk diet, with about 20% of their dry matter intake being fat, however after weaning the (NRC, 2001) recommends a diet with low to no dietary fat. Research has shown the increased dietary fat can increase energy and is a cheaper source of energy than crude protein sources. Machmuller et al. (2000), reported that the inclusion of fat into ruminant diets could help improve energy efficiency as it used the fatty acids directly in the metabolic pathway of fat synthesis instead of the need for glucose and acetate. Lipids, however, should be used in moderation as negative effects can occur including reduction of rumen fermentation and ammonia production.

Unlike the young calf, the lactating cow has a fully functioning rumen. Main areas of concern when feeding lactating cows is balancing the ration for maximum milk production, while still keeping the cow healthy and in good body condition. Dairy cows are typically fed a ration composed mostly of forages and concentrates. Forages typically make up the highest proportion of the dairy cows ration as the cow requires a minimum amount for fiber in the ration to optimize rumen fermentation and dry matter digestibility.

The interest of using lipids in dairy cow rations has increased in recent years Advantages of adding lipids include: potential increased energy intake for high milk production (Ostergaard et al., 1981; Ruesegger et al., 1985), energy utilization efficiency (Brumby et al., 1978), and aiding in the optimized starch to fiber ratio which improves rumen fermentation (Palmquist and Conrad, 1978). In addition to this the bioactivates from the fatty acids found in many plant-based oils have been known to have beneficial

effects in lactating cows. In regard to reproduction efficiency, Lucy et al., 1993; and Mattos et al., 2000, supplemented lipids in the rations of dairy cows and reported to increase the total number of follicles as well as stimulate the growth and size of the preovulatory follicle. Moreover, the increased fatty acid precursor availability in the diet is paired with increased steroid and eicosanoid secretion (Wang et al., 2005). Other potential effects of oils are a potential reduction in methane emissions. Nutritional study strategies have shown dietary lipids and used of poly-unsaturated fatty acids (PUFA) to decrease the hydrogen availability in the rumen (Beauchemin et al., 2008; and Hristov et al., 2013).

The utilization of newer plant-based oils, like flax, still requires continued research to determine inclusion rates and how to get the greatest benefits out of the highly unsaturated oil. Flax oil has the potential to work well in rations for all ages of dairy cattle. Alpha-linolenic acid is considered to be one of the most essential fatty acids as it can elongate into other essential fatty acids responsible for the most health benefits particularly in calves. In addition, if we can find a way to keep the alpha-linolenic acid from intense biohydrogenation in the rumen there is potential for increased omega-3 concentration in milk.

CHAPTER 2

EVALUATION OF FLAX OIL AND SOY OIL SUPPLEMENTATION ON HOLSTEIN HEIFER CALVES

ABSTRACT

Flax meal has been commercially used in the livestock industry as an alternative feeding ingredient; however, the oil extruded during milling has limited research in livestock feeding. The objective of this research was to investigate supplementing flax or soy oil in milk and then on starter pellets on growth performance, health, rumen fermentation and metabolic profile of dairy calves. Thirty-six female Holstein calves in individual hutches were used in a 12-wk randomized complete block design study. Treatments were: 1) control (**CON**) with no oil, 2) 80 g/d of flax oil (**FLAX**), and 3) and 80 g/d of soy oil (**SOY**). Pre-weaning the oils were fed with the milk and post-weaning the oils were top-dressed on starter pellets. Calves were fed 2.83 L of pasteurized milk 2×/d during wk 1 to 5 and 1×/d during wk 6. Pellets and water were fed ad libitum. Fecal scores (0 = firm, 3 = watery) and respiratory scores (healthy ≤ 3, sick ≥ 5) calculated from the sum of scores for rectal temperature, cough, ocular, and nasal discharge were recorded daily. Body weight (**BW**) and frame growth were measured weekly at 3 h post morning feeding. Once weekly at 4 h post-feeding blood was collected from the jugular vein for metabolite analyses. Rumen fluid was collected via esophageal tube on wk 12 for a subset of six calves per treatment. Results were analyzed using the MIXED procedure of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$. Dry matter intakes were greater ($P < 0.01$) in CON than FLAX with SOY similar to both. There was a treatment × wk interaction ($P < 0.01$) with calves on FLAX eating less in the

last two weeks of the study. Calf BW and gain: feed were similar ($P = 0.38$) but had treatment × wk interactions ($P < 0.05$). The ADG, body condition, withers height and other frame measures were similar ($P > 0.05$) among treatments. Fecal scores were similar overall but had an interaction of treatment × wk ($P < 0.01$) with SOY having greater fecal scores during weaning and the last two weeks. Body temperature and respiratory score were similar ($P > 0.05$). Plasma urea nitrogen concentrations were not different among treatments. Beta-hydroxyl butyrate was greater ($P < 0.01$) for the CON calves compared to the two oil treatments. Plasma concentrations of cholesterol were greater ($P < 0.01$) in the oil treatments compared to CON. Plasma triglycerides ($P < 0.01$) were greater in SOY. A tendency ($P = 0.05$) was observed for plasma glucose to be less in SOY. Total concentrations of plasma fatty acids were increased in SOY and FLAX compared to control and proportions of several individual fatty acids were altered. Supplementing flax and soy oil maintained growth performance compared to CON in the pre-weaning period when fed with milk, but decreased intake and BW during the last two weeks of the post-weaning period when fed with starter pellets. In addition, supplementing flax oil and soy oil maintained PUN concentrations, but changed several lipid and energy metabolites and fatty acid profiles. This study demonstrates that the supplementation of plant-based oils to young calves affects their metabolic profile, does not affect body growth, and decreases intake when compared to a diet with no oil supplementation.

Keywords: flax oil, dairy calf, growth performance, metabolic profile

INTRODUCTION

Lipids are bioactive compounds whose full role in the pre-ruminant animal is not fully understood. However, calves at an early age are exposed to a high-fat milk replacer or milk-based diet of roughly 20% or more fat on a dry matter basis. In addition, research on the use of products high in dietary fatty acid content to alter growth, performance, immune response, and average daily gain (ADG) are limited (Ballou and DePeters, 2008: Hill et al., 2011). Hill et al. (2007a), found increased ADG and feed efficiency in young calves transitioning from pre-ruminant to ruminant when diets included starters with sources of linoleic acid and alpha-linolenic acid.

Linoleic acid and linolenic acid, both highly unsaturated fatty acids undergo extensive biohydrogenation in the rumen yet have many health benefits. These are the only two essential fatty acids that elongate into other beneficial fatty acids (Klein, 2002). Some of these benefits include increased neural development and hormone synthesis. These essential fatty acids are typically found in lipids derived from plants including soybean and flax oil. Alpha-linolenic makes up roughly 55 % of the total fatty acid oil content of flax oil (Petit et al., 2002, 2003).

Lipids indirectly influence the fiber inaccessibility to rumen microorganisms by coating the feed particles. In addition, since there is a high rate of biohydrogenation of polyunsaturated fatty acids (PUFA), found primarily in oilseeds, it is possible that they reduce the substrates needed for production of archaea in the rumen (Czerkawski and Clapperton, 1984). Since calves preweaning utilize the esophageal groove to shunt nutrients past the rumen into the small intestine the highly unsaturated lipids could have

greater benefits to the young calf prior to the development of the rumen in order to avoid biohydrogenation.

The main objective of this study was to determine if flax oil, a byproduct of linseed meal, has potential as a feed supplement for dairy calves. It was hypothesized that feeding flax oil will improve feeding efficiency and energy metabolism resulting in encouragement of lean growth. Additionally, it is hypothesized that due to the high essential fatty acid profile and antioxidant properties that the flax oil will improve the health of the calves.

Materials and Methods

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee under welfare assurance number A3958-01 and protocol number 17-062E.

Experimental Design

Thirty-six newborn female Holstein calves were used in a 12-wk randomized complete block design experiment. Calves were blocked by birthdate and randomly assigned to 1 of 3 treatments. Treatments were: 1) control (**CON**) with no oil, 2) 80 g/d of flax oil (**FLAX**), and 3) and 80 g/d of soy oil (**SOY**). The study was conducted from August 2017 to February 2018 at the South Dakota State University Dairy Research and Training Facility in Brookings, SD. The calves were housed in individual hutches (Calf-Tec, Hampel Animal Care, Germantown, WI) measuring 223 cm long, 98 cm wide, and 133 cm high. In addition, each hutch had an outdoor exercise area. Hutches were placed

on a dirt pad and bedded with wheat straw. Calves overall average birthweight was 38.2 ± 4.4 kg.

Animal Care and Feeding

Calves were fed 3.78 L of their dams first colostrum immediately at birth. Another 2.83 L was offered at a second feeding, 6 hours later. The Brix 0 to 32% optical refractometer (JO351B; Jorgensen Laboratories Inc., Loveland, CO) was used to determine quality of the colostrum. The total serum protein (6.5 ± 0.7 g/dL) was checked with a refractometer (J-351; Jorgensen Laboratories Inc., Loveland, CO.) to provide an accurate reading of the passive transfer of the maternal immunoglobulins. All calves enrolled in the study had a total serum protein reading above 6.5 g/dL.

The soy oil had a density of 0.917 g/mL and was obtained through the South Dakota State University Feed Mill. The flax oil had a density of 0.93 g/mL and was received from Stengel Oils of Milbank, SD. Both oils were stored in a cool dark place to avoid rancidity and oxidation.

The experimental period was 12 wk consisting of a pre- (first 7 wk) and post- (last 6 wk) weaning periods. Week 0 was used as a covariate period. Due to the number of calves born each month and staggered start dates the total duration of the farm portion was seven months. Preweaning calves were fed oils with the milk and post-weaning the oils were top-dressed on the starter pellets. During the preweaning period, calves were fed 2.83 L of pasteurized waste milk 2 x/d during wk 1 to 5 and 1 x/d during week 6 to serve as a weaning period. Throughout the entire 12 wk period, calves were fed starter pellets and water ad libitum. All calves were fed the same starter pellet (Table 2.1).

Individual calf pellet and milk intakes were taken and recorded daily throughout the study.

Sample and Data Collection

A sample of the pasteurized milk was sampled once weekly and analyzed immediately for composition with DairySpecFT (Bentley Instruments, Inc. 4004, Peavey rd, Chaska, Minnesota). An additional milk sample was taken and stored at -20°C until further analysis of milk fatty acids. A starter pellet sample was collected for nutrient analysis once weekly and stored at -20°C for nutrient analysis.

Health scores were recorded daily before evening feeding and followed the criteria developed at the University of Wisconsin School and Veterinary Medicine (McGuirk, 2005). On a scale from 0 to 3 clinical signs were observed from normal to abnormal including rectal temperature, coughing, nasal and ocular discharge as well as fecal consistency (0=firm and 3= watery). The sum of scores for rectal temperature, cough, nasal and ocular discharge was calculated for the total respiratory score, as described by McGuirk (2005). Minimum total respiratory score of 0 for a completely healthy calf and a maximum of 12 for an abnormally sick calf. Rectal temperatures were measured using a digital probe thermometer (DeltaTrak, Pleasanton, Ca; model 11064).

Once weekly, 4 h post morning feeding, calves were measured for body frame size. Measurements included the hip width, withers height, hip height, heart girth, paunch girth, and body length. Body length was measured from the point of the withers to the end of the ischium. Calf body weight was also taken at the same time as growth measurements and was used to determine feed efficiency. Body condition score (BCS),

on a scale of 1 to 5, with 1 being emaciated and 5 being obese, was observed and recorded by 3 independent individuals in conjunction when growth measurements were taken (Wildman et al., 1982).

Blood samples were collected 3 to 4 h post morning feeding via venipuncture of the jugular vein using vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ). Three vacutainers contained potassium ethylene diamine tetra-acetic acid (K_2EDTA) and 1 vacutainer contained sodium fluoride (NaFl). The K_2EDTA samples were used to analyze plasma beta hydroxyl butyrate (BHB), cholesterol, triglyceride, and plasma urea nitrogen (PUN). The NaFl tube was used to analyze plasma glucose as described under laboratory analysis. After collection of the blood, samples were placed on ice immediately and processed within 2 h. The blood vacutainers were centrifuged at 1,000 x g for 20 min at 4°C (CR412 Jouan Inc., Winchester, VA). The plasma was transferred with a disposable plastic pipette into polystyrene storage tubes and frozen at -20°C until further analyses.

To analyze total tract digestibility of DM, CP, and fiber fecal grab samples were taken from the calves at wk 12. The samples were collected at 0430, 0630, 1200, 1630, and 1800 h over 3 d and frozen at -20°C until further processing and analyses.

On wk 12, rumen fluid from a subsequent group of calves, 6 from each treatment, was collected via esophageal tubing. The samples were collected at approximately 3-4 hours post morning feeding for analysis of pH, VFA, and ammonia concentrations. The first 50 mL of the rumen fluid was discarded in an attempt to minimize saliva contamination. In total over 15 mL of rumen fluid was collected into a stainless-steel cup and the pH of the sample was analyzed immediately (Waterproof pH Testr 30, Oakton

Instruments, Vernon Hills, IL.) An aliquot sample of 5 mL was mixed with 100 µl of 50% sulfuric acid for determination of rumen ammonia nitrogen (NH_3-N), and an aliquot sample of 5 mL was mixed with 1 mL of 25% meta-phosphoric acid for determination of VFA. Both samples were stored at -20°C until further analysis.

Laboratory Analysis

The weekly starter pellets were combined into monthly composites for nutrient analyses. The fecal grab samples that were collected at wk 12 were composited on and equal wet weight basis from each collection time point for each calf and each collection week. Starter pellet ingredients, starter composites, and fecal samples were dried in duplicate for 48 h at 55°C in a Dispatch Oven (V-23, Dispatch Oven co., Minneapolis, MN). The dried samples were ground to 2 mm using a Wiley Mill (model 3; Arthur H. Thomas Co., Minneapolis, MN), then ground again to 1 mm using an ultracentrifuge mill (Brinkman Instruments co. Westbury, NY). A 1 g aliquot of each sample were dried for 3 h in a 105°C oven (Model 28, Precision Scientific Co. Chicago, IL) to correct analyses to 100% DM (AOAC, 1998; method 935.29). Ash content of the samples was determined by incinerating 1 g samples for 8 h at 450°C (AOAC International, 2002; method 942.05) in a muffle furnace (Model F1730, Thermolyne Corp., Dubuque, IA; temperature controller Model Wheelco 293, Barber-Colman Co., Rockford, IL). After ash determination, the organic matter (OM) was calculated as OM = (100 - % ash). For analysis of NDF (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) the Ankom 200 fiber analysis system was used (Ankom Technology Corp., Fairport, NY). For the NDF procedure, heat stable sodium sulfite and alpha-amylase was used. For apparent total-tract digestibility calculations, acid detergent-insoluble ash (ADIA) was

used as an internal marker. The ADIA contents of the feed and fecal samples were determined by analyzing the samples for ADF, as previously described, and then obtaining the ash content which was determined by incinerating the Ankom F57 bags for 8 h at 450° C in a muffle furnace. The apparent total-tract digestibility was calculated as described by Merchen et al. (1988). Ether extracts were analyzed using petroleum ether (AOAC 1998; method 920.39) in Ankom XT10 fat analysis system (Ankom Technology corp., Fairport, NY). Non-fibrous carbohydrate (NFC) was calculated as % NFC= 100 - (% Ash + % CP + %NDF + % EE) according to the NRC (2001). Samples of monthly composited calf starter pellets were sent to a commercial laboratory for mineral and starch analysis (Dairyland Laboratories Inc., Arcadia, WI). Mineral analyses included Ca, P, Mg, K, Na (method 985.01), S (method 923.01), and Cl (method 915.01; AOAC International, 1998). Starch concentration was found using a modified method of glucose analysis (Bach Knudsen, 1997) completed on an YSI 2700 Select Biochemistry Analyzer (YSI Biochemistry Analyzer, YSI Inc., Yellow Spring, OH).

Blood metabolites, glucose, cholesterol, BHB, PUN, and triglycerides were analyzed using commercially available colorimetric and enzymatic assay kits on a spectrophotometer (Cary 50, Varian INC., Walnut Creek, CA) with a microplate reader. Glucose oxidase reagent used to analyze the serum glucose concentrations is described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed according to the procedure by Allain et al. (1974) using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI). Plasma BHB concentration was determined by using BHB dehydrogenase and diaphorase according to the method described by Williamson et al. (1962) (Cat. #:H7587; Pointe Scientific, Inc., Canton, MI).

To determine PUN diacetyl monoxime was used (procedure 0580; Stanbio Laboratory, Boeme, TX). Plasma triglyceride was analyzed with glycerol phosphate oxidase after hydrolysis of lipoprotein lipase as described by Fossati and Prencipe (1982) that paired the reaction with the classic Trinder (1959) reaction (Cat. #T7532; Pointe Scientific, Inc., Canton, MI). Plasma samples from wk 9 and 12 samples were analyzed for circulating concentrations of estradiol-17β using radioimmunoassay (RIA) as described by Perry et al., (2004).

For rumen fluid analysis, the samples were thawed and vortexed to completely mix contents before transferring 2 ml into a microcentrifuge tube which was centrifuged at 30,000 × *g* for 10 min in a micro centrifuge (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Concentration of ammonia-N was analyzed, according to the assay described by Chaney and Marbach (1962). Gas chromatography was used to analyze VFA concentrations, with the use of an automated gas chromatograph (Model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d × 15 m column (Nukol 24106-U, Supelco, Inc., Bellefonte, PA). Analysis of VFA was done using 2-ethylbutyrate as an internal standard. The gas flow rate was 1.3 ml/min of helium, and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Milk fatty acids were prepared as butyl esters according to Abdelqader et al. (2009) with some modifications. The milk samples were centrifuged at 3,500 g for 20 mins at 4°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.) Fifteen to 20 mg of fat from the fat pad was removed and added into a 13x 100 mm Pyrex extraction tube fixed with a Teflon-lined screw cap. The addition of 500 µL of butanol and 25 µL of internal standard (2-tridecenoic acid, Nu-Chek Prep Inc., Elysian, MN) was added to each

extraction tube. While adding 50 µL of acetyl chloride the samples were slowly vortexed. Tubes where then gases with N_2 for 4 seconds and placed on a dry heating block for 90 min at 60°C. Once samples had cooled to room temperature the addition of 5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 2,000 x g for 20 min at 4°C. After centrifugation, the bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 3x with 5 mL of distilled water and centrifuged in between each wash at 2,000 x g for 20 min at 4°C. After the final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

Blood fatty acids were prepared as butyl esters as according to Abdelqader et al. (2009) with some modifications. One milliliter of plasma was added into a 16x100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap. 300 µL distilled water, 1.5 mL chloroform and 3.0 mL methanol were added to extraction tube and vortexed for 2 min. Additionally, 1.5 mL of chloroform was added and vortexed. One and a half milliliters distilled water waster was added and vortexed. Samples were centrifuged for 20 min at 3000 g at 10°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.). Using a Pasteur pipette the upper layer of the sample was removed. Additionally, 2 mL of chloroform was added vortexed and again centrifuged for 20 min at 3000 g at 10°C. The bottom layer containing the chloroform and fat were removed and added to a clean 16 × 100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap. An additional 1 mL of chloroform is added to the 16x 100 mm extraction tube to remove any remaining fat

from the sample. The tube is vortexed and centrifuged for 20 min at 3000 g at 10°C. The lower portion of the tube is combined with the previous lower layer in the 13x100 mm tube. Using forced air, the samples in the 13x100 mm extraction tubes are dried, evaporating the chloroform and leaving behind the fat from the sample. After samples are dry the addition of 500 µL of butanol and 25 µL of internal standard (2-tridecenoic acid, Nu-Chek Prep Inc., Elysian, MN) was added to each extraction tube. While adding 50 µL of acetyl chloride the samples were slowly vortexed. Tubes where then gases with N_2 for 4 seconds and placed on a dry heating block for 90 min at 60°C. Once samples had cooled to room temperature the addition of 5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 2,000 x g for 20 min at 4°C. After centrifugation, the bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 3x with 5 mL of distilled water and centrifuged in between each wash at 2,000 x g for 20 min at 4°C. After the final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

Feed fatty acids were prepared using similar techniques to Sukhija and Palmquist (1988) with some modifications. The concentration of lipid is adjusted so that 20 to 25 mg of lipid is concentrated in 750 µl of butanol. The adjusted concentration of feed was then added into a 16x100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap and of 750 µl of butanol was added. The addition of 25 µl of the internal standard, C19:0, as well as 75 µl of acetyl chloride while mixing the extraction tube at a low speed. Tubes

where then gases with N_2 for 4 seconds and placed on a dry heating block for 90 min at 100°C. Once samples had cooled to room temperature the addition of 7.5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 1,500 x g for 20 min at 10°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.). Using a Pasteur pipette the upper layer of the sample was transferred to a clean 13x100 mm tube fixed with a Teflon-lined screw cap. 5 mL of distilled water was added to the 13x100 and vortexed before centrifuged for 20 min at 1,500 x gat 10°C. After centrifugation, the bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 4x with 5 mL of distilled water and centrifuged in between each wash at 1,500 x g for 20 min at 10°C. After the final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

The milk, blood and feed fatty acid samples were analyzed using the GC (model 6890, Hewlett-Packard, Palo Alto, CA). The injector port was at 230°C with a split ratio of 100:1 and helium as the carrier gas at 2.0 mL/min column flow-rate. The 100 m in length column had an inside diameter of 0.25 mm (Supelco 2560, Supelco Inc). Initial column temperature was set to 50 °C and held constant for 5 min, then the temperature was increased to 145°C at a rate of 5°C/min. The temperature of 145°C was held for 30 min and then increased to 190°C at a rate of 10°C/min and held for 30 min. The final temperature increase was 210°C with a rate of increase of 5°C/min and held for a final 40 min. The analyzed fatty acids were identified through the order of elution and comparison

to known standards that were commercially prepared (GLC-60, GLC-68D, and GLC-566, Nu-Chek Prep Inc.)

Statistical Analysis

Data for growth measurements, intakes, blood and rumen fluid analyses were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC) as a randomized complete block design experiment with week as the repeated measure and calf (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, and the interactions of all terms. Changes over time for the frame growth measurements and ADG were calculated by the differences for the one-wk intervals. Gain to feed (G:F) ratio was calculated as the ratio of ADG to DMI for each treatment. For all variables analyzed with repeated measures 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 models. MIXED procedures of SAS were used for analysis of data for the total tract digestibility of nutrients. The model included only treatment with block included as a random variable. For all results, significant differences between treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Least square means for each treatment are reported in the tables and were separated using Tukey's test.

RESULTS AND DISCUSSION

Feed Analysis

All treatments were fed the same basal pellet and pasteurized milk with the only difference being the additional oil supplemented to the SOY and FLAX assigned calves.

Calf starter pellet formulation on a DM basis can be found in Table 2.1. The composition of nutrients of the basal pellets is presented in Table 2.2. The pellets fed are primarily corn and soybean-based. Previous research looking at palatability of protein in calf pellets suggested that calves prefer soybean meal when compared to other protein sources, including canola meal (Montoro and Bach, 2012; Miller-Cushon et al., 2014). Drackley et al., (2008), reported that since there is such a high importance in encouraging solid feed intake at an early age, palatability of feed is a top priority when formulating rations for young calves. Studies have suggested that there is little to no advantage in providing a starter pellet with a CP greater than 18 to 20 % (Hill et al., 2007a; Stamey at al., 2012). Comparing the nutrient composition of pellets fed to calves on Hill et al., (2009), the CP is slightly higher than the control pellet they fed. The NDF in the current studies ration is also greater than all treatment pellet diets reported in Miller-Cushon et al. (2014).

The composition of pasteurized milk is found in Table 2.3. Since newborn calves primarily live off a liquid diet for the first few weeks of their life, it is highly important to ensure the nutrient density of the milk is meeting the demands of the growing calf. The total solid content of milk ranges anywhere between 5 to 15 %, Foley and Otterby, (1978) reported that feeding pasteurized milk above 12 % total solids is suitable for newborn calves. Comparing the milk used in the current study at 12.80 % total solids the milk is suitable for use in calves. Research by Butler at al. (2000) and Stabel et al. (2001) showed that pasteurization of waste milk was effective to kill and destroy harmful bacteria including Mycoplasma sp. and Mycobacterium paratuberculosis.

Growth Performance

Through the random treatment assignments within blocks, calves from all three treatments had similar body weights at wk 0 which is found in Table 2.4. Overall, there was no treatment effect for BW and the mean BW were similar between treatments. However, during wk 12 calves on the CON treatment tended ($P = 0.09$) to have a greater BW (104.1, 99.4, and 101.8 kg/BW ± 1.51) respectively for CON, FLAX, and SOY. Mean ADG was 0.78 kg/d for CON calves, 0.73 kg/d for FLAX and 0.76 kg/d for SOY. Calves fed similar diets to the CON and FLAX treatments in Hill et al. (2009) reported lower ADG than calves of the current study. They did show an increase in ADG as the flax oil increased in the starter. Hill et al. (2007), observed that as the flax oil inclusion increased in the starter, intake also increased. Results in the current study contradicted their results in that DMI (1792, 1568 and 1637 g/d; SEM = 77.56 for CON, FLAX, and SOY, respectively) were greater ($P = 0.11$) in CON than FLAX with SOY similar to both. Any increase in intake from adding flax oil is unlikely the common response of decreased intake and ADG when larger quantities of oil are added to calf starters (Caffrey et al., 1998; Doppenberg and Palmquist, 1991).

Body Frame Growth

Calf body frame sizes and growth were similar among all treatments (Table 2.5). During wk 12 CON calves tended to have a greater body length ($P = 0.11$) than the FLAX and SOY calves. For all previous weeks they were comparable to one another. Body condition score BCS (Table 2.5) tended to be greater ($P = 0.14$) for CON calves than the FLAX and SOY. Previous research by Senevirathne et al. (2018), feeding a similar diet to dairy calves, and similar age range are comparable to the calf frame growth

measurements of the current study. In a study by Karcher et al., (2014), when feeding calves milk replacer with 2% flax oil, compared to a control diet, no significant BW gain or BCS were different between treatments. Their results are similar to the current study, where no differences among treatments were reported for BW and BCS.

Fecal Scores, Rectal Temperatures, Respiratory Scores

Overall, fecal scores tended ($P = 0.07$) to be lesser (firmer) for calves on the CON diet when compared with calves on the FLAX and SOY diet (Table 2.6). In addition, there was a treatment by week interaction ($P < 0.01$) at wk 3, 4, and 7 where SOY-fed calves had greater fecal scores than CON and FLAX. Weekly fecal scores slowly decreased from wk 1 to wk 6 over the preweaning period (Figure 2.2). During wk 7, fecal scores from FLAX and SOY treatments increased during the first week of post weaning. This could be the result of changing the oil addition from in the milk to top dressing the starter pellets. For the remainder of the post weaning period, all treatment fecal scores decreased and were similar to one another. Hill et al. (2009), observed that when feeding calves different percentages of flax or fish oil compared to a control diet there were not differences between fecal scores. However, the reported scores from their study are greater than the calves in the current study. Results contrasted findings of Ballou and DePeters (2008), who fed a mix of corn and canola oil, fish oil, and a control diet. Body rectal temperature was similar between all treatments over the duration of the study. Temperatures were similar to Hill, et al. 2011, who also noticed no differences among treatments for rectal temperature. Due to the increase in fecal scores from the oil fed calves total respiratory scores tended to be greater ($P = 0.09$) during the pre-weaning and post-weaning periods. Calved on FLAX had slightly greater respiratory scores than the

SOY and CON calves during the pre-weaning period. Overall, all calves were healthy for the duration of the study.

Rumen Fermentation

The method of collection of rumen fluid via esophageal tube is recognized to be less than ideal for a variety of reasons; however, since this is preliminary research looking at rumen fermentation with the supplementation of different plant-based oils, this method was utilized. Rumen fermentation data is presented in Table 2.7. The CON calves had a greater pH ($P < 0.01$) than the FLAX and SOY fed calves. Rapidly fermentable carbohydrates, like the ones found in calf starter, can cause a decrease in ruminal pH which is also associated with inflammation (Kleen et al., 2003; Gozho et al., 2005). For total VFA, CON calves had significantly greater ($P < 0.01$) VFA production than calves on FLAX and SOY. This is possibly due to the greater intake in the CON calves causes leading to higher VFA production (Quigley et al., 1991). Increased ruminal concentration of VFA and absorptive metabolic activity due to increased feed intake indicated greater use of the VFA during the transition to ruminant digestion (Anderson et al., 1987; Quigley et al., 1985; and Sutton et al., 1963). Total VFA production was similar to results of Terre et al. (2013), when calves were fed a commercial milk replacer and starter pellet. Calves on the SOY and CON treatment had greater butyrate concentrations ($P = 0.02$) than the FLAX calves. Increased butyrate production in the developing rumen has been researched and proven to be responsible for functional ruminal epithelial tissue development (Sander et al., 1959). The FLAX and SOY calves had greater ($P < 0.01$) isovalerate concentrations than the CON calves. Calves supplemented with FLAX tended ($P = 0.05$) to have greater concentrations of valerate than CON and SOY calves. Other

VFA measured including acetate, propionate and the acetate to propionate ratio were similar amongst all treatments.

Apparent Total-Tract Digestion of Nutrients

During the final wk of the study, wk 12, fecal samples were collected to determine apparent total-tract digestibility of nutrients, these results can be found in Table 2.8. Crude protein (CP) digestion was significantly greater ($P = 0.04$) for the FLAX calves. Dry matter digestibility tended to be greater ($P = 0.13$) for FLAX calves in addition to OM ($P = 0.14$) and NDF ($P = 0.13$) over that of the CON and SOY calves. The ADF digestibility was similar among all treatments. The apparent total-tract digestion of OM, CO, ADF and NDF in the CON calves were similar to the control calves of Khan et al. (2007) and Senevirathne et al. (2017). Ghasemi et al. (2017), fed calves either palm oil, soybean oil, tallow, a mix or a control diet. Similar results were reported that the palm oil fed calves had greater CP and DM digestion compared to the control and soybean treatment. Moreover, Hill et al. (2015), reported that calves fed starter grain supplemented with soybean oil had reduced intake and digestibility.

Blood Metabolites

Blood metabolite concentrations are reported in Table 2.9. Concentrations of PUN were similar among all treatments. Calves on the current study had greater concentrations of PUN than calves researched by Hayashi et al., (2006). Calves on their study showed an increase of PUN over the duration of 24 wk study. A similar trend was observed in the current study as calves in post-weaning stage exhibited a greater PUN concentration than pre-weaning. Significant difference among treatments was observed for BHBA with

calves on the CON treatment having greater ($P < 0.01$) plasma concentrations than calves on the oil treatments. Figure 2.5 displays the change of plasma BHBA concentrations over the duration of the study. Butyrate is the main volatile fatty acid responsible for rumen development, as it provides the energy needed to thicken the developing rumen wall and as well as form papillae (Govil, 2017). Since calves on the CON had greater concentrations of BHBA throughout the study this could mean that they have greater rumen development than calves on the oil treatments. Quigley et al., (1994) reported an increase in BHBA over time when feeding dairy calves but his reported concentrations were less than the concentrations of the current study. Calves on FLAX and SOY treatments had greater ($P < 0.01$) plasma concentrations of triglycerides over that of the CON calves. Plant-based oils are made up of highly unsaturated fats, typically medium-chain triglycerides, are not limited to oxidation in the mitochondria as long-chain triglycerides (Graulet et al. 2000). Since calves on the FLAX and SOY treatments were given greater amount of lipids in their diet, it makes sense that they have greater plasma concentration of triglycerides. In addition, FLAX and SOY calves also had greater ($P < 0.01$) concentrations of cholesterol than the CON calves. Figure 2.3 compares the plasma cholesterol concentrations throughout the study among treatments. In young calves, high fat diets have stimulated the secretion of lipoproteins (Piot et al., 2000). In addition, cholesterol is primarily synthesized from products of lipid metabolism in the liver of calves (Drackley et al., 2005). Cholesterol is the starting material for biosynthesis of steroid hormones, including estradiol, progesterone, and cortisol (Hu et al., 2010). Increasing the plasma cholesterol concentration could potentially increase steroid production and mammary development. Sinha and Tucker (1969), reported that prior to 3

months of age the calf's mammary gland grows similar to the body in an isometric growth. In contrast, Brown et al. (2005) reported that mammary epithelial cells could be influenced as early as the preweaning stage. Since calves on the oil fed diets had increased cholesterol, it could be the reason why we observed a numerically greater concentration in plasma estradiol concentration in week 9 and 12. The CON and FLAX calves tended ($P = 0.05$) to have greater concentrations of glucose than the SOY calves. The pre-ruminant calf is reliant on glucose as the primary energy substrate, increased plasma glucose can correlate with increases ADG (Quigley et al., 1991). A similar trend was observed in Akbarian-Tefaghi, et al. (2017), with CON calves having greater concentrations of glucose compared to calves supplemented with essential oils or monensin.

Plasma Fatty Acid Analysis

Results for plasma fatty acid concentrations of samples collected in wk 0, 6, and 12 can be found in Table 2.10. Typically, calf starters are low in C18:2 and C18:3 which have shown improvements in ADG and feed efficiency in previous research (Hill et al., 2007). Alpha-linolenic acid is an essential fatty acid that is converted into omega-3 fatty acids which produce high health benefits in livestock (Albin, 2015). The only two essential fatty acids that have been able to elongate into other functional fatty acids which are important for development and hormone synthesis are C18:2 and C18:3 (Klein, et al., 2002). The primary fatty acid in flax oil is α-linolenic, making up 55 % of the total fatty acids in the oil (Petit, 2002, 2003), which could potentially attribute to effects of development, feed efficiency, hormone synthesis and increased ADG. In the current study for linoleate, C18:2; was significantly different among treatments with SOY calves

having greater ($P < 0.01$) proportions than the FLAX and CON calves. In relation to previous statements made for alpha-linolenic, FLAX calves had statistically greater ($P < 0.01$) C18:3 proportions compared to the SOY and CON calves. A statistical increase ($P < 0.01$) in C18:3 is observed at wk 6 for the FLAX treatment. This can be explained by where the inclusion of the oil was fed to the calves. During the first six weeks of the study, the flax oil was fed directly in with the milk. This would cause the oil to flow through the esophageal grove shunting into the small intestine and avoiding the biohydrogenation that takes place in the rumen. During the post-weaning period, although still statistically greater ($P < 0.01$), the concentration of C18:3 in the FLAX-fed calvesdecreases. This is due to the biohydrogenation in the developing rumen taking the highly unsaturated fat and breaking it down for utilization. Since plant-based oils are comprised mainly of unsaturated fat (Jenkins, 1997) it makes sense that the FLAX and SOY calves had statistically greater ($P < 0.01$) PUFA content compared to the CON calves. In contrast to this, CON calves had statistically greater ($P < 0.01$) proportions for the saturated fatty acids. There was a statistical treatment effect in that SOY and FLAX calves had greater ($P < 0.01$) concentrations of total fatty acids in their plasma over that of CON. In addition, during wk 6 calves on the FLAX treatment had statistically greater ($P = 0.01$) concentrations of total fatty acids found in the plasma over that of the other two treatments. In wk 12 SOY calves tended to have greater ($P = 0.08$) total fatty acid concentrations than the other two treatments. Plant-based oils are made up of numerous fatty acids which makes sense why calves on the two oil treatments exhibited higher concentrations of total fatty acids in their blood plasma.

CONCLUSIONS

Supplementing flax and soy oil maintained growth performance compared to CON in the pre-weaning period when fed with milk, but decreased intake and BW during the last two weeks of the post-weaning period when fed with starter pellets. In addition, supplementing flax oil and soy oil maintained PUN concentrations, increased cholesterol concentrations, decreased BHBA, but caused different responses compared to CON for triglycerides and glucose. Calves fed flax oil also had greater DM and CP digestibility which is potentially due to the low intake, allowing feed to ferment longer in the developing rumen with a slower passage rate. The addition, of flax oil to the pre ruminants diet showed increased α-linolenic acid through the duration, especially during the weaning period. Since α-linolenic, found in plasma fatty acids, leads to numerous health benefits this could potentially help the immune system of the calf during the weaning period. The weaning period is typically a high-stress period as the calf transitions from a highly palatable liquid diet where a majority of energy is derived from the milk fats and proteins into glucose for energy to a ruminant consuming greater amounts of starter pellets responsible for rumen development and the production of VFA. This study demonstrates that the supplementation of plant-based oils to young calves affects their metabolic profile, does not affect body growth, and decreases intake when compared to a diet with no oil supplementation. Overall, the hypothesis that calves would have increased feed efficiency and encouragement over that of the CON fed calves was proven wrong, since all treatments had similar feed efficiency and growth performance.

ACKNOWLEDGEMENTS

This research was funded by Stengel oils of Milbank, SD with additional support by the SDSU Ag Experiment Station. In addition, the authors would like to thank the South Dakota State University Dairy Research and Training Facility farm crew and fellow students for their assistance during the farm trial. This research contributes to the goals of the USDA North Central Cooperative Research project NC-2042: management Systems to improve Economic and Environmental Sustainability of Dairy Enterprises. The research also contributes to goals of the USDA HATCH project SD00H461-13: Evaluation of different dietary energy sources on growth performance and metabolism of replacement dairy heifers.

Table 2.1. Pellet formulation on DM basis

Ingredients	**% of DM**
Wheat Middlings	40.05
Corn Grain, Ground, Dry	26.96
Soybean Meal	21.11
Soybean Hulls	6.25
Molasses, dried	3.41
Mineral Mix (PGC 20-10)	1.77
Salt	0.44

Table 2.2. Nutrient composition of basal pellets fed to dairy calves on a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil

Nutrients[1]	**Pellets**
DM, %[2]	89.39 ± 0.09
Ash[2]	6.69 ± 0.05
OM[2]	93.30 ± 0.51
CP[2]	19.58 ± 0.19
EE[2,4]	3.42 ± 0.07
ADF[2]	13.02 ± 1.55
NDF[2]	23.95 ± 0.71
NFC[2,3]	49.94 ± 1.26
Starch[2]	27.98 ± 0.50
Ca[2]	0.70 ± 0.06
P[2]	0.92 ± 0.02
Mg[2]	0.33 ± 0.002
K[2]	1.40 ± 0.01
S[2]	0.26 ± 0.002
Na[2]	0.27 ± 0.01
CI[2]	0.55 ± 0.02
DCAD, mEq/100g[2]	15.36 ± 0.51

[1] % of DM, unless otherwise indicated.
[2] Results from analysis of weekly composites.
[3] % NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[4] Ether extract, analyzed with petroleum ether.

Table 2.3. Nutrient composition of pasteurized milk fed to dairy calves on acontrol diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Nutrient[1]	**Pasteurized milk, %**
Fat	3.84 ± 0.63
Protein	3.22 ± 0.34
Total Protein	3.41 ± 0.35
Lactose	4.77 ± 0.14
Total Solids	12.80 ± 0.81
Total Solids Non-fat	8.91 ± 0.32

[1] Analyzed on DairySpec FT (Bently Insruments, Inc. Chaska, MN)

Table 2.4. Body weights, ADG and Gain:Feed of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Item	Treatment				*P*-values		
	CON	FLAX	SOY	SEM	Trt	wk	Trt × wk
BW, kg							
Mean	69.8	68.0	68.2	1.20	0.50	<0.01	0.43
Wk 0	36.3	38.6	39.6	1.30	0.17		
Wk 6	65.5	65.1	64.5	1.51	0.92		
Wk 12	104.1	101.8	99.4	1.51	0.09		
DMI, kg							
Mean	1791.96	1568.29	1637.35	77.57	0.11	< 0.01	< 0.01
Wk 0	685.47	762.66	749.44	137.88	0.91		
Wk 6	1152.76	1060.95	1058.44	118.98	0.80		
Wk 12	3408.31	3025.43	3215.55	123.44	0.08		
ADG, kg/d	0.78	0.76	0.73	0.03	0.43	<0.01	0.66
Gain:Feed	0.561	0.550	0.542	0.014	0.67	<0.01	<0.01

Table 2.5. Frame growth measurements of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Item	Treatment				*P*-values		
	CON	FLAX	SOY	SEM	Trt	wk	Trt × wk
Wither Height, cm							
Mean	82.09	82.44	82.96	0.68	0.68	<0.01	0.58
Wk 0	70.75	71.87	73.24	0.84	0.11		
Wk 6	82.58	82.93	83.08	1.33	0.96		
Wk 12	85.89	90.82	92.46	1.33	0.85		
Change, cm/d	0.164	0.220	0.244	0.05	0.49		
Hip Height, cm							
Mean	86.07	86.22	87.01	0.68	0.58	<0.01	0.69
Wk 0	75.92	76.73	77.25	1.08	0.66		
Wk 6	86.44	86.97	86.98	1.26	0.94		
Wk 12	95.34	93.85	96.30	1.26	0.63		
Change, cm/d	0.230	0.211	0.238	0.013	0.34		
Body Length, cm							
Mean	73.32	72.19	73.59	0.63	0.25	<0.01	0.95
Wk 0	59.75	62.09	62.41	1.01	0.12		
Wk 6	72.64	72.66	72.44	0.94	0.98		
Wk 12	85.32	82.60	84.55	0.94	0.11		
Change, cm/d	0.296	0.244	0.269	0.015	0.07		
Heart Girth, cm							
Mean	92.40	92.86	93.29	0.61	0.58	<0.01	0.68
Wk 0	75.92	76.72	77.25	1.09	0.66		
Wk 6	92.63	93.79	92.74	1.57	0.95		
Wk 12	107.22	105.84	106.84	1.57	0.81		
Change, cm/d	0.367	0.340	0.357	0.009	0.17		
Paunch Girth, cm							
Mean	101.58	100.02	102.40	0.91	0.18	<0.01	0.46
Wk 0	75.42	77.36	77.58	1.41	0.47		
Wk 6	97.31	97.14	99.22	1.38	0.49		
Wk 12	125.06	122.14	125.30	1.38	0.20		
Change, cm/d							
Hip Width, cm							
Mean	18.93	18.87	20.70	0.89	0.29	<0.01	0.46
Wk 0	14.66	15.24	15.61	0.36	0.17		
Wk 6	18.52	18.60	18.98	2.89	0.99		
Wk 12	23.38	23.20	23.79	2.89	0.99		
Change, cm/d	0.101	0.095	0.099	0.004	0.59		
BCS							
Mean	2.44	2.41	2.49	0.027	0.141	<0.01	0.31
Wk 0	1.74	1.75	1.80	0.046	0.460		
Wk 6	2.35	2.43	2.35	0.046	0.345		
Wk 12	3.08	2.96	2.98	0.046	0.136		

Table 2.6. Fecal scores, temperatures and respiratory scores of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Item	Treatment				*P*-values		
	CON	FLAX	SOY	SEM	Trt	wk	Trt × wk
Fecal score[2]							
Mean	0.57	0.61	0.68	0.07	0.48	<0.01	<0.01
Preweaning	0.73	0.82	0.86				
Postweaning	0.46	0.46	0.57				
Temperature, °C							
Mean	39.38	39.44	39.42	0.06	0.39	<0.01	0.25
Preweaning	39.43	39.48	39.44	0.10			
Postweaning	39.37	39.40	39.41	0.09			
Respiratory score[1]							
Mean	2.45	2.55	2.52	0.51	0.30	<0.01	0.24
Preweaning	2.45	2.58	2.53	0.09			
Postweaning	2.45	2.52	2.51	0.09			

[1] Respiratory score = temperature + nasal score + eye score + cough score (McGurik, 2005)
[2] Scale of 0 to 3 with 0 being firm (normal) and 3 being watery

Table 2.7. Rumen fermentation characteristics of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Item	Treatment				*P*-values
	CON	FLAX	SOY	SEM	Trt
pH	5.52	6.72	6.47	0.082	<0.01
Ammonia-N, mg/dL	17.38	17.26	18.29	1.836	0.90
Total VFA, mM	154.78	102.96	92.65	10.71	<0.01
Acetate	71.79	51.46	42.88	6.309	0.01
Propionate	64.29	40.04	37.48	3.207	<0.01
Butyrate	16.73	9.27b	10.20	1.656	<0.01
Isovalerate	1.726	1.876	1.860	0.248	0.88
Valerate	0.246	0.316	0.232	0.047	
VFA, mM/100mM					
Acetate	46.50	48.70	46.24	1.181	0.26
Propionate	41.59	40.21	40.61	1.344	0.54
Butyrate	10.66	8.84	10.86	0.684	0.02
Isovalerate	1.08	1.933	2.046	0.204	<0.01
Valerate	0.161	0.318	0.241	0.041	0.05
Acetate:Propionate	1.119	1.240	1.147	0.078	0.38

Table 2.8. Week 12 apparent total tract digestion of nutrients dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

	Treatment				*P*-values
Item, % digested	CON	FLAX	SOY	SEM	Trt
DM	68.19	75.60	69.19	2.307	0.13
OM	70.64	75.66	71.56	2.178	0.14
CP	68.06	75.57	71.49	2.585	0.04
NDF	33.01	40.41	41.61	4.077	0.13
ADF	40.55	47.48	38.46	4.857	0.28

Table 2.9. Blood metabolites of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

Item	Treatment				*P*-values		
	CON	FLAX	SOY	SEM	Trt	wk	Trt × wk
Triglycerides, mg/dL	26.27	27.04	32.14	1.25	<0.01	<0.01	0.74
Glucose, mg/dL	106.93	105.61	99.95	2.15	0.05	<0.01	0.63
Cholesterol, mg/dL	36.93	52.26	55.97	2.29	<0.01	<0.01	<0.01
BHBA[1], mg/dL	57.39	49.83	50.95	1.70	<0.01	<0.01	0.04
PUN[2], mg/dL	11.38	10.48	10.70	0.36	0.21	<0.01	0.13
Estradiol[3] ng/mL	1.77	2.01	1.74	0.11	0.17	<0.01	0.80

[1]BHBA = Beta Hydroxyl butyrate
[2]PUN = Plasma Urea Nitrogen
[3]Only analyzed for week 9 and 12 samples

Table 2.10. Proportions of plasma fatty acids of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

	Treatments				*P*-values		
Fatty Acid	CON	FLAX	SOY	SEM	Trt	wk	Trt × wk
C4:0							
Mean	2.021	1.656	1.552	0.094	<0.01	<0.01	0.18
Wk 0	2.607	2.532	2.795	0.204	0.63		
Wk 6	1.711	1.320	1.371	0.113	0.03		
Wk 12	2.331	1.992	1.733	0.113	<0.01		
C5:0							
Mean	6.719	5.248	4.908	0.328	<0.01	<0.01	0.14
Wk 0	8.046	7.541	8.626	0.700	0.54		
Wk 6	5.621	4.158	4.455	0.420	0.04		
Wk 12	7.812	6.338	5.362	0.420	<0.01		
C14:0							
Mean	1.058	0.659	0.627	0.058	<0.01	<0.01	<0.01
Wk 0	3.494	3.029	3.204	0.244	0.41		
Wk 6	1.621	0.955	0.927	0.085	<0.01		
Wk 12	0.496	0.364	0.387	0.085	0.42		
C16:0							
Mean	12.986	10.841	11.641	0.280	<0.01	<0.01	<0.01
Wk 0	20.928	20.334	21.901	0.792	0.36		
Wk 6	15.179	12.149	12.996	0.324	<0.01		
Wk 12	10.613	9.533	10.286	0.324	0.06		
C16:1 cis 9							
Mean	0.848	0.661	0.472	0.051	<0.01	<0.01	<0.01
Wk 0	2.174	2.319	2.549	0.232	0.51		
Wk 6	1.113	0.655	0.488	0.061	<0.01		
Wk 12	0.583	0.667	0.456	0.061	0.05		
C18:0							
Mean	12.009	11.087	11.710	0.255	0.04	<0.01	0.08
Wk 0	10.546	10.336	9.978	0.268	0.31		
Wk 6	11.192	10.124	10.378	0.303	0.04		
Wk 12	12.825	12.049	13.041	0.303	0.06		
C18:1 cis 9							
Mean	9.046	8.258	7.545	0.367	0.02	<0.01	<0.01
Wk 0	20.856	20.583	21.921	0.859	0.50		
Wk 6	11.712	8.625	7.550	0.619	<0.01		
Wk 12	6.381	7.891	7.540	0.619	0.20		
C18:1 cis 11							
Mean	4.222	3.627	3.692	0.209	0.10	<0.01	0.28
Wk 0	2.647	2.934	2.933	0.192	0.48		
Wk 6	1.352	1.175	1.071	0.278	0.76		
Wk 12	7.092	6.080	6.314	0.278	0.03		
C18:2 cis 9, 12							
Mean	35.984	37.186	45.608	0.682	<0.01	0.02	<0.01
Wk 0	13.317	13.639	12.152	1.465	0.74		
Wk 6	35.726	35.236	49.853	0.843	<0.01		
Wk 12	36.243	39.137	41.363	0.843	<0.01		
C18:3 alpha							
Mean	1.742	12.059	1.354	0.946	<0.01	<0.01	<0.01
Wk 0	2.049	4.903	1.210	0.823	<0.01		
Wk 6	2.858	17.760	1.857	1.057	<0.01		
Wk 12	0.625	6.359	0.851	1.057	<0.01		

C20:3							
Mean	0.950	0.535	0.725	0.061	<0.01	<0.01	0.2
Wk 0	0.965	1.028	1.189	0.117	0.37		
Wk 6	0.818	0.444	0.489	0.070	<0.01		
Wk 12	1.083	0.627	0.960	0.070	<0.01		
C20:4							
Mean	2.654	1.692	2.106	0.147	<0.01	<0.01	<0.01
Wk 0	2.651	2.644	3.054	0.241	0.38		
Wk 6	3.341	2.269	2.403	0.161	<0.01		
Wk 12	1.967	1.115	1.808	0.161	<0.01		
C20:5							
Mean	1.783	1.389	1.220	0.336	0.48	<0.01	0.28
Wk 0	0.584	0.476	0.446	0.078	0.42		
Wk 6	0.284	0.728	0.362	0.510	0.80		
Wk 12	3.282	2.051	2.079	0.510	0.16		
Others[1]							
Mean	7.325	6.228	6.458	0.356	0.10	<0.01	0.56
Wk 0	9.079	7.705	8.042	0.560	0.21		
Wk 6	6.730	5.531	5.448	0.453	0.09		
Wk 12	7.920	6.926	7.469	0.453	0.32		
Saturated							
Mean	37.036	31.379	32.330	0.763	<0.01	0.06	0.02
Wk 0	48.173	45.932	48.803	0.935	0.09		
Wk 6	37.517	30.310	31.627	0.855	<0.01		
Wk 12	36.555	32.447	33.044	0.855	<0.01		
MUFA							
Mean	17.023	15.603	14.508	0.525	<0.01	<0.01	<0.01
Wk 0	30.063	29.215	31.054	1.185	0.54		
Wk 6	16.970	13.193	11.603	0.748	<0.01		
Wk 12	17.076	18.012	17.413	0.748	0.67		
PUFA							
Mean	45.719	53.507	52.199	0.964	<0.01	<0.01	<0.01
Wk 0	21.764	24.854	20.142	1.906	0.21		
Wk 6	45.291	56.985	56.537	1.172	<0.01		
Wk 12	46.147	50.030	49.300	1.172	0.05		
Total ug/ml							
Mean	1267.91	1589.97	1615.08	87.9793	0.01	<0.01	0.36
Wk 0	983.70	959.05	910.48	79.0858	0.79		
Wk 6	1578.14	2011.59	1930.41	107.36	0.01		
Wk 12	957.68	1168.36	1299.76	107.36	0.08		

1 Others = sum of C6:0, C10:0, C12:0, C14:1, C15:0, C15:1, C16:1 trans, C16:1 cis 12, C17:0, C17:1, C18:1 trans 6, C18:1 trans 9, C18:1 trans 10, C18:1 trans 11, C19:0, C18:3 gamma, C20:0, C20:1, C20:2, C22:0, C22:1, C22:2, C22:3, C22:4, C22:5, C22:6.

Figure 2.1. Pellet intakes of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

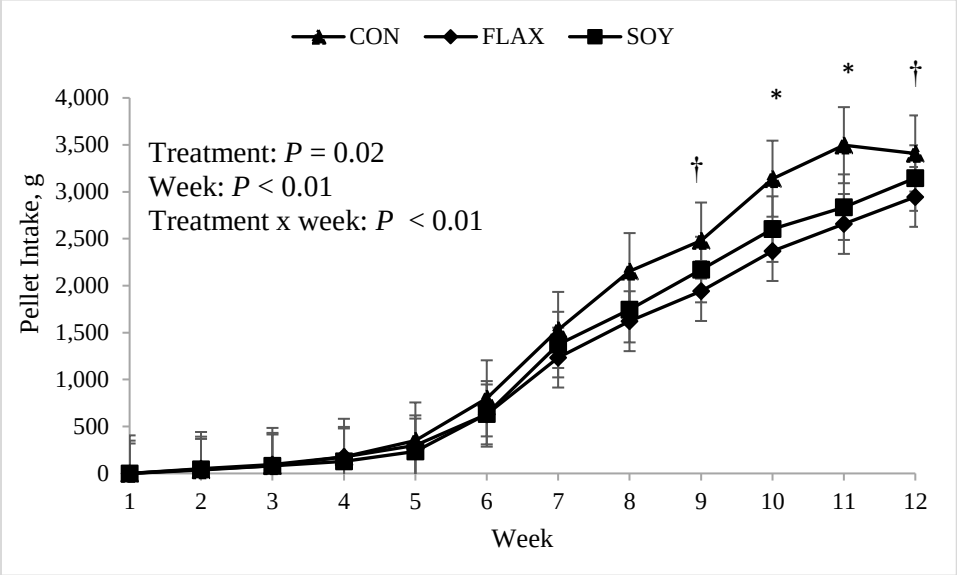


Figure 2.2. Fecal consistency of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

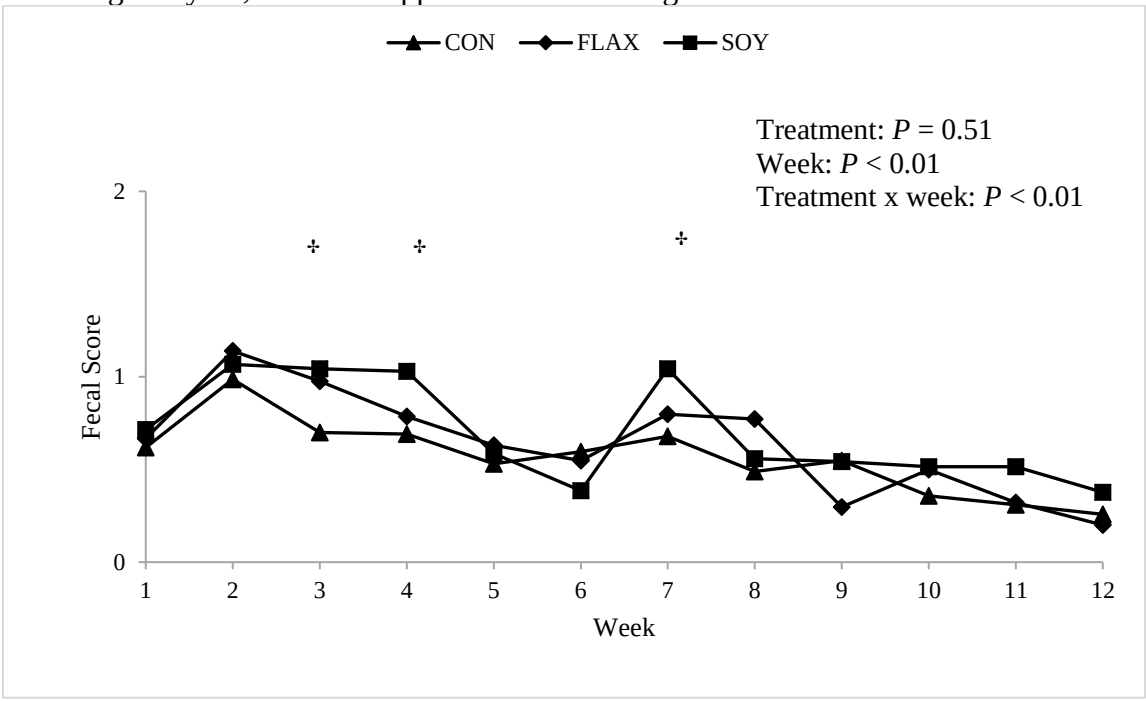


Figure 2.3. Plasma cholesterol concentrations of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

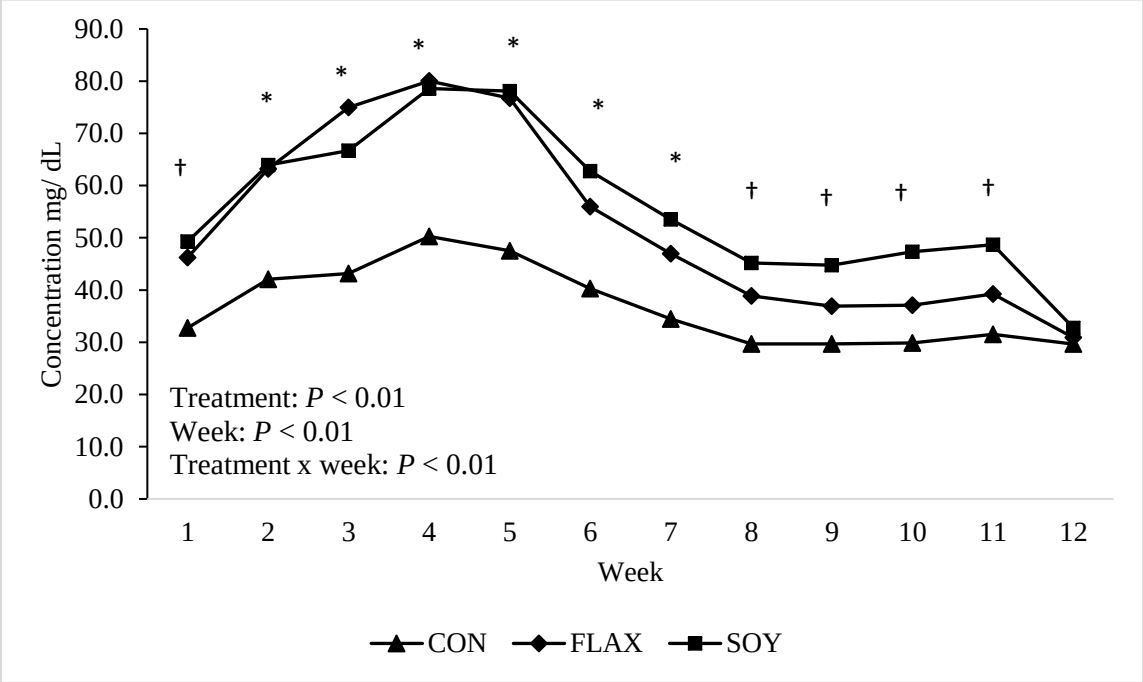


Figure 2.4. Plasma triglyceride concentration of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.

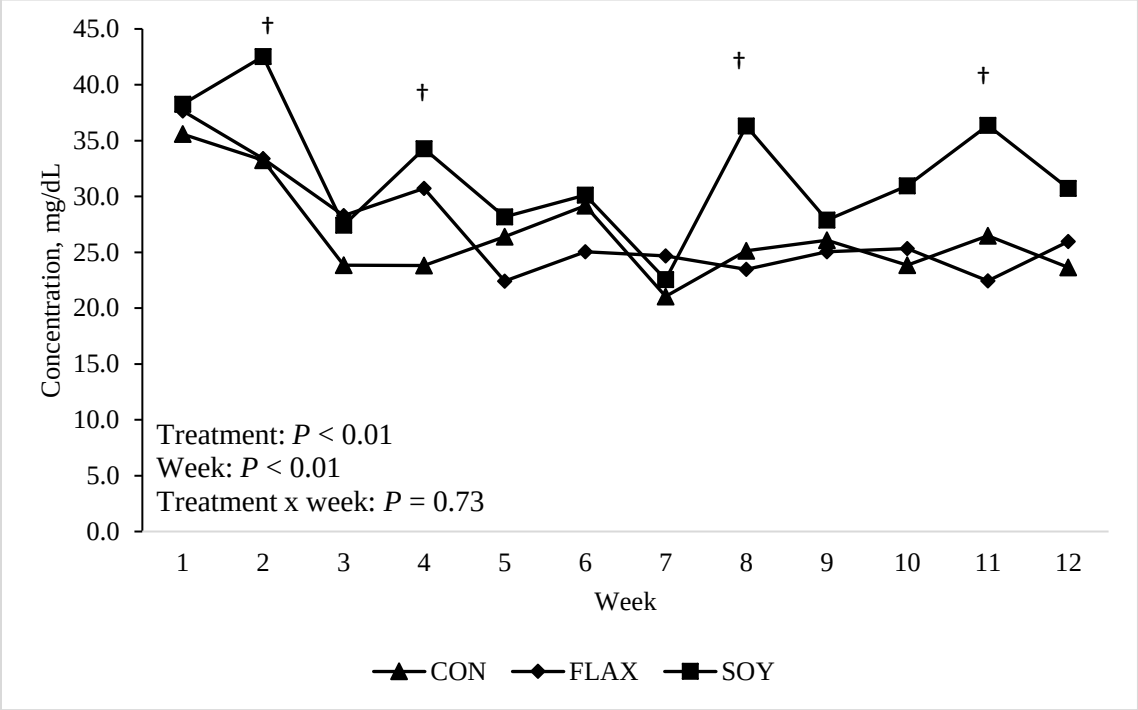
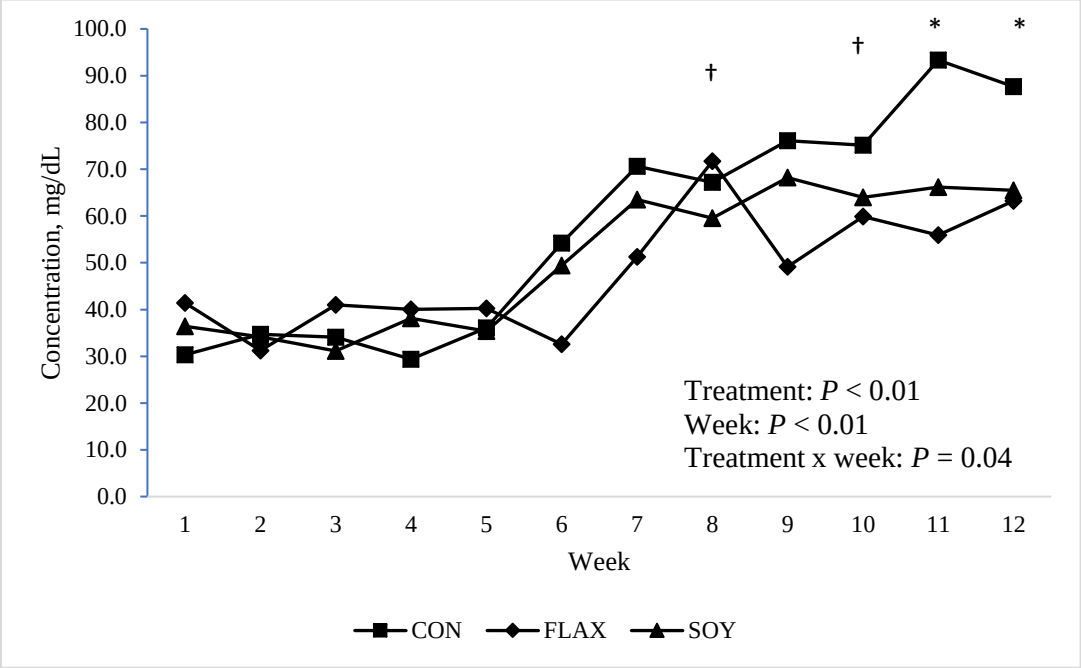


Figure 2.5. Plasma BHBA concentration of dairy calves fed either a control diet, diet supplemented with 80 g/d soy oil, or a diet supplemented with 80 g/d flax oil.



CHAPTER 3

UTILIZATION OF NUTRI-FLAX COATED BY LACTATING DAIRY CATTLE

ABSTRACT

The inclusion of oilseeds in dairy cattle diets has shown potential for increasing milk fat. The objective was to investigate potential benefits of supplementing an encapsulated flax oil to high producing cows and how it is utilized. Eight multiparous and 4 primiparous Holstein cows (73 ± 40 d in milk, 694 ± 71 kg of BW) were used in a 3-wk randomized complete block design study. Cows were fed a basal diet containing 52% forage on a dry matter basis. Treatments were: 1) control (**CON**) with no oil, and 2) 250 g/d of encapsulated flax oil (**FLAX**). Cows were fed once daily individually, and daily intakes for each cow were measured using a Calan gate feeding system at 0700h and water was given ad libitum. The encapsulated flax product was supplemented individually into the FLAX cows feed boxes. Coccygeal blood samples were collected once weekly for metabolite and long-chain fatty acid analysis. Rumen fluid was collected via esophageal tube on two consecutive days during wk 0 and 3 for analysis of volatile fatty acid (VFA) and ammonia production. Milk weights were recorded daily and on two consecutive days in wk 0 and 3 and one day in wk 1 and 2 of the study, milk samples were taken at each milking for compositional, and fatty acid analysis. Results were analyzed using the MIXED procedures of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$. Dry matter intakes were not affected by inclusion of the encapsulated flax oil. Milk production, milk fat and milk protein had no differences between treatments.

Plasma concentrations of glucose, triglyceride, and cholesterol were similar among both treatments. Rumen ammonia tended to be less in the FLAX cows ($P = 0.10$). All major VFA were similar between treatments. During the short 3 wk study, minimal milk fatty acids differences were observed between treatments. However, since the cows on the FLAX treatment produced an average of 0.12 kg/d more of fat in their milk the total amount of the fatty acids was numerically greater than the CON treatment. Supplementing encapsulated flax oil, maintained lactation performance when compared to CON and increased milk fat percentage.

Keywords: encapsulated flax oil, lactating cows, milk production

INTRODUCTION

The inclusion of oilseeds in lactating rations have shown to enhance beneficial fatty acids in milk (Chilliard et al., 2007; Glasser et al., 2008). The most common oilseeds incorporated into lactating rations are soybeans and cottonseed. In addition, meals from canola, flaxseed, and safflower are fed. The meals are fed instead of whole seeds due to the value and economics of the oil.

Flaxseed contains a high amount of oil, about 40 percent of the total seed weight (Mustafa et al., 2002). When feeding the flaxseed to dairy cows the concentration of short-chain fatty acids and medium-chain fatty acids were reduced, yet the long-chain fatty acids in milk fat were increased. (Mustafa et al., 2003). Flax oil is primarily made up of α-linolenic acid, which accounts for roughly 55 percent of the total fatty acids in the oil (Petit et al., 2002, 2003). Even though the flax is primarily made up of α-linolenic acid, the increase in milk was slight. This suggested that the α-linolenic from the diet was being used by the rumen bacteria through extensive biohydrogenation (Chilliard et al., 2000). Therefore, it is important to protect the flax oil from rumen biohydrogenation in order to see a concentration increase of α-linolenic acid in the milk through milk fat synthesis.

Vegetable oils, in general are being used as a vehicle for encapsulation of lipids. Since flax oil is roughly 89 percent unsaturated fat (Jenkins et al., 1997) the lipid is highly susceptible to oxidation resulting in rancidity and negative effects on nutritional quality. In order to prevent negative effects from the lipid and decrease biohydrogenation, it is important to encapsulate the highly unsaturated flax oil with a nonessential oil including that of corn oil, soybean oil, and other popular vegetable oils. Encapsulated flax

meal goes through a process in which the end-product is a bead. One study compared an un-encapsulated oil to an encapsulated flax oil in which the encapsulated fat did not undergo oxidation (Can Karaca et al., 2013). To our knowledge limited research on the encapsulated flax oil incorporation in dairy cattle rations has been published. Therefore, the objective of this study was to evaluate the potential benefits of supplementing high producing lactating cows with Nutri-flax coated™ and determine how it was utilized. The hypothesis was the supplementing 250 g/d of Nutri-flax coated could improve feed efficiency, milk production and milk fatty acid composition.

MATERIALS AND METHODS

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee. Approval number 18-003E and assurance number A3958-01.

Experimental Design

Twelve high producing Holstein cows (73 ± 40 d in milk, 694 ± 71 kg of body weight) were used in a 3-wk randomized complete block design. Eight multiparous and four primiparous cows were used in this study. Cows were blocked by milk production, parity, and days in milk. After assignment to block cows were randomly assigned to one of the two treatments. The feeding study was completed in 5 wks. Week -1 was used as a training period to familiarize cows to the new pen and Calan gate door feeding system (American Calan, Inc., Northwood, NH). Week 0 was used as the covariate period. Week 1 to 3 was the treatment period.

Treatments included: 1) a control diet (**CON)** with no supplementation of encapsulated flax oil, 2) a diet supplemented with 250 g/d of Nutri- Flax coated (**FLAX**).

A basal TMR (Table 3.1) was fed to both treatment groups. The diet was formulated using NRC (2001) and contained 27% corn silage, 25% grass hay, and 10% trace mineral mix. The remainder of the diets were comprised of ground corn, soybean meal, soybest, soybean hulls, and calcium carbonate to meet nutrient requirements (Table 3.1).

Animal Care and Feeding

The feeding portion of the experiment was conducted at the South Dakota State University Dairy Research and Training Facility (SDSU DRTF) in Brookings, SD from February to March of 2018. Daily observations of cows were conducted for any signs of physical or disease problems, and if found, treatment was administered according to the normal herd management practices at the SDSU DRTF. Cows were housed in sixteen free-stall pen and bedded with mattresses. Mattresses were bedded with straw once a day in the AM. Fresh water was provided ad libitum. Bales of alfalfa hay were ground every week using a vertical tub grinder (Haybuster 1330, DuraTech Industries International, Inc., Jamestown, ND). Corn silage was grown and harvested by the SDSU farm department. Cows were fed once daily individually, and daily intakes for each cow were measured using a Calan gate feeding system (American Calan, Inc., Northwood, NH). Rations were fed ad-libitum to allow for 10% refusal rate and were adjusted weekly based on DM of feeds. A base forage mix was prepared by the farm crew in a larger mixer wagon and the basal grain mix was mixed into the TMR with the Calan Data Ranger (American Calan, Inc., Northwood, NH). The flax was mixed in individually in the designated feed boxes of treatment cows. Refusals were weighed and recorded once daily, and the diet was adjusted to ensure 10% feed refusal. Cows were fed experimental

diets for 3 weeks, and the normal herd diet was fed before the experimental period during the training and covariate periods.

Sample and Data Collection

Feed samples of alfalfa hay, corn silage, and TMR were taken and stored at -20°C for nutrient analysis weekly on 2 consecutive days and composited by week. Grain mixes were sampled every week, stored at -20°C and composited by week for analysis later.

Milk weights were recorded daily and on two consecutive days in wk 0 and 3 and one day in wk 1 and 2 of the study, milk samples were taken at each milking for compositional analysis and sent to a DHI laboratory (Manhattan, KS) for analysis. During wk 0, 1, 2 and 3 extra milk samples were collected at each milking and samples were frozen at -20°C for fatty acid and Mojonnier milk fat analysis.

On wk 0 and 3 on two consecutive days and on wk 1 and 2 on one day cows were weighed, and body condition scored (BCS) by three individuals on a scale of 1 to 5 with 1 being emaciated and 5 being obese (Wildman et al.,1982). During wk 0 and 3 collection of rumen fluid via esophageal tubing was collected on two consecutive days at approximately 3-4 post feeding for analysis of pH, VFA, and ammonia concentrations. The first 200 mL of the rumen fluid was discarded in an attempt to minimize saliva contamination. In total over 20 mL of rumen fluid was collected into a stainless-steel cup and the pH of the sample was analyzed immediately (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL.) An aliquot sample of 10 mL was mixed with 200 µl of 50% sulfuric acid to determine rumen ammonia nitrogen (NH_3-N), and an aliquot sample of 10 mL was mixed with 2 mL of 25% meta-phosphoric acid for determination

of VFA. Both samples were stored at -20°C until further analysis. On days 0, 7, 14, and 21 (1 day each week) of the feeding period, at approximately 3-4 hours post feeding blood samples were collected via coccygeal vein venipuncture into vacutainer tubes (Becton, Dickinson, and company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) for glucose analysis (Cat. #: 367729) or potassium ethylene diamine tetra-acetic acid (K_2EDTA) for all other analyses (Cat #: 366643). Right after blood collection, samples were placed on ice and brought into the laboratory within 2 hr for processing and storage. Blood collections tubes were centrifuged at 1000 x g for 20 min at 4°C (Centrifuge: CR412 Jouan, Inc., Winchester, VA.). Plasma was separated from whole blood cells with a serum separator (Cat #: 268151) and transferred using a plastic pipette into polystyrene storage tubes and frozen at -20°C until further analysis.

Laboratory Analysis

Samples of feed ingredients were dried for 24 h at 105°C every wk for DM analysis, to check ingredient inclusion rates in the ration and determine DMI. At processing, feeds were thawed, and samples were analyzed for DM, ash, CP, ether extract (EE), NDF, and ADF. The samples composited were dried in duplicate for 48 h at 55°C in a Despatch oven (Style V-23, Despatch Oven co. Minneapolis, MN). Composited forages were ground first to a 4-mm particle with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). The ground forages and grain mix then are ground to a 1-mm particle using a ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To check and correct the analysis to 100% DM (AOAC, 1998; method 935.29), a 1 g sample of the feed ingredients was dried for 4 h in a 105°C oven (Model 28, Precision Scientific Co. Chicago, IL). To analyze ash content (AOAC 1998; method 942.05) 1 g of sample

was incinerated overnight in a muffle furnace at 450°C (Model F1730, Thermolyne Corp., Dubuque, IA; temperature controller Model Wheelco 293, Barber-Colman Co., Rockford, IL). Organic matter was calculated as OM=(100-%Ash). For analysis of NDF (Van Soest et al., 1991) and ADF (Robesrtson and van Soest, 1981) the Ankom 200 fiber analysis system was used (Ankom Technology Corp., Fairport, NY). For the NDF procedure, heat stable sodium sulfate and alpha-amylase were used. Ether extracts were analyzed using petroleum ether (AOAC 1998; method 920.39) in Ankom XT10 fat analysis system (Ankom Technology corp., Fairport, NY). Samples of TMR were sent to a commercial laboratory for mineral and starch analysis (Dairyland laboratories Inc., Arcadia, WI). Mineral analyses included Ca, P, Mg, K, Na (method 985.01), S (method 923.01), and Cl (method 915.01; AOAC International, 1998). Starch concentration was found using a modified method of glucose analysis (Bach Knudsen, et al. 1997) completed on an YSI 2700 Select Biochemistry Analyzer (YSI Biochemistry Analyzer, YSI Inc., Yellow Spring, OH).

Blood metabolites, glucose, cholesterol, and triglycerides were analyzed using commercially available colorimetric and enzymatic assay kits with a micro-plate reader (Cart 50, Varian INC., Walnut Creek, CA). A glucose oxidase reagent was used to analyze concentrations of serum glucose as described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed according to the procedure described by Allain et al. (1974) using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI). Total triglyceride concentration was analyzed with the glycerol phosphate oxidase after hydrolysis of lipoprotein lipase as

described by Fossati and Prencipe (1982) that paired the reaction with the classic Trinder et al. (1959), reaction.

For rumen fluid analysis, the samples were thawed and vortexed to completely mix contents before transferring 2 ml into a micro-centrifuge tube which was centrifuged at 30,000 × *g* for 10 min in a micro centrifuge (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Concentration of NH_3-N was analyzed, according to the assay described by Chaney and Marbach (1962). Gas chromatography was used to analyze VFA concentrations, with the use of an automated gas chromatograph (Model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d × 15 m column (Nukol 24106-U, Supelco, Inc., Bellefonte, PA). Analysis of VFA was done using 2-ethylbutyrate as an internal standard. The gas flow rate was 1.3 ml/min of helium, and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Mid-infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN: AOAC International 2002) was used to analyze milk protein, and lactose. Milk urea nitrogen (MUN) concentration was determined using a chemical methodology based on the modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments). Somatic cell counts were determined using a flow cytometer laser (Somacount 500, Bentley Instruments, AOAC International, 2002).

Milk fat was analyzed using the Roese-Gottlieb ether extraction method with some modifications. Milk samples were thawed in a 37°C water bath and then vortexed on low speed. Using a Mojonnier flask 10 g of milk was weighed and added to the flask. The addition of 1.5 mL Ammonia hydroxide, 10 mL ethyl alcohol, 25 mL of ethyl ester, 25 mL petroleum ether and 2 drops of phenol indicator is added to the flask. Mixed and

then centrifuged for 5 min. The ether with fat is poured off into a previously weighed aluminum dish. The second extraction has the addition of 5 mL ethyl alcohol, 15 mL ethyl ester and 25 mL petroleum ether. Mixed and centrifuged for an additional 5 min and then poured off with the ether from the first extraction. The dish is then put on a hot plate to evaporate the ether and leave the remaining fat from the milk behind.

Milk fatty acids were prepared as butyl esters according to Abdelqader et al. (2009) with some modifications. The milk samples were centrifuged at 3,500 g for 20 mins at 4°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.) Fifteen to Twenty mg of fat from the fat pad was removed and added into a 13x 100 mm Pyrex extraction tube fixed with a Teflon-lined screw cap. The addition of 500 µL of butanol and 25 µL of internal standard (2-tridecenoic acid, Nu-Chek Prep Inc., Elysian, MN) was added to each extraction tube. While adding 50 µL of acetyl chloride the samples were slowly vortexed. Tubes where then gases with N_2 for 4 s and placed on a dry heating block for 90 min at 60°C. Once samples had cooled to room temperature the addition of 5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 2,000 x g for 20 min at 4°C. After centrifugation, the bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 3x with 5 mL of distilled water and centrifuged in between each wash at 2,000 x g for 20 min at 4°C. After the final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

Blood fatty acids were prepared as butyl esters as according to Abdelqader et al. (2009) with some modifications. 1 mL of plasma was added into a 16x100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap. 300 µL distilled water, 1.5 mL chloroform and 3.0 mL methanol were added to extraction tube and vortexed for 2 min. Additionally, 1.5 mL of chloroform was added and vortexed. 1.5 mL distilled water waster was added and vortexed. Samples were centrifuged for 20 min at 3000 g at 10°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.). Using a Pasteur pipette the upper layer of the sample was removed. Additionally, 2 mL of chloroform was added vortexed and again centrifuged for 20 min at 3000 g at 10°C. The bottom layer containing the chloroform and fat were removed and added to a clean 16x100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap. An additional 1 mL of chloroform is added to the 16x 100 mm extraction tube to remove any remaining fat from the sample. The tube is vortexed and centrifuged for 20 min at 3000 g at 10°C. The lower portion of the tube is combined with the previous lower layer in the 13x100 mm tube. Using forced air, the samples in the 13x100 mm extraction tubes are dried, evaporating the chloroform and leaving behind the fat from the sample. After samples are dry the addition of 500 µL of butanol and 25 µL of internal standard (2-tridecenoic acid, Nu-Chek Prep Inc., Elysian, MN) was added to each extraction tube. While adding 50 µL of acetyl chloride the samples were slowly vortexed. Tubes where then gases with N_2 for 4 seconds and placed on a dry heating block for 90 min at 60°C. Once samples had cooled to room temperature the addition of 5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 2,000 x g for 20 min at 4°C. After centrifugation the

bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 3x with 5 mL of distilled water and centrifuged in between each wash at 2,000 x g for 20 min at 4°C. After the final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

Feed fatty acids were prepared using similar techniques to Sukhija and Palmquist (1988) with some modifications. The concentration of lipid is adjusted so that 20 to 25 mg of lipid is concentrated in 750 µl of butanol. The adjusted concentration of feed was then added into a 16 x 100 mm Pyrex extraction tube fixed with a Teflon- lined screw cap and of 750 µl of butanol was added. The addition of 25 µl of the internal standard, C19:0, as well as 75 µl of acetyl chloride while mixing the extraction tube at a low speed. Tubes were then gassed with N_2 for 4 seconds and placed on a dry heating block for 90 min at 100°C. Once samples had cooled to room temperature the addition of 7.5 mL of 6% K_2CO_3 was added and the samples were again vortexed for 30 s. Next 1 mL of Hexane was added to each tube, and the samples were vortexed once more before being centrifuged at 1,500 x g for 20 min at 10°C (CR412 centrifuge, Jouan, Inc., Winchester, VA.). Using a Pasteur pipette the upper layer of the sample was transferred to a clean 13x100 mm tube fixed with a Teflon-lined screw cap. 5 mL of distilled water was added to the 13x100 and vortexed before centrifuged for 20 min at 1,500 x gat 10°C. After centrifugation, the bottom layer of the sample was removed with a 9-inch Pasteur pipette and discarded. The remaining hexane layer was then washed 4x with 5 mL of distilled water and centrifuged in between each wash at 1,500 x g for 20 min at 10°C. After the

final wash, the upper layer that contains the hexane and butyl esters of fatty acids was removed with a Pasteur pipette and placed into injection vials for GC analysis.

The milk, blood and feed fatty acid samples were analyzed using the GC (model 6890, Hewlett-Packard, Palo Alto, CA). The injector port was at 230°C with a split ratio of 100:1 and He as the carrier gas at 2.0 mL/min column flow-rate. The 100 m in length column had an inside diameter of 0.25 mm (Supelco 2560, Supelco Inc. Bellefonte, PA). Initially, column temperature was set to 50 °C and held constant for 5 min, then the temperature was increased to 145°C at a rate of 5°C/min. The temperature of 145°C was held for 30 min and then increased to 190°C at a rate of 10°C/min and held for 30 min. The final temperature increase was 210°C with a rate of increase of 5°C/min and held for a final 40 min. The analyzed fatty acids were identified through the order of elution and comparison to known standards that were commercially prepared (GLC-60, GLC- 68D and GLC-566, Nu-Chek Prep Inc., Waterville, MN).

Statistical Analysis

Cow intakes and milk production were analyzed in a randomized complete block design with repeated measures using MIXED procedures of SAS 9.4 (Littell et al., 2008). The model included treatment, wk and treatment by wk interactions. Initial body size, days in milk, and milk production were included as covariates within the model. Least square means for each treatment are reported in the tables. Significant differences between treatments were declared at $P \leq 0.05$ and a tendency was declared at $0.05 < P \leq 0.10$.

Feed data analysis and standard errors were calculated using the MEANS procedure in SAS version 9.4. Total dietary nutrient values were calculated based on

analysis of forages and concentrate mixes for the basal diet throughout the entire feeding period.

MIXED procedures of SAS 9.4 (Littell et., 2008) were used to analyze the cow plasma metabolites and rumen fermentation characteristics using repeated measures by week. Main effects in the model were treatment, week and treatment by week interactions. For repeated measures, Akaike's criterions were used for each parameter to determine a suitable covariance structure. Least square means for each treatment are reported in the tables and were compared among treatments.

RESULTS AND DISCUSSION

Feed Analysis

For the formulations of the basal diet, pre-trial samples of the forages were used, and the diet compositions are presented in Table 3.1. These diets are very typical for the Midwestern region of the United States.

Nutrient composition of the covariate diet and the treatment diet are found in Table 3.2. The DM % of the treatment diet was greater than that of the covariate treatment; however, is still within an acceptable range. The treatment diet also had a greater CP content than the covariate diet which was expected since the formulated CP was 17.0% and analysis resulting in a CP of 17.88%. Since this is a fat supplementation study, the removal of whole cotton seed was done so that any changes in milk composition and rumen fermentation would solely be potentially from the effect of the encapsulated flax oil addition. The formulated treatment ration for this study is similar in nutrient analysis to the control diet fed in Anderson, et al. (2006).

Lactation Performance

Table 3.3 compares the DMI and the milk for both treatments over the entire study. Cows on both treatments had similar DMI at 24.71 vs 24.53 kg/d. Overall milk production was also similar between treatments at 46.85 vs 46.02 kg/d. Milk production is greater than the findings of Leduc, et al. (2017) when cows were feed flax seed meal and oil. In addition, milk production is also greater than the results of feeding linseed meal to Holstein dairy cows in Jahani-Moghadam, et al. (2015). Overall, supplementing the diet with the encapsulated flax did not alter DMI or total milk production.

Table 3.4 displays the wk 3 lactation performance and composition. Cows on the CON treatment were producing numerically higher quantities of milk, however the cows on the FLAX had a numerically greater average fat percentage. The protein for CON cows was significantly greater at 3.29% with a ($P = 0.01$) over that of the FLAX cows at 3.00%. Supplementing fat in feed is a common technique to increase fat percentage in milk and generally increase milk yield. However, with adding fat to the diet the milk protein concentration typically will see a decrease by 0.1 to 0.2 %. The decrease in protein concentration could be due to increased fat fed to the FLAX cows as they go through the metabolic process the attributes to decrease in protein as described by (Schingoethe, et. al 1996: Wu and Huber, 1994). There was no statistical difference between the lactose and solids non-fat in the milk. Cows on the FLAX treatment had a significantly greater MUN concentration at 9.71 mg/dL with ($P < 0.01$) when compared to the CON cows with a value of 7.34 mg/dL. Since MUN is a useful indicator of protein metabolism in the dairy cow (Roseler et al., 1993) it is important that value is within the target value range of 10 to 16 mg/dL which is dependent on milk production (Jonker et

al., 1998). Since cows on the study were at peak lactation, lower MUN values are expected, however the CON concentration 7.34 mg/ dL is slightly concerning and is a good indication that these cattle are deficient in dietary intake protein.

Rumen Fermentation Characteristics

The method of collection of rumen fluid via esophageal tube is recognized to be less than ideal for a variety of reasons. Rumen fermentation characteristics are presented in Table 3.6. No statistical difference was found between the two treatments for rumen pH (P =0.2). Total volatile fatty acid (VFA) concentration was also not affected by the fat supplementation. Supplementation of fat typically has no impact on total VFA and rumen pH (Pantoja et al, 1994). All major VFA (acetate, propionate, and butyrate) were similar between treatments. Iso-valerate and valerate, as well as the acetate to propionate ratio had no significant differences. Similar results for butyrate and isovalerate were found in (Cortes et al., 2010) paper on feeding Holstein lactating cows whole flax seed and calcium salts of flaxseed oil. The ammonia-N concentration tended to be less in the FLAX fed cows (P = 0.10) when compared to the CON treatment cows. Lower ammonia-N concentration was also demonstrated in other research in which cows were fed a diet with increased fat (Onetti et al., 2001). Increased fat concentration in dairy rations has an association with reducing rumen protozoa numbers and decreasing the microbial nitrogen that is being recycled (Ikwuegbu and Sutton, 1988; Broudiscou et al., 1994). This will lead to a decrease in rumen ammonia-N concentrations that were observed in the results of this study.

Blood Metabolites

Blood metabolite concentrations are presented in Table 3.7. Plasma triglycerides concentration were similar between treatments ($P = 0.97$). In contrast to the current results in a study where diets were enriched with α-linolenic acid, through the use of flax seed, plasma triglyceride concentrations were significantly higher for the FLAX cows (Ambrose et al., 2006). Yet, when results are compared to another study triglycerides concentrations are slightly less than the CON group in research by Sevinc et al. (2003). Plasma triglyceride concentrations are not derived from indigested fat, instead most is a result of de novo synthesis (Holtenius et al., 1989). Reasons for variation in triglyceride concentrations between studies could stem from the levels of albumin in the cattle. Cows with low triglyceride concentrations typically are found to have increased free fatty acid concentrations (Herdh et al., 1988).

Plasma glucose concentrations were also similar between treatments ($P = 0.12$). When compared to cows fed a similar diet which included linseed instead of the encapsulated flax oil the glucose concentrations are comparable to one another (Jahani-Moghadam et al., 2015). There was a treatment by week interaction ($P < 0.01$) for glucose at wk 2.

No statistical difference was observed between treatments for plasma cholesterol concentrations ($P = 0.53$). Plasma cholesterol concentrations reported in the current study are similar to 0 hr concentration found in Chen et al. (1995), prior to their addition of the bile salts to lactating cows.

Overall, no significant treatment differences were observed for all blood metabolites analyzed.

Milk Fatty Acid Analysis

Table 3.8 presents the fatty acids identified in milk samples during wk 3. A full table with fatty acid composition by week can be found in Table 1 of the appendix. The triglycerides found in milk fat are synthesized into numerous fatty acids; however, only about 15 fatty acids are found to have quantities greater than 1% (Palmquist et al., 1993). Overall, during the short three week study, minimal differences between treatments were observed between the CON cows and FLAX cows. As a percentage of total fatty acids, there were minimal treatment effects. Since the cows on the FLAX treatment produced a numerical average of 0.12 kg/d more fat in their milk over the CON cows the total amount of fatty acids is greater for the FLAX cows. Butyric acid (C4; $P = 0.03$) and caproic acid, (C6; $P = 0.06$) and stearate (C18:0; $P = 0.08$) all have effects or tendencies for differences between treatements, where FLAX fed cows had a greater percentage of fatty acid than the CON cows. Myristoleate acid, C14:1; ($P = 0.06$), pentadecaenoic acid, C15; ($P = 0.10$) and palmitoleate acid, C16:1 cis; ($P = 0.03$) all had a treatment effect or tendency to be greater in CON fed cows than FLAX cows.

CONCLUSIONS

Supplementation of encapsulated flax oil to lactating dairy cows is a new approach to improve milk fat yield, essential fatty acids in milk and potentially increase health benefits. Given that it takes roughly twenty one days for a dairy cows rumen to adjust to a diet this study could have been too short to detect the full potential of the encapsulated flax product. Dry matter intake was not affected by the inclusion of the encapsulated flax oil. In addition, milk production for both treatments increased throughout the study but is most likely accredited to the increase in crude protein in the basal diet compared to the diet that was fed to the cows previous to enrollment on the

study. The FLAX fed cows did have a numerically greater percentage of milk fat, but it was not significant. Rumen fermentation was not affected by the encapsulated flax which means that the encapsulation process possibly helped avoid biohydrogenation of the poly-unsaturated fatty acids primarily found in flax oil. Rumen ammonia was affected by the addition of encapsulated flax in that concentrations were less than the CON treatment. Serum glucose, triglycerides, and cholesterol were similar between treatments. Major milk fatty acids were similar between treatments. Alpha-linolenic acid was numerically greater during wk 3 for cows fed the encapsulated flax. Potentially the low number of cows per treatment and the short duration of the study period could have affected the results. Further evaluation of the product that this preliminary study should be conducted to confirm results. Overall, contrary to our hypothesis, minimal differences were observed when supplementing encapsulated flax to high producing cows.

ACKOWLEDGEMENTS

This research was funded by Nutriad Inc of Schietstandlann, Belgium with additional support by the SDSU Ag Experiment Station. In addition, the authors would like to thank the South Dakota State University Dairy Research and Training Facility farm crew and fellow students for their assistance during the farm trial. This research contributes to the goals of the USDA North Central Cooperative Research project NC-2042: Management Systems to improve Economic and Environmental Sustainability of Dairy Enterprises.

Table 3.1. Ingredient composition of Basal TMR

Ingredient	% of DM
Corn Silage	27.0
Alfalfa Hay	25.0
Ground Corn	25.0
Soybean Meal, 48%	4.0
Soybest L	8.0
Soybean Hulls	8.5
Calcium Carbonate	0.5
Trace Mineral Mix	0.10
Vitamin Mix	0.15
Magnesium Oxide	0.05
Salt-White	0.70
Energy Booster	1.00

Table 3.2. Nutrient composition of Covariate and Basal Diet

Nutrient[1]	Covariate Diet	Basal Diet
DM, %[2]	49.29	60.00 ± 5.17
CP[2]	13.78	17.88 ± 0.88
NDF[2]	27.69	26.68 ± 2.05
ADF[2]	28.00	15.59 ± 1.88
NFC[2,3]	47.06	45.03 ± 1.12
Starch[2]	29.37	31.60 ± 1.71
EE[2,4]	4.58	3.86 ± 0.57
Ash[2]	6.89	6.54 ± 0.10
Ca[2]	0.84	0.92 ± 0.17
P[2]	0.44	0.35 ± 0.04
S[2]	0.23	0.19 ± 0.02
ME[5], Mcal/kg DM	-	2.49
NEg[5], Mcal/kg DM	-	1.57

[1] % of DM, unless otherwise indicated.
[2] Results from analysis of weekly composites.
[3] % NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[4] Ether extract, analyzed with petroleum ether.
[5]Based on formulation predictions of NRC (2001) when analyses values for samples were entered into the program.

Table 3.3. Lactation Performance of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item	Treatment			*P*-values		
	CON	FLAX	SEM	Trt	wk	Trt × wk
DMI[1], kg/d						
Mean	25.33	24.52	0.776	0.48	0.89	0.21
Wk 0	34.56	33.52	1.140	0.24		
Wk 1	25.66	23.80	0.991	0.20		
Wk 2	25.62	24.30	0.991	0.36		
Wk 3	24.70	25.47	0.991	0.59		
Milk, kg/d						
Mean	46.85	46.02	1.062	0.60	0.52	0.54
Wk 0	45.91	47.46	1.817	0.23		
Wk 1	46.21	46.20	1.177	0.99		
Wk 2	46.82	45.67	1.177	0.50		
Wk 3	47.50	46.20	1.177	0.45		

[1]DMI = Dry matter intake

Table 3.4. Week 3 Lactation Performance of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

	Treatment			*P*-values
Item	CON	Flax	SEM	Treatment
DMI, kg/d	24.71	25.47	0.99	0.59
Milk, kg/d	47.50	46.19	1.18	0.45
Fat, %	2.66	3.00	0.22	0.29
Protein, %	3.29	3.00	0.06	0.01
Lactose, %	4.98	4.98	0.03	0.83
SNF, %	9.31	9.10	0.10	0.03
Fat yield, kg/d	1.32	1.33	0.09	0.96
Protein yield, kg/d	1.56	1.40	0.06	<0.01
Lactose yield, kg/d	2.36	2.32	0.08	0.55
SNF yield, kg/d	4.43	4.21	0.15	0.11
MUN, mg/dL	7.34	9.71	0.54	<0.01
SCC	23.05	24.02	5.96	0.91

Table 3.5. BW and BCS dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item	Treatment			*P*-values		
	CON	FLAX	SEM	Trt	wk	Trt × wk
BW, kg						
Mean	710.03	702.05	4.54	0.24	0.03	0.32
Initial	665.01	666.45	5.88	0.93		
Final	712.80	711.82	5.12	0.89		
BCS[1]						
Mean	2.81	2.74	0.04	0.21	0.17	0.09
Initial	2.96	3.00	0.14	0.41		
Final	2.84	2.76	0.05	0.19		

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

Table 3.6. Rumen fermentation characteristics of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item	Treatment			*P*-values
	CON	FLAX	SEM	Trt
pH	6.52	6.31	0.12	0.20
Ammonia-N, mg/dL	8.57	6.18	1.30	0.10
Total VFA[1], mM	117.3	134.2	8.26	0.11
VFA, mM/100mM				
Acetate	55.15	54.82	0.92	0.75
Propionate	32.27	32.28	1.33	0.99
Butyrate	10.64	11.02	0.47	0.39
Isovalerate	1.74	1.47	0.16	0.14
Valerate	0.30	0.30	0.02	0.99
Acetate: Propionate	1.74	1.73	0.10	0.97

[1]VFA = Volatile Fatty Acids

Table 3.7. Blood metabolites of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item mg/dL	Treatment		SEM	*P*-values		
	CON	FLAX		Trt	wk	Trt ×wk
Triglyceride	19.30	19.32	0.43	0.97	0.19	0.42
Glucose	59.35	57.51	0.77	0.12	<0.01	0.24
Cholesterol	72.80	79.66	6.86	0.53	0.41	0.62

Table 3.8. Proportions of milk fatty acids of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item	Treatment			
	CON	FLAX	SEM	*P*-values
C4:0	3.226	4.076	0.311	0.01
C5:0	0.992	0.635	0.173	0.06
C6:0	1.149	1.453	0.117	0.04
C8:0	0.954	1.180	0.122	0.10
C10:0	3.227	3.339	0.318	0.68
C12:0	3.533	3.630	0.351	0.76
C14:0	10.457	10.429	0.458	0.94
C14:1	0.931	0.728	0.141	0.15
C15:0	1.324	1.116	0.150	0.24
C15:1	1.314	1.156	0.133	0.26
C16:0	33.136	34.676	1.642	0.39
C16:1 *trans*	0.318	0.509	0.116	0.07
C16:1 *cis*	1.571	1.286	0.244	0.15
C17:0	0.602	0.575	0.050	0.58
C17:1	0.227	0.155	0.032	0.01
C18:0	6.154	7.392	0.630	0.08
C18:1 *trans 10*	0.270	0.331	0.091	0.53
C18:1 *trans 11*	0.392	0.504	0.208	0.48
C18:1 *cis 9*	13.725	13.884	0.939	0.85
C18:1 *cis 11*	1.829	1.720	0.327	0.73
C18:2 *cis 9, 12*	2.759	2.989	0.312	0.50
C18:3 *gamma*	0.455	0.550	0.066	0.15
C18:3 *alpha*	0.124	0.269	0.151	0.26
C20:0	0.027	0.054	0.076	0.69
C18:2 *CLA trans 10 cis12*	0.010	0.017	0.010	0.50
C18:2 *CLA cis 9 trans 11*	0.024	0.036	0.017	0.44
C20:5	8.244	5.534	2.277	0.17
Others[6]	2.484	2.743	0.820	0.78
SCFA[1]	45.11	45.89	1.373	0.48
LCFA[2]	54.88	54.11	1.371	0.48
Saturated FA[3]	66.39	70.20	2.410	0.09
MUFA[4]	21.01	20.08	1.522	0.49
PUFA[5]	12.39	10.05	2.125	0.21

[1]SCFA = Short chain fatty acid
[2]LCFA = Long chain fatty acid
[3] Saturated FA = Saturated fatty acid
[4] MUFA = Monounsaturated fatty acid
[5] PUFA = Polyunsaturated fatty acid
[6] Others = sum of C18:1 trans 9, C19:0, C20:1, C20:2, C22:0, C22:1, C22:2, C22:3, C22:4, C22:5, C22:6.

OVERALL SUMMARY AND CONCLUSIONS

The research presented in this thesis met the overall objectives, which were to evaluate the potential benefits of flax oil on performance of dairy calves and lactating cows. Results from Chapter 2, when flax oil was fed directly into the milk of calves preweaning and then on the starter pellets post weaning showed that supplementing flax and soy oil maintained growth performance compared to CON in the pre-weaning period when fed with milk, but decreased intake and BW during the last two weeks of the post-weaning period when fed with starter pellets. In addition, supplementing flax oil and soy oil maintained PUN concentrations, increased cholesterol concentrations, decreased BHBA, but caused different responses compared to CON for Triglycerides and Glucose. Plasma fatty acids were also impacted by treatment and week of the study. This study demonstrates that the supplementation of plant-based oils to young calves affects their metabolic profile, does not affect body growth, and decreases intake when compared to a diet with no oil supplementation.

In Chapter 3, it was determined that supplementing encapsulated flax oil did not have main effects between FLAX fed cows and CON fed cows. Dry matter intake was not affected by the inclusion of the encapsulated flax oil. In addition, milk production for both treatments increased throughout the study but is most likely accredited to the increase in crude protein in the basal diet compared to the diet that was fed to the cows previous to enrollment on the study. The FLAX fed cows did have a numerically greater percentage of milk fat, but it was not significantly different. Rumen fermentation was not affected by the encapsulated flax which means that the encapsulation process possibly helped avoid biohydrogenation of the poly-unsaturated fatty acids primarily found in flax

oil. Rumen ammonia was affected by the addition of encapsulated flax in that concentrations were less than the CON treatment. Serum glucose, triglycerides, and cholesterol were similar between treatments. Major milk fatty acids were similar between treatments. Alpha-linolenic acid was numerically greater during wk 3 for cows fed the encapsulated flax. Potentially the low number of cows per treatment and the short duration of the study period could have affected the results.

In conclusion, the results from these two studies demonstrate that there is potential to use lipids as an energy source in dairy calves and lactating dairy cows. The high fatty acid profile in the FLAX oil showed improvement in plasma fatty acids during the preweaning and post weaning period. In addition, feeding flax oil maintained growth, and was comparable to soy oil when fed to young calves. The use of encapsulated flax oil in cows maintained lactation performance and DMI was not affected by the inclusion of the supplementation. Further research on flax oil in calves should be focused on delivery method and further analysis of fatty acid profile and lipid metabolites. For cows, further analysis with a larger subset of cows and longer study period should be the focus to see if any changes are visible.

APPENDIX

Table 1. Milk Fatty Acid Composition by week of dairy cows fed ad libitum the control (CON) or the diet supplemented with 250g/d Nutri-Flax coated.

Item	Treatment		SEM	*P-Values*		
	CON	FLAX		Treatment	week	Treatment ×week
C4:0						
Mean	3.492	4.163	0.196	0.03	0.04	0.39
Wk 0	5.237	5.466	0.195	0.10		
Wk 1	3.993	4.322	0.311	0.39		
Wk 2	3.256	4.091	0.311	0.05		
Wk 3	3.226	4.076	0.311	0.01		
C5:0						
Mean	0.699	0.618	0.117	0.64	0.04	0.06
Wk 0	1.096	1.642	0.156	0.01		
Wk 1	0.580	0.587	0.173	0.97		
Wk 2	0.524	0.632	0.173	0.64		
Wk 3	0.992	0.635	0.173	0.06		
C6:0						
Mean	1.243	1.492	0.088	0.06	0.05	0.30
Wk 0	1.804	1.833	0.062	0.54		
Wk 1	1.487	1.547	0.117	0.73		
Wk 2	1.094	1.476	0.117	0.05		
Wk 3	1.149	1.453	0.117	0.04		
C8:0						
Mean	1.028	1.213	0.084	0.13	0.08	0.34
Wk 0	1.383	1.372	0.068	0.82		
Wk 1	1.243	1.262	0.122	0.91		
Wk 2	0.885	1.198	0.122	0.08		
Wk 3	0.954	1.180	0.122	0.10		
C10:0						
Mean	3.137	3.342	0.167	0.38	0.15	0.26
Wk 0	3.455	3.465	0.145	0.93		
Wk 1	3.588	3.392	0.318	0.61		
Wk 2	2.595	3.294	0.318	0.10		
Wk 3	3.227	3.339	0.318	0.68		
C12:0						
Mean	3.664	3.763	0.199	0.72	0.20	0.32
Wk 0	3.601	3.532	0.140	0.51		
Wk 1	4.163	3.822	0.351	0.43		
Wk 2	3.295	3.836	0.351	0.24		
Wk 3	3.533	3.630	0.351	0.76		
C14:0						
Mean	10.736	10.410	0.238	0.33	0.21	0.56
Wk 0	10.422	10.219	0.292	0.35		
Wk 1	11.325	10.592	0.458	0.20		

Wk 2	10.425	10.210	0.458	0.72		
Wk 3	10.457	10.429	0.458	0.94		
C14:1						
Mean	1.043	0.794	0.088	0.06	0.12	0.75
Wk 0	0.530	0.504	0.044	0.67		
Wk 1	0.993	0.790	0.141	0.25		
Wk 2	1.204	0.863	0.141	0.07		
Wk 3	0.931	0.728	0.141	0.15		
C15:0						
Mean	1.403	1.113	0.117	0.10	0.37	0.63
Wk 0	0.871	0.846	0.057	0.57		
Wk 1	1.490	1.162	0.150	0.11		
Wk 2	1.393	1.060	0.150	0.11		
Wk 3	1.324	1.116	0.150	0.24		
C15:1						
Mean	1.375	1.153	0.091	0.11	0.45	0.76
Wk 0	0.816	1.498	0.224	0.03		
Wk 1	1.455	1.202	0.133	0.14		
Wk 2	1.355	1.100	0.133	0.16		
Wk 3	1.314	1.156	0.133	0.26		
C16:0						
Mean	34.575	33.648	1.152	0.58	0.19	0.02
Wk 0	35.635	32.881	0.616	0.002		
Wk 1	32.992	33.331	1.642	0.87		
Wk 2	37.600	32.938	1.642	0.05		
Wk 3	33.136	34.676	1.642	0.39		
C16:1 *trans*						
Mean	0.388	0.366	0.064	0.80	0.69	0.07
Wk 0	0.262	0.257	0.028	0.83		
Wk 1	0.395	0.301	0.116	0.50		
Wk 2	0.453	0.288	0.116	0.28		
Wk 3	0.318	0.509	0.116	0.07		
C16:1 *cis*						
Mean	1.712	1.429	0.107	0.07	0.10	0.93
Wk 0	1.331	1.442	0.079	0.07		
Wk 1	1.640	1.259	0.244	0.20		
Wk 2	1.949	1.742	0.244	0.51		
Wk 3	1.571	1.286	0.244	0.15		
C17:0						
Mean	0.528	0.461	0.031	0.15	<0.01	0.31
Wk 0	0.393	0.412	0.017	0.13		
Wk 1	0.398	0.354	0.050	0.48		
Wk 2	0.584	0.454	0.050	0.05		
Wk 3	0.602	0.575	0.050	0.58		
C17:1						
Mean	0.266	0.222	0.015	0.07	<0.01	0.09
Wk 0	0.297	0.327	0.015	0.0005		

Wk 1	0.331	0.359	0.032	0.49		
Wk 2	0.241	0.155	0.032	0.05		
Wk 3	0.227	0.155	0.032	0.01		
C18:0						
Mean	5.959	7.356	0.451	0.05	0.17	0.15
Wk 0	11.510	11.156	0.370	0.24		
Wk 1	6.700	7.317	0.630	0.45		
Wk 2	5.024	7.359	0.630	0.009		
Wk 3	6.154	7.392	0.630	0.08		
C18:1 *trans 10*						
Mean	0.332	0.358	0.065	0.78	0.05	0.82
Wk 0	0.485	0.379	0.072	0.06		
Wk 1	0.427	0.431	0.091	0.97		
Wk 2	0.299	0.311	0.091	0.92		
Wk 3	0.270	0.331	0.091	0.53		
C18:1 *trans 11*						
Mean	0.637	0.568	0.079	0.51	0.24	0.52
Wk 0	0.294	0.181	0.134	0.17		
Wk 1	0.827	0.565	0.208	0.30		
Wk 2	0.693	0.636	0.208	0.83		
Wk 3	0.392	0.504	0.208	0.48		
C18:1 *cis 9*						
Mean	13.890	14.577	0.514	0.34	0.35	0.13
Wk 0	16.295	16.429	0.580	0.76		
Wk 1	15.072	14.445	0.939	0.58		
Wk 2	12.874	15.401	0.939	0.04		
Wk 3	13.725	13.884	0.939	0.85		
C18:1 *cis 11*						
Mean	1.886	1.549	0.207	0.26	0.88	0.57
Wk 0	1.009	0.652	0.394	0.17		
Wk 1	1.858	1.510	0.327	0.39		
Wk 2	1.970	1.417	0.327	0.20		
Wk 3	1.829	1.720	0.327	0.73		
C18:2 *cis 9, 12*						
Mean	2.887	2.971	0.224	0.79	0.33	0.04
Wk 0	2.483	2.536	0.143	0.56		
Wk 1	3.357	2.845	0.312	0.21		
Wk 2	2.544	3.080	0.312	0.20		
Wk 3	2.759	2.989	0.312	0.50		
C18:3 *gamma*						
Mean	0.233	0.236	0.042	0.97	<0.01	0.14
Wk 0	0.017	0.019	0.003	0.54		
Wk 1	0.044	0.015	0.066	0.73		
Wk 2	0.202	0.141	0.066	0.48		
Wk 3	0.455	0.550	0.066	0.15		
C18:3 *alpha*						
Mean	0.313	0.527	0.075	0.05	0.002	0.12

Wk 0	0.391	0.367	0.031	0.29		
Wk 1	0.622	0.594	0.151	0.88		
Wk 2	0.193	0.719	0.151	0.01		
Wk 3	0.124	0.269	0.151	0.26		
C20:0						
Mean	0.151	0.214	0.040	0.25	<0.01	0.24
Wk 0	0.133	0.134	0.046	0.99		
Wk 1	0.244	0.218	0.076	0.77		
Wk 2	0.182	0.372	0.076	0.06		
Wk 3	0.027	0.054	0.076	0.69		
C18:2 *CLA trans 10 cis12*						
Mean	0.018	0.014	0.005	0.60	0.003	0.34
Wk 0	0.007	0.012	0.003	0.02		
Wk 1	0.042	0.026	0.010	0.21		
Wk 2	0.001	0.000	0.010	0.87		
Wk 3	0.010	0.017	0.010	0.50		
C18:2 *CLA cis 9 trans 11*						
Mean	0.042	0.058	0.009	0.27	<0.01	0.93
Wk 0	0.058	0.054	0.009	0.59		
Wk 1	0.073	0.086	0.017	0.51		
Wk 2	0.030	0.051	0.017	0.35		
Wk 3	0.024	0.036	0.017	0.44		
C20:5						
Mean	6.497	6.740	1.187	0.88	0.50	0.10
Wk 0	0.000	1.810	1.822	0.17		
Wk 1	3.193	7.579	2.277	0.12		
Wk 2	8.052	7.108	2.277	0.75		
Wk 3	8.244	5.534	2.277	0.17		
Others[6]						
Mean	1.839	1.762	0.582	0.93	0.01	0.78
Wk 0	1.102	1.449	0.073	0.001		
Wk 1	1.815	1.400	0.820	0.70		
Wk 2	1.216	1.144	0.820	0.95		
Wk 3	2.484	2.743	0.820	0.78		
SCFA[1] (De novo synthesis)						
Mean	45.76	44.88	0.577	0.27	0.49	0.34
Wk 0	46.55	46.23	1.874	0.75		
Wk 1	47.07	44.89	1.373	0.19		
Wk 2	45.09	43.97	1.373	0.49		
Wk 3	45.11	45.89	1.373	0.48		
LCFA[2]						
Mean	54.24	55.12	0.58	0.27	0.49	0.34
Wk 0	53.45	53.77	1.873	0.75		
Wk 1	52.93	55.11	1.373	0.19		
Wk 2	54.91	56.13	1.373	0.49		
Wk 3	54.88	54.11	1.371	0.48		
Saturated FA[3]						

Mean	67.73	68.76	1.378	0.59	0.63	0.31
Wk 0	75.26	72.50	2.147	0.05		
Wk 1	69.55	68.82	2.410	0.81		
Wk 2	67.25	67.26	2.410	0.99		
Wk 3	66.39	70.20	2.410	0.09		
MUFA[4]						
Mean	21.32	20.51	0.810	0.46	0.77	0.48
Wk 0	21.38	21.84	0.893	0.46		
Wk 1	22.47	20.13	1.522	0.21		
Wk 2	20.49	21.32	1.522	0.67		
Wk 3	21.01	20.08	1.522	0.49		
PUFA[5]						
Mean	10.65	11.03	1.122	0.81	0.40	0.15
Wk 0	2.81	5.07	1.792	0.10		
Wk 1	7.69	11.33	2.125	0.17		
Wk 2	11.87	11.71	2.125	0.95		
Wk 3	12.39	10.05	2.125	0.21		

[1]SCFA = Short chain fatty acid
[2]LCFA = Long chain fatty acid
[3] Saturated FA = Saturated fatty acid
[4] MUFA = Monounsaturated fatty acid
[5] PUFA = Polyunsaturated fatty acid
[6] Others = sum of C18:1 trans 9, C19:0, C20:1, C20:2, C22:0, C22:1, C22:2, C22:3, C22:4, C22:5, C22:6.

LITERATURE CITED

Abdelqader, M.M., Hippen, A.R., Kalscheur, K.F., Schingoethe, D.J., and Garcia, A.D. Isolipidic additions of fat from corn germ, corn distillers grains, or corn oil in dairy cow diets. (19841215) *J. Dairy Sci.* 2009; 92: 5523–5533 http://dx.doi.org/10.3168/jds.2008-1867

Agerholm, J.S., A. Basse, and H.V. Krogh. 1993. Abortion and calf mortality in Danish cattle herds. Acta Vet Scand. 34:371-377.

Akbarian-Tefaghi, M., Ghasemi, E., and Khorvash, M. 2017. Performance, rumen fermentation and blood metabolites of dairy calves fed starter mixtures supplemented with herbal plants, essential oils or monensin. J. Anim. Physiol. Anim. Nutr. 102: 630-638.

Ambrose, D.J., Kastelic, J.P., Corbett, R., Pitney, P.A., Petit, H.V., Small, J.A., and Zalkovic, P. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in α-linolenic acid. J. Dairy Sci. 89:3066-3074.

Anderson, J. L., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. J. Dairy Sci. 89: 3133-3142. http://dx.doi.org/10.3168/jds.S0022-0302(06)72587-5

Anderson, E. L., T. G. Nagaraja, and J. L. Monill. 1987. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci. 70: 1OOO

AOAC. 1998. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists. Gaithersburg, MD.

AOAC. 2002. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists. Gaithersburg, M. D.

Araujo, G., Terré, M., and Bach, A. 2014. Interaction between milk allowance and fat content of the starter feed on performance of Holstein calves. *J. Dairy Sci.* 97: 6511–6518

Arieli, A., Z. Shabi, I. Bruckental, H. Tagari, Y. Aharoni, S. Zamwell, and H. Voet. 1996. Effect of the degradation of organic matter and crude protein on ruminal fermentation in dairy cows. J. Dairy Sci. 79:1774–1780.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319– 338. https: / / doi .org/ 10 .1016/ S0377 -8401(97)00009 -6.

Ballou, M.A., and E.J. DePeter 2008. Supplementing milk replacer with omega-3 fatty acids from fish oil on immunocompetence and health of jersey calves. J. Dairy Sci.91:3488-3500.

Barnoiun, J., and Chassagne, M. 1991. A aetiological hypothesis for the nutrition-induced association between retained placenta and milk fever in dairy cow. Ann Rech. Vet. 22:331-343.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76:3864-3881.

Bauman, D.E., and Lock, A.L. 2006. Concepts in lipid digestion and metabolism in dairy cows. Tri-state dairy nutrition conference. April 25 and 26, 2006.

Bauman, D.E., Mather, I.H., Wall, R.J., and Lock, A.L. 2006. Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89: 1235-1243.

Bauman, D.E., and Griinari, J.M. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23: 203-227.

Bauman, D.E., Corl, B.A., and Peterson, D.G. 2003. The biology of conjugated linoleic acids in ruminants, pages 146-173 in Advances in Conjugated Linoleic Acid Research, Vol. 2. J. -L. Sebedio, W.W. Chrisrie, and R.O. Adlof, ed. AOCS Press, Champaign, IL.

Bauman, D.E. and Grinari, J.M, 2000. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Mol, J.A. Clegg,R.A. eds. Biology of the Mammary Gland: 209-216 Kluwer Academic/Plenum Publishers New York, NY.

Beauchemin, K.A., Kruzer, M., O'Mara, F., and McAllister, T.A. 2008. Nutritional management for enteric methane abatement: a review. Anim. Prod. Sci. 48, 21-27. doi:10.1071/EA07199

Becker, R., P. T. D. Arnold, and S. P. Marshall. 1951. Development of the bovine stomach during fetal life. J. Dairy Sci. 34:329.

Beede, D., Moore, S., Burns, C., and Bucholtz, H. 1996. Mineral and vitamin nutrition and feeding management practices. In: Manging the dry cow for more profit notebook, Michigan Sate University, dairy Programs Group.

Bellows, R.A., and Short R.E. 1978. Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. J. Anim. Sci. 46: 1522-1528.

Berends, H., Vidal, M., Terre, M., Leal, L.N., Martin-Tereso, J. and Bach, A. 2018. Effect of fat inclusion in starter feeds for dairy calves by mixing increasing levels of high-fat extruded pellet with a conventional highly fermentable pellet. J. Dairy Sci. 101:10962-10972.

Bisphlinghoff, F.D. 1990. Fat in dairy cow rations. Fat and Protein Research Foundation, No. 196. Ft Myers Beach, FL.

Broudiscou, L., S. Pochet, and C. Poncet. 1994. Effect of linseed oil supplementation on feed degradation and microbial synthesis in the rumen of ciliate-free and refaunated sheep. Anim. Feed Sci. Technol. 49:189–202.

Bruckmaier, R.M. and Gross, J.J. 2017. Lactational challenges in transition dairy cows. Anim. Prod. Sci. 57:1471-1481.

Brumby, P.E., Storry, J.E., Bines, J.A., and Fulford, R.J. 1978. Utilization of energy for maintenance and production in dairy cows given protected tallow during early lactation. J. Agric. Sci. Camb. 91:151.

Butler, J.E. Sickles, S.A., Johanns, C.J., and Rosenbusch, R.F. Pasteurization pf discard mycoplasma mastitic milk used to feed calves: Thermal effect on various mycoplasma. J. Dairy Sci. 2000. 83: 2285-2288.

Byers, F.M. and Schelling, G.T. 1988. Lipids in ruminant nutrition. Pages 298-312 In: Ruminant Animal, D.C. Church, Waveland Press, Prospect Heights, III.

Caffrey, P.J., C. Mill, P.O. Bropy, and D.L. Kelleher. 1988. The effects of method of processing of starter, tallow inclusion and roughage supplementation on the performance of early-weaned calves. Anim. Feed Sci. Technol. 19:231-246.

Cakaloglu, B., Ozyurt, V.H., and Otles, S. Cold press in oil extraction. A review. 2018. Ukrainian Food J. 7:640-654.

Can Karaca, A., Low, N., and Nickerson, M. 2013. Encapsulation of flaxseed oil using a benchtop spray dryer for legume protein-maltodextrin microcapsule preparation. J. Agric. Food Chem. 61:5148-5155.

Chen, Z., Herdt, T.H., Liesman, J.S., Ames, N.K., and Emery, R.S. 1995. Reduction of bovine plasma cholesterol concentration by partial interruption of enterohepatic circulation of bile salts: a novel hypocholesterolemic model. J Lipid Research. 36:1544-1556.

Chilliard, Y., Ferlay, A., Mansbridge, R.M., and Doreau, M. 2000. Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49:181-205.

Chilliard, Y., Glasse, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. 2007. Diet, rumen biohydrogenation, cow and goat milk fat nutritional quality: A review. Eur J. Lipid Sci. Technol, 109: 828-855.

Christen, K.A., Schingoethe, D.J., Kalscheur, K.F., Hippen, A.R., and Krages, K.K. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. J. Dairy Sci. 93: 2095-2104.

Cressman, S. G., D. G. Grieve, G. K. Macleod, E. E. Wheeler, and L. G. Young. 1980. Influence of dietary protein concentration on milk production by dairy cattle in early lactation. J. Dairy Sci. 63:1839–1847.

Coppock, C.E., Everett, R.W., Natzke, R.P., and Ainsle, H.R. 1974. Effect of dry period length on Holstein milk production and selected disorders at parturition. J. Dairy Sci. 57:712.

Corah, L., A. Fleck, M. McKee, and R. Schalles. 1978. Effect of energy level during late gestation on the performance of heifers calving for the first time. Kansas Agr. Exp. Sta. Rep. of Progress 320.

Cribier S. Morrot, G., and Zachowski, A. 1993. Dynamics of the membrane lipid phase. Prostaglandins Leukot Essent Fatty Acids. 48:27-32.

Cunningham, K. D., M. J. Cecava, T. R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79: 620–630.

Czerkawski, J.W., and Clapperton, J.L. 1984. Fats as energy yielding compounds in the ruminant diet. In Fats in Animal Nutrition, ed. J. Wiseman. London, Butterworths, 249-263.

Daun, J.K., V.J. Barthet, T.L. Chornick and S. Duguid. 2003. Structure, composition, and variety development of flaxseed. In: Flaxseed in Human Nutrition, 2nd ed., AOCS Press, Champaign, Ill., pp. 1-40.

Daun, J.K., and R. Przybylski. 2000. Environmental effects on the composition of four Canadian flax cultivars. Proc. 58th Flax Institute, March 23-25, 2000, Fargo, N.D. pp 80-91.

Davis, C.L., and Brown, R.E. 1970. Low-fat milk syndrome. Pages 545-565 in Physiology of Digestion and Metabolism in the Ruminant, A.T. Phillipson, ed. Oriel Press Ltd., Newcastle upon Tyne, UK.

Doppenberg, J., and D.L. Palmquist. 1991. Effectof dietary fat level on feed intake, growth, plasma metabolites and hormones of calves fed dry or liquid diets. Livest. Prod. Sci. 29:151-166.

Doreau, M, and Ferlay, A. 1994. Digestion and utilization of fatty-acids by ruminants. Anim. Feed Sci. Technol. 45: 379-396.

Drackley, J.K. 2015. Interorgan lipid and fatty acid metabolism in growing ruminants. in: D.G. Burrin, H.J. Mersmann (Eds.) Pages 323–350 in Biology of Metabolism in Growing Animals. Elsevier Limited, Edinburgh, UK

Drackley, J. K. 2008. Calf nutrition from birth to breeding. Vet. Clin. North Am. Food Anim. Pract. 24:55–86.

Drackley, J.K. 2005. Early growth effects on subsequent health and performance of dairy heifers. Chapter 12 in Calf and Heifer Rearing. P.C. Garnsworthy, ed. Nottingham University Press, Nottingham, UK.

Drackley, J.K., Heather, M., Dann, G., Janovick Guretzky, N.A. Literland, N.B., Underwood, J.P. and Loor, J.J. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Ital. J. Anim. Sci. 4: 323-344.

Drackley, J.K., Overton, T.R., and Douglas, G.N. 2001. Adaptation of glucose and long chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84: E100-E112.

Drackley, J.K. 1999. ADSA Foundation Scholar Award. Biology of dairy cow during the transition period: the final frontier. J. Dairy Sci. 82: 2259-2273.

Drouillard, J.S., E.J. Good, C.M. Gordon, T.J. Kessen, M.J. Sulpizio, S.P. Montgomery and J.J. Sindt. 2002. Flaxseed and flaxseed products for cattle: Effects on health, growth performance, carcass quality, and sensory attributes. Proc. 59th Flax Institute, March 21-23, 2002, Fargo, N.D. pp 72-87.

Dyk, P. and Emery, r. 1996. Reducing the incidence of peripartum health problems. Proc. Tristate Nutrition Conference, Ft. Wayne, IN, 9. 41.

Erb, H.N., Smith, R.D., Hillman, R.B., Powers, P.A., Smith, M.C., White, M.E., and Pearson, E.G. 1984. Rates of diagnosis of six diseases of Holstein cows during 15-day and 21-day intervals. Am. J. Vet. Res. 45:333-335.

Foa, Carlo. Sull'Origine del lattosio, Della Caseina e del Grasso Della Latte. Arch Fisiol., 10:402. 1912

Foley, J. A., and D. E. Otterby. 1978. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A review. J. Dairy Sci. 61:1033–1060.

Forster, R. J., D. G. Grieve, J. G. Buchanan-Smith, and G. K. Macleod. 1983. Effect of dietary protein degradability on cows in early lactation. J. Dairy Sci. 66:1653–1662.

Fossati, P., and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28:2077–2080.

Funk, D.A., Freeman, A.E., and Berger, P.J. 1987. Effects of previous days open, previous days dry, and present days open on lactation yield. J. Dairy Sci. 70: 2366.

Glasser, F., Ferlay, A., and Chillard, Y. 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. J. Dairy Sci. 91:4687-4703.

Ghasemi, E., Azad-Shahraki, M., and Khorvash, M. 2017. Effect of different fat supplements on performance of dairy calves during cold season. J. Dairy Sci. 100: 5319-5328.

Godden, S., J. Fetrow, J. Feirtag, L. Green, and S. Wells. 2005. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. JAVMA. 226(9): 1547-1554.

Govil, K., Yadav, D.S., Patil, A.K., Nayak, S., Baghel, R.P.S., Yadav, P.K., Malapure, C.D., and Thakur, D. 2017. Feeding management for early rumen development in calves. J. Entomology and Zoology Studies. 5:1132-1139.

Gozho, G.N., Plizer, J.C., Krause, D.O., Kennedy, A.D., and Wittenberg, K.M. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88: 1399-1403.

Grant, R.J., and Weidner, S.J. 1992. Digestion kinetics of fiber: Influence of the in vitro buffer pH varied within observed physiological range. J. Dairy Sci. 75: 1060.

Graulet, B., Gruffat-Mouty, D., Durand, D., and Bauchart, D. 2000. Effects of milk diets containing beef tallow or coconut oil on the fatty acid metabolism of liver slices from preruminant calves. Br. J. Nutr. 84:309-318.

Gross, J.J., and Bruckmaier, R.M. 2019. Metabolic challenges and adaptation during different functional stages of the mammary gland in dairy cows: Perspectives for sustainable milk production. J. Dairy Sci. 102:2828-2843.

Godden, S. 2008. Colostrum management for dairy calves. Vet Clin Food Anim 24:19-39. Goff, J.P., and Horst, R.L. 1995. Dietary potassium, but not calcium (Ca), induces milk fever in dairy cows. J. Dairy Sci. 78:185.

Goodridge, J., J.R. Ingalls and G.H. Crow. 2001. Transfer of omega-3 linolenic acid and linoleic acid to milk fat from flaxseed or Linola protected with formaldehyde. Can. J. Anim. Sci. 81: 525-532.

Guilloteau, P., Corring, T., Toullec, R., and Guilhermet, 1980. R. Enzyme potentialities of the abomasum and pancreas of the calf. II- Effect of weaning and feeding a liquid supplement to ruminant animals. Reprod Nutr Dev. 25:481-493.

Guilloteau, P., Zabielski, R., and Blum, J.W. 2009. Gasterointestional tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulations. J. Phys and Pharm. 60:37-46.

Hafez, E.S.E, and Lineweaver, L.A. 1968. Suckling behavior in material and artificially fed neonate calves. Z. Tierpsychol. 25: 187-198.

Han can der Kolk, J.H., Gross, J.J., Gerber. V., and Bruckmaier, R.M. 2017. Disturbed bovine mitochondrial lipid metabolism: A review. Vet. Q. 37: 262-273.

Harfoot, C.G., and G.P. Hazlewood, G.P. 1997. Lipid metabolism in the rumen. In: P.N. Hobson and C. S. Stewart (ed.) The Rumen Microbial Ecosystem. Pp 382-426. Chapman and Hall, London, UK.

Heinrichs, J., P. Tozer, M. Gabler,and T. Schriefer. 2016a. Heifer economics. PennState Extension.

Heinrichs, J., and Jones, C.M. 2016b. Colostrum management tools: hydrometers and refractometers. PennState Extension.

Heinrichs, J., and Kmicikewycz, A. 2016c. Total Mixed Rations for Dairy Cows. PennState Extension

Heinrichs, A. J. 1993. Raising dairy replacements to meet the needs of the 21st century. J. Dairy Sci. 76:3179-3187.

Heinrichs, A.J. and G.L. Hargrove. 1991. Standards of weight and heifer of Holstein heifers. J. Dairy Sci. 70:653-660.

Heinrichs, A.J. and Losinger, W.C. 1998. Growth of Holstein dairy heifers in the United States. J. Anim. Sci. 76:1254-1260.

Heinrichs, A.J., Wells, S.J., and Losinger, W.C. 1995. A study of the use of milk replacers for dairy calves in the United States. *J. Dairy Sci.* 78: 2831–2837

Herdt, T.H.: Fatty Liver in Dairy Cows. Vet. Clin. North Am. Food Anim. Pract. 1988; 4: 269-287.

Hill, T.M., Bateman, H.G., Aldrich, J.M., Quigley, J.D., and Schlotterbeck, R.L. 2015. Inclusion of tallow and soybean oil to calf starters fed to dairy calves from birth to four months of age on calf performance and digestion. *J. Dairy Sci.* 98: 4882–4888

Hill, T.M., H.G. Batemann II., Aldrich, J.M., and R.L. Schlotterbeck. 2011. Impact of various fatty acids on dairy calf performance. Prof, Anim. Sci. 27: 167-175.

Hill, T.M., H.G. Batemann II., Aldrich, J.M., and R.L. Schlotterbeck. 2009. Effects of changing the essential and functional fatty acid intake of dairy calves. J. Dairy Sci. 92:670-676. doi:10.3168/jds.2008-1368.

Hill, T. M., J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman. 2007a. Protein concentrations for starters fed to transported neonatal calves. Prof. Anim. Sci. 23:123–134.

Hill, T.M., J.M. Aldrich, R.L. Schlotterbeck, and H.G, Bateman. 2007b. Effects of changing the fatty acid composition of calf starters. Prof. Anim. Sci. 23: 665-671

Hill, T.M., H.G. Bateman, II, J.M. Aldrich, and R.L. Schlotterbeck. 2007c. Effects of feeding rate of milk replacers and bedding material for calves in a cold, naturally ventilated nursery. Prof. Anim. Sci. 23:656-664.

Hill, T.M., J.M. Aldrich, R.L. Schlotterbeck, and H.G. Bateman II. 2006. Effects of feeding calves different rates and protein concentrations of 20 percent fat milk replacers on growth during the neonatal period. Prof. Anim. Sci. 22:252-260.

Hoffman, P.C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836-845.

Hoffman, P.C. Funk, D.A. 1992. Applied dynamics of dairy replacement growth and management. J. Dairy Sci. 75:2504-2516.

Hoffman, R.; Gerber, M. 2014. Can rapeseed oil replace olive oil as part of a Mediterranean-style diet? Br. J. Nutr.112, 1882–1895.

Holtenius, P. 1989. Plasma lipids in normal cows around partus and in cows with metabolic disorders with and without fatty liver. Acta Vet. Scand. 30: 441-445.

Hristov, A. N., Oh, J., Firkins, J.L., Dijkstra, J., Kereab, E., and Waghorn, G. 2013. Mitigation of methane and nitrous oxide emissions from animal operations. I. A review of enteric methane mitigation options. J. Anim. Sci. 91: 5045-5069. doi:10.2527/jas.2013-6583.

Hu, J., Zhang, Z., Shen, W.J., and Azhar, S. 2010. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr. Metab. 7:47. doi.org/10.1186%2F1743-7075-7-47

Ikwuegbu, O. A. and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365–375.

Ingvartsen, K.L. 2006. Feeding and management related diseases in the transition cow: physiological adaptations around calving and strategies to reduce feeding related diseases. Anim. Feed Sci. Technol. 126: 175-213.

Ingvartsen, K.L., Dewhurst, R.J., and Friggens, N.C. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest Prod. Sci. 83: 277-308.

Ingvartsen, K.L., and Andersen, J.B. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83: 1573-1597.

Institute of Medicine. 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academies Press, p 8-1-8-97.

Ishler, V.A. 2017. Dairy Sense: Know your herd's feed cost. Dairy Herd management: Farm journal inc.

Jahani-Moghadam, M., Mahjoubi, E., and Dirandeh, E. 2015. Effect of linseed feeding on blood metabolites, indidence of cystic follicles, and productive and reproductive performance in fresh Holstein dairy cows. J. Dairy Sci. 98: 1828-1835. http://doi.org/10.3168/jds.201-8789

Jasper, J., and D.M. Weary. 2002. Effects of *ad libitum* milk intake on dairy calves. J. Dairy Sci. 85:3054-3058.

Jenkins, K.J., Kramer, J.K.G., Sauer, F.D., and Emmons, D.B. 1995. Influence of triglycerides and free fatty acids in milk replacers on calf performance, blood plasma and adipose lipids. J. Dairy Sci. 68:669.

Jenkins, T. 1997. Success of fat in dairy rations depends on the amount. Feedstuffs Feb 13, 1997, issue: 11-12

Jenkins, T.C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851.

Jenkins, T.C., and Jenny, B.F. 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. J. Dairy Sci. 72: 2316.

Jenkins, K.J., Kramer, J.K.G., and Emmons, D.B. 1986. Effect of lipid in milk replacers on calf performance and lipids in blood plasma, liver, and perirenal fat. J. Dairy Sci. 69:447-459.

Jenkins, K.J., Kramer, J.K.G., Sauer, F.D., and Emmons, D.B. 1985. Influence of triglycerides and free fatty acids in milk replacers on calf performance, blood plasma, and adipose lipids. J. Dairy Sci.68: 669–680

Johnson, D. Jr., Dolge, K.L., Rousseau, J.E. Jr., Teichman, R., and Eaton, H.D. 1956. Effect of addition of inedible tallow to a calf starter fed to Holstein calves. *J. Dairy Sci.* 39: 1268–1279.

Jonker, J.S., Kohn, R.A., and Erdman, R.A. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81: 2681-2692.

Kalscheur, K.F., Vandersall, J.H., Erdman, R.A., Kohn, R.A., and Russek-Cohen, E. 1999. Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. J. Dairy Sci. 82: 545-554.

Karcher, E.L., Hill, T.M., Bateman II, H.G., Shlotterbeck, R.L., Vito, N., Sordillo, L.M., and VandeHaar, M.J. 2014. Comparison of supplementation of n-3 fatty acids from fish and flax oil on cytokine gene expression and growth of milk-fed

Holstein calves. J. Dairy Sci. 97:2329-2337. https://doi.org/10.3168/jds.2013-7160

Kassa, T., Ambrose, J.D., Adams, A.L., Risco, C., Staples, C.R., Thatcher, M.J., Van Horn, H.H., Garcia, A., Head, H.H., and Thatcher, W.W. 2002. Effects of whole cottonseed diet and recombinant bovine somatotropin on ovarian follicles in lactating dairy cows. J. Dairy Sci. 85:2823-2830.

Kehoe, S.I., Dechow, C.D., and Heinrichs, A.J. 2007. Effects of weaning age and milk feeding frequency on dairy calf growth, health and rumen parameters. Livestock Sci. 110: 267-272.

Kelley, N.S., Hubbard, N.E., and Erickson, K.L. 2007. Conjugated linoleic acid isomers and cancer. J. Nutr. 137: 2599-2607.

Kennelly, J.J., and G.R. Khorasani. 1992. Influence of flaxseed feeding on fatty acid composition of cows' milk. Proc. 54th Flax Institute, Jan. 30-31, 1992, Fargo, N.D. pp 99-105.

Kertz, A.F., Prewitt, L.R., and Everett, J.P. 1979. A early weaning calf program: summarization and review. J. Dairy Sci. 62:1835-1843.

Khan, M.A., H.J. Lee, W.S. Lee, H.S. Kim, S.B. Kim, K.S. Ki, J.K. Ha, H.G. Lee, and Y.J. Choi. 2007a. Pre- and post-weaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876-885.

Khan, M.A., H.J. Lee, W.S. Lee, H.S. Kim, K.S. Ki, T.Y. Hur, G.H. Suh, S.J. Kang, and Y.J. Choi. 2007b. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:3376-3387.

Kleen, J.L., Hooijer, G.A., Rehage, J., and Noordhuizen, J.P.T.M. 2003. Subacute ruminal acidosis (SARA): a review. J. Vet. Med. A 50:406-414.

Klein, C.J. 2002 Nutrient requirements for preterm infant formulas. *J. Nutr.*132: 1395S–1577S

Klein, R.D., Kincaid, R.L., Hodgson A.S., Harison, J.H.,Hiller, J.K., and Cronrath, J.D. 1987. Dietary fiber and early weaning on growth and rumen development of calves. J. Dairy Sci. 70:2095-2104.

Kuehn, C.S., Otterby, D.E., and Linn, J.G. 1994. The effect of dietary energy concentration on calf performance. *J. Dairy Sci.* 77: 2621–2629

Kumar, S., Choudhury, P.K., Carro, M.D., Griffith, G.W., Dagar, S. S., and Puniya, M. 2014. New aspects and strategies for methane mitigation from ruminants. Appl. Microbiol. Biotechnol. 98:31-44. doi:10.1077/s00253-013-5365-0.

Kung, L., Jr., and J. T. Huber. 1983. Performance of high producing cows in early lactation fed protein of varying amounts, sources and degradability. J. Dairy Sci. 66:227–234

Lambert, M.R., N. L. Jacobson, R. S. Allen, and M. R. Bell. 1955. The relationship of growth, feed consumption, and certain blood constituents to changes in the dietary of young calves. J. Dairy Sci. 38:6.

Lardy, G.P., and V.L. Anderson. 1999. Alternative feeds for ruminants. North Dakota State University Extension Service publication AS1182

Leat, W.M.F., and Harrison, F.A. 1975. Digestion, absorption and transport of lipids in sheep. Page 481 in Proc. IV int. Sym Ruminant Physiol. I. W. McDonald and A.C.I. Warner, ed. Univ. New England Publ. Unit, Armidale, NSW, Aust.

Leduc, M., Letourneau-Montiminy, M.P., Gervais, R., and Chouinard, P.Y. 2017. Effect of dietary flax seed and oil on milk yield, gross composition, and fatty acid profile in dairy cows: A meta-analysis and meta-regression. J. Dairy Sci. 100: 8906-8927. http://doi.org/10.3168/jds.2017-12637

Le Huerou-Luron, I., Guilloteau, P., And Wicker-Planquart, C. 1992. Gastric and pancreatic enzyme activities and their relationship with some gut regulatory peptides during postnatal development and weaning in the calf. J Nutr. 122: 1434-1445.

Lock, A.K., Harvatine, K.J., Drackley, J.K. and Bauman, D.E. 2006, Concepts in fat and fatty acid digestion in ruminants. Proc. Intermoutain Nutr, Conf pp. 85-100.

Lock, A.l., Harvatine, K.J., Ipharraguerre, I.R., Van Amburgh, M.E., Drackley, J.K. and Bauman, D.E. 2005. The dynamics of fat digestion in lactating dairy cows: what does the literature tell us? Pro. Cornell Nutr. Conf. pp. 83-94.

Looper, M. 2014. Use of commodity feeds in dairy rations. University of Arkansas Cooperative Extension. FSA4013

Loosli, J.K., Elliot, J.M., and Warner, R.G. 1960. Comparative value of corn distillers dried grains with solubles, soybean oil meal, and linseed oil meal for milk production. J. Dairy Sci. 43: 816-820.

Lundy, F.P., Block, E., Bridges, W.C., Bertrand, J.A., and Jenkins, T.C. 2004. Ruminal biohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci.87: 1038-1046.

Lucy, M.C., De La Sota, R.L., Staples, C.R., and Thatcher, W.W. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine

somatotropin (sometribove) or saline and fed diets differing in fat content and energy. J. Dairy Sci. 76:1014-1027.

Machmuller, A., Ossowski, D.A., and Kreuzer, M. 2000. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Anim. Feed Sci. Technol. 85: 41–60

Madison, R.J., Schingoethe, D.J., and Brouk, M.J. 1994. Lactational response of primiparous and multiparous cows to supplemental fat. P86. Abstr. ASA and ADSA Midwestern Branch meeting, Des Moines, March 21-23, 1994.

Mattos, R., Staples, C.R., and Thatcher, W.W. 2000. Effects of dietary fatty acids in reproduction in ruminants. Rev Reprod. 5:38-45.

Mattson, F.H., Benedict, J.H., Martin, J.B., and Beck, L.W. 1952. Intermediates formed during digestion of triglycerides. Journal of Nutrition 48: 335-344.

McGuirk, S.M. 2008. Disease management of dairy calves and heifers. Vet Clin Food Anim. 24:139-153.

McGuirk, S. M. 2005. Otitis media in calves. Pages 228-230 in Proc. 23rd Am. Coll. Vet. Intern. Med., Baltimore, MD. American College of Veterinary Internal Medicine, Medical Forum, Greenwood Village, Co.

Mellenberger, R., Sears, P., Erskine, R., and McFadden, M. 1996. Dry cows: Health and mastitis management. In: Managing the dry cow for more profit notebook. Michigan State University, Dairy Programs Group.

Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. In: D.C. Church (Ed) The Ruminant Animal: Digestive Physiology and Nutrition. Prentice Hall Inc., Englewood Cliffs, NJ, 182-189.

Miller, W.J., Carmon, J.L., and Dalton, H.L. 1959. Influence of high levels of plant and animal fats in calf starters on growth, feed consumption, and palatability. *J. Dairy Sci.* 42: 153–158.

Miller-Cushon, E. K., C. Montoro, I. Ipharraguerre, and A. Bach. 2014. Dietary preference in dairy calves for feed ingredients high in energy and protein. J. Dairy Sci. 97:1634–1644. http://dx.doi. org/10.3168/jds.2013-7199.

Miller-Cushon, E.K., M. Terre., T.J. DeVries., and A. Bach. 2014. The effect of palatability of protein source on dietary selection in dairy calves. J. Dairy Sci. 97: 4444-4454. https://doi.org/10.3168/jds.2013-7816

Mohamed, O.E., Satter, L.D., Grummer, R.R., and Ehle, F.R. 1988. Influence of dietary cottonseed and soybean on milk production and composition. J. Dairy Sci. 71: 2677

Moody, M.L., Zanton, G.I., Daubert, J.M., and Heinrichs, A.J. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. J. Dairy Sci. 90: 5580-5586.

Montoro, C., and A. Bach. 2012. Voluntary selection of starter feed ingredients offered separately to nursing calves. Livest. Sci. 149:62– 69.

Morris, D.H., 2007. Flax: A health and nutrition primer. Winnipeg, MB: Flax Council of Canada.

Mustafa, A.F., Mckinnon, J.J., Christensen, D.A, and He, T. 2002. Effects of micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. Anim, Feed Sci. Technol. 95: 123-132.

Mustafa, A.F., Chouinard, P.Y., and Christensen, D.A. 2003. Effects of feeding micronized flaxseed on yield and composition of milk from Holstein cows. J. Sci. Food Agric. 83:920-926.

Nonnecke, B.J., M.R. Foote, J.M. Smith, B.A. Pesch, T.H. Elsasser, and M.E. Van Amburgh. 2003. Composition and functional capacity of blood mononuclear leukocyte populations from neonatal calves on standard and intensified milk replacer diets. J. Dairy Sci. 86:3592-3604. 10

Nikitin, B.N. 1949. Some new views in biochemistry of lactation. Biolhimiya, 14:211.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl.Acad. Sci., Washington, DC.

Oelke, E. Johnson, S., Ehrhardt, P. and Comstock, V. 1987. From flax straw to linin fiber. Agricultural Fact Sheet 3339. Minnesota Extension Service, University of Minnesota, St. Paul.

Ohajuruka, O.A., Wu, Z., and Palmquist, D.L. 1991. Ruminal metabolism, fiber and protein digestion by lactating cows fed calcium soap or animal-vegetable fat. J. Dairy Sci. 74:2601.

Onetti, S.G., Shaver, R.D., McGuire, M.A., and Grummer, R.R. 2001. Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. J. Dairy Sci. 84: 2751-2759. http://doi.org/10.3168/jds.S0022-0302(01)74729-7

Oplinger, E.S., Oelke, E.A., Doll, J.D., Bundy, L.G., and Schuler, R.T. 1989. Flax: Alternative Field Crops Manual. University of Wisconsin Extension, Madison, WI.

Ostergaard, V., Danfaer, A., Dangaard, J., Hindhede, J., and Thysen, I. 1981. The effect dietary lipids on milk production in dairy cows. Berenting Fra Statens Hudyr-brugs Forsog No. 508, Copenhagen, DN.

Overton, T.R., and Waldron, M.R. 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. J. Dairy Sci. 87: E105-E119.

Palmquist, D.L. 2006. Milk fat: origin of fatty acids and influence of nutritional factors thereon. In: P.F. Fox and P.L.H McSweeney (Eds.) Advanced Dairy Chemistry, Volume 2: Lipids, 3rd Edition. Pp. 43-92. Kluwer Academic/ Plenum Publishers, New York, USA.

Palmquist, D.L., Beaulieu, A.D., and Barbano D.M. 1993. Feed and animal factors influencing milk fat composition. J Dairy Sci. 1993;76:1753–71.

Palmquist, D.L., and Conrad, H.R. 1978. High fat rations for dairy cows. Effects on feed intake, milk and fat production, and plasma metabolites. J. Dairy Sci. 61:890.

Palmquist, D.L. 1987. Adding fat to dairy diets. Animal Health and Nutrition, 42:32.

Palmquist, D.L. and Jenkins, T.C. 1982. Calcium soaps as fat supplement in dairy cattle. Pages 477-481 in Proc. XII World Cong. Dis. Cattle.

Pantoja, J., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. J. Dairy Sci. 77:2341–2356.

Perry, G.A., Smith, M.F., and Geary, T.W. 2004. Ability to intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. J. Anim. Sci. 82:695-704. doi.org/10.2527/2004.823695x

Petit, H.V. 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J. Dairy Sci. 86:2637-2646.

Petit, H.V. 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. J. Dairy Sci. 85: 1482-1490.

Petit, H.V., R.J. Dewhurst, J.G. Proulx, M. Khalid, W. Haresign and H. Twagiramungu. 2001.Milk production, milk composition, and reproductive function of dairy cows fed different fats. Can. J. Anim. Sci. 81: 263-271.

Piot, C., Hocquette, J., Herpin, P., Veerkamp, J.H., and Bauchart, D. 2000. Dietary coconut oil affects more lipoprotein lipase activity than the mitochondria oxidative capacities in muscles of preruminant calves. J. Nutr. Biochem. 11: 231–238

Quigley, J.D. III, Rejman, J.J., and Martin, K.R. 1994. Response of peripheral blood mononuclear cells to mitogenic stimulation in young calves. J. Dairy Sci. 77: 259-263. https://doi.org/10.3168/jds.S0022-0302(94)76949-6

Quigley, J.D. III., Smith, Z.P., and Heitmann, R.N. 1991. Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. J. Dairy Sci. 74:258-263.

Quigley. J. D. III, C. G. Schwab, and W. E. Hyltoa 1985. Development of rumen function in calves: nature of protein reaching the abomasum. J. Dairy Sci. 68: 794.

Raven, A.M. 1970. Fat in milk replacers for calves. *J. Sci. Food Agric.* 21: 352–359

Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123-158 in The Analysis of Dietary Fiber in Food. W. P. T. James and O. Theander, ed. Marcel Dekker Inc. New York, NY.

Robinson, P. H., and J. J. Kennelly. 1988. Influence of intake of rumen undegradable protein on milk production of late lactation Holstein cows. J. Dairy Sci. 71:2135–2142.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525.

Ruesseger, G.J., and Schultz, L. H. 1985.Response of high producing cows in early lactation to the feeding of heat treated whole soybeans. J. Dairy Sci. 68: 3272.

Ruthig, D.J.; Meckling-Gill, K.A. 1999. Both (n-3) and (n-6) fatty acids stimulate wound healing in the rat intestinal epithelial cell line, IEC-6. J. Nutr. 129, 1791–1798.

Sander, E.G., Warner, R.G., Harrison, H.N., and Loosli, J.K. 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42:1600-1605.

Savoire R., Lanoisellé J. L., Vorobiev E. (2013), Mechanical continuous oil expression from oilseeds: a review, *Food and Bioprocess Technology*, 6(1), pp. 1‑16.

Schingoethe, D.J., kalscheur, K.F., Hippen. A.R., and Garcia, A.D. 2009. Invited Review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 95: 5802-5813.

Schingoethe, D.J., Brouk, M.J., and Birkelo, C.P. 1999. Milk production and composition from cows fed wet corn distillers grains. J. Dairy Sci. 82: 574-580.

Schingoethe, D.J. 1996. Dietary influence on protein level in milk and milk yield in dairy cows. Anim. Feed Sci. Tech. 60:181-190.

Scott, M.C., R.E. James, and M.L. McGilliard. 2006. Performance of calves fed whole milk and milk replacer in different sequences. J. Dairy Sci. 89(Suppl.1):32.

Senevirathine, N.D., Anderson, J.L., and Rovai, M. 2018. Growth performance and health of dairy calves given water treated with a reverse osmosis system compared with municipal city water. J. Dairy Sci. 101:8890-8901.

Senevirathine, N.D., Anderson, J.L., W.R. Gibbions, and J.A. Clapper. 2017. Growth performance of calves fed microbially-enhanced soy protein in pelleted starters. J. Dairy Sci. 100:199-212.

Sevinc, M., Basoglu, A., and Guzelbektas, H. 2003. Lipid and lipoprotein levels in dairy cows with fatty liver. Turk J Vet Anim Sci. 27:295-299.

Shaw, J.C., and Knodt, C.B. 1941. The utilization of β-Hydroxybutyric acid by the lactating mammary gland. J. Biol. Chem., 138:287.

Singh J., Bargale P. C. (2000), Development of a small capacity double stage compression cold press for oil expression, *Journal of Food Engineering*, 43(2), pp. 75‑82.

Skaar, T.C., Grummer, R.R., Detine, M.R., and Stauffacher, R.F. 1989. Seasonal effects of pre and postpartum fat and niacin feeding on lactation performance and lipid metabolism. J. Dairy Sci. 72: 2028.

Stabel, J.R. On farm batch pasteurization destroys Mycobacterium paratuberculosis in waste milk. J. Dairy Sci. 2001. 84: 524-527.

Stamey, J. A., N. A. Janovick, A. F. Kertz, and J. K. Drackley. 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. J. Dairy Sci. 95:3327–3336.

Stamey, J.A., Janovick Guretzky, N.A., and Drackley, J.K. 2005. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. J. Dairy Sci. 88:254.

Stanton, T.L., Jones, L.R., Everett, R.W. and Kachman, S.D. 1992. Estimating milk, fat, and protein lactation curves with a test day model. J. Dairy Sci. 75:1691-1700.

Staples, C.R., Burke, J.M., and Thatcher W.W. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Staples, C.R. 1992. Large Dairy Heard management: Forage section, harvesting, sorting and feeding. P. 347. Book. American Dairy Science Association, Champaign, IL.

Stern, M.D., Santos, K.A., and Satter, L.D. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating cattle fed heat treater soybeans. J. Dairy Sci. 68: 45.

Sukhija, P.S., and Palmquist, D.L. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36 (6):1202-1206.

Sutton, J. D., A. D. Mcoilliard. and N. L. Jacobson. 1963. Pnnctional development of ramen mucosa I. Absorptive ability. J. Dairy Sci 46:426.

Svensson, C., A. Linder, and S. O. Olsson. 2006. Mortality in Swedish dairy calves and replacement heifers. J Dairy Sci. 89:4769-4777.

Swali, A., and D.C Wathes. 2006. Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. Theriogenolgy 66:1173-1184.

Tekerli, M., Akinci, Z., Dogan, I., and Akcan, A. 2000. Factors affecting the shape of lactation curves of Holstein cows from the balikesir province of turkey. J. Dairy Sci. 83:1381-1386.

Terre, M., Castells, L., Fabregas, F., and Bach, A. 2013. Comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. J. Dairy Sci. 8: 5290-5294. https://doi.org/10.3168/jds.2012-5921

Tessman, N.J., Radloff, H.D., Dhiman, T.R., Kleinmans, K., and Satter, L.D. 1988. Effect of dietary forage: grain ratio on performance of lactating dairy cows. P. 46 In 1987 Research summaries, US Dairy Forage Research Center, Madison, WI.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem. 6:24–27.

Uitterhaegen E., Evon P. (2017), Twin‑cold extrusion technology for vegetable oil extraction:A review, *Journal of Food Engineering*, 212, pp. 190‑200.

Van De Stoet, D.L., Calderin Diaz, J.A., Stalder, K.J., Heinrichs, A.J., and Dechow, C.D. 2016. Association of calf growth traits with production characteristics in dairy cattle. J. Dairy Sci. 99:8347-8355.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal

nutrition. J. Dairy Sci. 74: 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Van Soest, P.J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency, A review, J, Dairy Sci. 46: 204.

Virtala, A.M., G. D. Mechor, and Y. T. Grohn. 1996. Morbidity from nonrespiratory diseases and mortality in dairy heifers during first three months of life. J Am Vet Med Assoc. 208:2043-2046.

Wang, S., Giller, K., Kreuzer, M., Ulbrich, S.E., Braun, U., and Schwarm, A. 2017. Contribution of ruminal fungi, archaea, protozoa, and bacteria to the methane suppression caused by oilseed supplemented diets. Frontiers in Microbio. 8: 1864. Doi.10.3389/frnicb.2017.01864

Wang, H., and Dey, S.K. Lipid signaling in embryo implantation. Prostaglandins other Lipid Mediat. 77:84-102.

Wathes, D.C., J. C. Brickell, N. E. Bourne, A. Swali, and Z. Cheng. 2008. Factors influencing heifer survival and fertility on commercial dairy farms. Animal 2:1135-1143.

Wildman, E. E., G.M. Jones, P.E. Wagner, R.L. Bowman, H.F. Troutt Jr., and T.N. Lesch. 1982. A dairy cow body condition scouring system and its relationship to standard production characteristics. J. Dairy Sci. 65:495-501.

Williamson, D.D., Mellanby, J., and Krebs, H.A. 1962. Enzymatic determination of D (-) β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J, 82:90-96

Winder, C.B., Bauman, C.A., Duffield, T.F., Barkema, H.W., and Keefe, G.P. 2018. Canadian National Dairy Study: heifer calf management. J. Dairy Sci. 101:10565-10579.

Wells, S.J., and D. A. Dargatz. 1996. Factor associated with mortality to 21 days of life in dairy heifers in the United States. Prev Vet Med. 29:9-19.

Wu, Z., and J.T. Huber. 1994. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: a review. Livest. Prod. Sci. 39:141-155.