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Evaluation of Feeding Alternative Feedstuffs Including Hydroponic Barley Sprouts and Carinata Meal to Dairy Cattle

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EVALUATION OF FEEDING ALTERNATIVE FEEDSTUFFS INCLUDING HYDROPONIC BARLEY SPROUTS AND CARINATA MEAL TO DAIRY CATTLE

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Biological Sciences degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADG	Average daily gain
AFC	Age first calving
BCS	Body condition score
BW	Body weights
Ca	Calcium
Cl	Chloride
cm	centimeters
CRM	Carinata meal
dL	deciliter
DDGS	Dried distillers grains with solubles
DM	Dry matter
DMI	Dry matter intake
DIM	Days in milk
EAA	Essential amino acid
EE	Ether extract
FE	Feed efficiency
HYD	Hydroponic barley sprouts
K	Potassium
kg	kilogram

K_2EDTA	potassium ethylene diamine tetra-acetic acid
Mcal	Mega calories
ME	Metabolizable energy
Mg	Magnesium
mg	milligram
mL	milliliter
µg	micrograms
N	Nitrogen
Na	Sodium
NaFl	Sodium fluoride
NDF	Neutral detergent fiber
NEAA	Nonessential AA
NFC	Non-fibrous carbohydrate
ng	nanograms
NE_g	Net energy gain
NE_L	Net energy of lactation
OM	Organic matter
P	Phosphorus
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RIA	Radioimmunoassay
RUP	Rumen undegradable protein

S	Sulfur
SBM	Soybean Meal
SEM	Standard error of the mean
T3	Triiodothyronine
T4	Thyroxin
wk	week

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ABSTRACT

EVALUATION OF FEEDING ALTERNATIVE FEEDSTUFFS INCLUDING HYDROPONIC BARLEY SPROUTS AND CARINATA MEAL TO DAIRY CATTLE

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The purpose of this dissertation research was to examine alternative feed ingredients not typically found in dairy cattle diets. In total, four studies were conducted to evaluate feedstuffs such as hydroponic barley sprouts and carinata meal and how they affect cattle performance. To determine how feeding hydroponic barley sprouts would affect growing dairy heifer and lactating cow performance two feeding studies were conducted. In the first study, inclusion of 14 % (DM basis) hydroponic barley sprouts (HYD) was evaluated in an ad libitum total mixed ration (TMR) compared to a control (CON) diet on dairy heifer performance during a 12 wk randomized complete block design study using 24 growing heifers. Results indicated that replacing ground corn and some soybean meal with hydroponic fresh barley sprouts maintained rumen fermentation, metabolic profile and heifer body frame growth with slightly decreased gain: feed. To further evaluate barley sprouts 20 mid-lactation Holsteins were used in a 6 wk randomized complete block design study. Milk production, metabolic profile, rumen fermentation, and nutrient utilization were evaluated. The HYD treatment consisted of a typical mid-lactation TMR with 8 % (DM basis) hydroponic barley sprouts and the control (CON) had corn and soybean meal as major concentrates. Lactation performance was not affected by supplementing HYD and plasma cholesterol and digestion of dry

matter and organic matter tended to be greater for the HYD cows. Hydroponic barley sprouts can replace a portion of the grain mix and maintain rumen fermentation and lactation performance. As hydroponic barley sprouts are not available for commercial purchase and must be grown by the dairy producer, efforts were refocused in evaluating carinata meal, a potential alternative protein source in the dairy industry in the third and fourth studies. Carinata meal (CRM) is a brassica oilseed that is newly developed in the United States. In study three, 10 % (DM basis) carinata meal was fed in dairy heifer diets for ad libitum consumption. A randomized complete block trial conducted with 24 heifers evaluated a control treatment (CON) and a 10 % CRM treatment. Feeding CRM decreased dry matter intake; however, growth was similar between treatments. Metabolic profile, thyroid hormone concentration, rumen fermentation and total tract digestion of nutrients were not affected by feeding CRM. Overall, CRM could potentially serve as an alternative protein source for growing dairy heifers. For the fourth study, the first lactation trial in the U.S. was conducted to determine effects of CRM. It was found that cows fed 10 % CRM maintained milk production, composition, and fatty acid profile comparable to the control (CON) diet using 10 % canola meal. Metabolic profile and rumen fermentation were not altered when CRM was fed in a lactating TMR, similarly, thyroid hormone concentration did not differ between treatments. Amino acid composition of treatment diets and plasma was evaluated, and no differences were found. Solvent extracted carinata meal is a viable protein source for the dairy industry, for use in both lactating cow and growing heifer diets.

INTRODUCTION

Climate changes and a decreasing arable land make it difficult for producers to grow high-quality feedstuffs (Cross, 2015). Inclusion of grain is typical in ruminant diets; however, variability of grain prices affects how producers use these ingredients. Alternative feeding strategies dairy producers implement include decreasing grain supplementation and using byproducts from the biofuel industry in cattle diets. Additionally, there is a need to research non-conventionally grown feeds.

A strategy to decrease grain supplementation and provide fresh forage year round is to supplement hydroponically sprouted barley (Rodriguez-Muela et al., 2004). Hydroponically grown feeds have not been well researched for dairy cattle. Recent research concerning feeding hydroponic barley in an organic system found similar milk production between treatments (Soder et al., 2018). To determine the feasibility of feeding hydroponic barley sprouts in a conventional dairy system we conducted two feeding trials to analyze the effects on growing dairy heifer and lactating cow performance.

Inclusion of byproduct meals from the biofuel industry is a more commonly adopted feeding strategy. The oilseed carinata (*Brassica carinata*) is currently being researched as a new feedstock for biofuels (Marillia et al., 2014). Carinata has unique agronomic benefits that appeal to crop and livestock producers (Cardone et al., 2003). The byproduct left after extraction of the oil is carinata meal (CRM), and it is a quality protein (30-40% CP) source for livestock, it does contain some anti-nutritional factors such as glucosinolates and erucic acid.

Carinata meal can be fed at 10% of the diet (DM basis) to growing beef and dairy cattle without negatively affecting performance (Brake, 2017; Rodriguez-Hernandez and

Anderson, 2018; Schulmeister et al., 2019; Rosenthal, 2018). In recent research, apparent total tract digestibility was decreased for heifers fed cold pressed CRM and rumen fermentation was similar between treatments (Rodriguez-Hernandez and Anderson, 2018). Dairy heifers fed solvent extracted CRM had similar digestibility of nutrients and thyroid hormone concentrations to control diets (Rodriguez-Hernandez, 2018). Feeding CRM in a TMR to dairy cattle is of particular interest in the current research, especially since the TMR would contain more moisture than previous trials feeding CRM, and the glucosinolates (sinigrin) within CRM are degraded in the presence of myrosinase which is released during mastication and enzyme reactions may vary due to water and pH (Duncan and Milne, 1993; Peng et al., 2014). Thus, it is imperative that this alternative feedstuff be evaluated in the dairy sector for different feeding scenarios and different stages of life. The availability and low cost of CRM, along with the 2020 goal of the aviation industry to use 50% biofuel makes CRM a sustainable protein source in dairy rations (Biofuels Digest, 2015). The objective of the current research is to determine the effects of feeding 10 % CRM to growing heifers and lactating cows when ad-libitum fed in total mixed rations with corn silage.

Hydroponic barley sprouts and CRM could potentially improve the economic and environmental sustainability of dairy operations. Main objectives of the dairy industry include reducing the cost of raising replacement heifers and decreasing feed cost in lactating cow feeding regimens without impacting milk production. The overall goal of this research is to evaluate the use of these two alternative feedstuffs in dairy heifer and lactating cow diets. It is hypothesized they will maintain cattle growth and production performance when fed in replacement of more conventional or traditional feedstuffs.

CHAPTER 1:

LITERATURE REVIEW

The ongoing research into dairy cattle nutrition is constantly changing and addressing new issues related to economic feasibility of the dairy operation. The increased availability of alternative feedstuffs allows for incorporation of less expensive feeds into dairy rations. These feedstuffs allow producers to reduce the cost of raising replacement heifers and cost of milk production through incorporation of newly developed feeds.

Current Challenges in Lactating Cows

The world's population is estimated to increase to 10.5 billion people by 2067 (United Nations, 2017). The demand for dairy products will grow over the next 50 years due to an increase in per capita income worldwide and due to dairy products fulfilling nutritional needs more efficiently than other agricultural practices (Britt et al., 2018). To meet the demand for the growing population an estimated 600 billion kg more of milk is needed in 2067 (Britt et al., 2018). Dairy cattle produce 82.4% of the world's milk and in 2014 the estimated number of dairy cows was 274 million (FAO, 2017). The average dairy cow would need to double its annual yield to produce the additional milk anticipated in 50 years (Britt et al., 2018). This is an unrealistic situation, due to the fact that the lowest annual production per cow is found in countries that have the most cow numbers. However, in the Unites States milk production has seen a 13% increase over the past 10 years (USDA-NASS, 2018). Milk is a commodity and even in small household farms, the price paid to farmers is driven by global demand and supply. Dairy farm profitability is influenced by the balance between milk supply and demand, especially

since farmers are able to increase output quickly when demand increases but reduce output slowly when supply exceeds demand (Britt et al., 2018).

Profitability is also highly influenced by cost of feed, which is the greatest cost of producing milk. Crop yield is one of the main factors that has driven the price of commodity feeds such as grain and the decreasing availability of arable land and changes in climate drastically affect overall feed cost (Nickerson et al., 2011; Griffin et al., 2014; Britt et al., 2018). The decline in arable land will shift production toward the human food market, leading to a decrease in quality forages and grains for dairy cattle. Drought and declining water levels also affect how dairy producers meet lactating cow demands (Cross, 2015). Water usage for crops to feed cattle accounts for approximately 90% of the water for milk production (Innovation Center for US Dairy, 2013). The production of forage crops such as alfalfa and corn silage commonly used in total mixed rations (TMR) is becoming more difficult with depletion of ground water and well reserves (Saylor et al., 2018). Improving feed efficiency of dairy cattle through technological advancements to combat these issues with feed shortages will increase profitability of the dairy operation.

The focus of dairy feeding until recently was on conventional systems and how the offered TMR affects milk production and metabolic status (Gillespie et al., 2009). Typically pasture-based feeding or grazing is more common in temperate regions where the growing season is longer, whereas TMR are more extensively used in arid regions and is composed of silage, grain, and vitamin/mineral supplements. Past research has found that pasture-based systems reduce feed, facility, labor, and equipment requirements leading to an increase on net return per cow (Tucker et al., 2001; White et al., 2002).

The profitability of pasture-based versus conventional systems depend on the extent of pasture use, most producers have small-scale operations, lower debt and are thought of as “extensive” grazing operations (Gillespie et al., 2009). Large operations may not incorporate grazing as easily, due to increased land cost and the longer distances cows must travel to the parlor. Due to the need for increased milk yield to meet the growing population the Midwest and Great Lakes region are more suitable for dairy expansion (Britt et al., 2018). The projected growth of dairy farms in the region of the 1-29 corridor may affect how operations raise replacement heifers, especially since most pastures are reserved for growing heifers. Smaller paddocks and more intensive rotational grazing may alleviate these issues, also incorporation of legumes into grazing systems will allow for a more nutrient dense feeding regimen (Pembleton et al., 2016). In lieu of current trends in the industry many producers along the I-29 corridor focus on TMR feeding in a conventional dairy system. This requires adequate balancing of forages and concentrates to meet the increased demands of lactating cows (Schingoethe, 2017).

Common starch sources in lactating diets

Starch is considered to be contained in the polysaccharide component encompassed in the non-fiber carbohydrate (NFC) fraction of a plant. A lactating dairy cow diet will most likely contain 70-80 % carbohydrates (Weiss and Firkins, 2007). Starch accounts for nearly 35-40% of carbohydrates while NDF accounts for 50-45 % and the rest is comprised of simple sugars, and soluble fiber (Weiss and Firkins, 2007). Ensiled forages are the most common source of dietary starch for dairy cattle. Typically, corn silage contains 35 % starch, this value is dependent upon the corn hybrid, harvest maturity, chop length, and overall proportion of grain (Aoki et al., 2013, Ferrareto and

Shaver, 2015; Khan et al., 2012). Corn hybrids may vary starch content from 26-35 %, as was reported when a total of 38 corn hybrids were evaluated within a similar location and silage processing techniques (Lauer et al., 2015). Sorghum silage is another option for dietary starch and the concentration varies greatly from 4-15% (Weiss and Firkins, 2007). Small grain silages include wheat, barley and oats, however the species, maturity, and processing method affect digestibility (Khorasani et al., 2000). Wheat has been found to include the greatest starch content at 72 %, next are corn and sorghum at 70% and followed by barley and oats which only contain 57-58 % (Aimone and Wagner, 1977; Hatfield et al., 1993). Various in-situ, in-vitro and in-vivo experiments have found that starch degradation is greatest for wheat, followed by barley, corn, and lastly sorghum (Galloway et al., 1993; Herrera-Saldana et al., 1990; Lanzas et al., 2007). Sources of starch are commonly processed though either rolling, grinding, cracking, crimping, pelleting, or treating with heat and pressure, which improves digestibility (Huntington, 1997). The grain source as well as processing method should be evaluated before utilizing in cattle diets.

Protein for lactating dairy cattle

As protein supplements are the most expensive ingredient in any dairy ration, accurate diet formulation and efficient use of protein supplements is key to any nutritional program (McGuffey, 2017). Prior to 1917, crude protein was a “proximate” analysis and numerous proteins and compounds were going unnoticed (Schwab and Broderick, 2017). Earlier methods which rely on dietary crude protein (CP) concentration (dietary nitrogen (N) × 6.25) as the main predictor of dietary N adequacy, it was recognized 60 years ago that using only CP had many disadvantages (Schwab and

Broderick, 2017). Protein nutrition of dairy cows has moved past the use of dietary CP as a targeted nutrient and is now focused on meeting the ammonia and AA needs of ruminal fermentation for microbial protein synthesis and the AA requirements of the cow. Currently, the protein in rations is formulated to meet rumen bacteria and dairy cow requirements. Virtanen (1966) was able to determine that ruminal microflora were able to completely synthesize all essential amino acids (EAA) when fed non-protein N (NPN) (urea and ammonium sulfate) as the only dietary N source and cows produced over 4,200 kg of milk in 1 lactation (McGuffey, 2017; Schwab and Broderick, 2017).

The 20 amino acids that comprise proteins, include both essential amino acids (EAA) and nonessential AA (NEAA), in which the body can synthesize NEAA, but the EAA most be supplemented in the diet. Even if providing adequate AA, the cow may not be able to utilize AA due to their requirements for absorbable AA and adequate fermentable energy to promote microbial synthesis. Metabolizable protein (MP) is comprised of ruminally synthesized microbial crude protein (MCP), rumen undegradable protein (RUP) and endogenous CP contributions (ECP) from sloughage of epithelial cells of the gastrointestinal tract. Each proportion of MP has an intestinal digestibility coefficient that allows for determination of MP from each fraction. Although advancements in genetic potential of dairy cattle has pushed this even farther to focus on AA demands for production, instead of focusing solely on ruminally synthesized MCP (Brito et al., 2007). The overall goal is to balance protein to optimize the supply of RDP and NPN to prevent limitation of microbial function (NRC, 2001).

Past research was able to accurately provide information for identification of key production-limiting EAA and frequently identifies lysine (LYS) and methionine (MET)

as first limiting AA for milk protein production (Schwab et al., 1992). Due to most ration ingredients coming from plant based sources. Formulation of lactating diets relies on adequate selection of primary protein sources to meet MP needs are largely based on cost, nutrient concentrations, and degradability characteristics of the individual feedstuffs (McGuffey, 2017).

Current Challenges in Replacement Heifers

The main objective of heifer management is to minimize investment inputs while also maximizing profitable output (Hoffman and Funk, 1992). The major factors that affect the cost of raising heifers is the cost directly associated with growing the heifers and the number of heifers raised (Tozer and Heinrichs, 2001). Raising replacement heifers is 15% to 20% of the cost of producing milk (Whitlock et al., 2002). Cost is associated with feed, labor, materials, and disease management (Heinrichs, 1993). One possible way in which producers could reduce the input costs would be to alter rearing time or reduce the age at first calving (AFC) (Hoffman and Funk, 1992). The lifetime producing ability of the replacement heifer is highly affected by the rate of growth from birth to parturition and the productive integrity of the heifer must be maintained (Hoffman and Funk, 1992; Heinrichs, 1993). It is recommended that when trying to implement these management practices the main goal would not be the fastest rate of gain, but the optimal rate of gain for the heifer to reach her full milk production potential (Swanson, 1960; Hoffman and Funk, 1992; Heinrichs, 1993).

Heifer feeding strategies

The recommended weight for replacement heifers is between 580 and 635 kg of body weight (BW) at calving (Hoffman, 1997). Hoffman (1997) also found that heifers above 660 kg of BW or that received body condition scores (BCS) of 3.5 or greater did not increase milk production and could potentially be predisposed to metabolic disorders. The ideal calving age is 23-24 months of age, even though heifers may calve at 15 to 16 months (Sejrsen and Purup, 1997; Tozer and Heinrichs, 2001). Early calving at less than 23 months has been associated with reduced milk yield, and an increase in reproductive problems (Hoffman et al., 1996; Ettema and Santos, 2004).

The recommended ADG and BW at calving can be accomplished through different feeding strategies. Typically, heifers are fed for ad libitum consumption a TMR consisting of a greater forage: concentrate ratio. Decreased fiber digestibility might affect growth efficiency, depending on the fiber quality and energy: protein (Moody et al., 2007; Heinrichs et al., 2017). Heifers fed high forage (72-76%) diets for ad libitum consumption contained corn stover residue, alfalfa haylage, cracked corn, supplemented with urea or consuming a diet without urea were only able to achieve a 0.65 kg/d ADG (Lopez-Guisa et al., 1991). Hoffman et al. (2007) fed an ad libitum (control) diet or more nutrient-dense diets at 80 or 90% of ad libitum DMI and found no difference in weight gain, structural growth, or 150-d milk production. Limit-fed heifers had higher feed efficiency and lower manure excretion. Feeding behavior of 1,049 (5-9 months old) heifers were analyzed and results indicated that the more efficient heifers (lower residual feed intake) consumed less feed, ate more slowly, spent less time eating, had longer meals, and consumed more feed during the night and less during the afternoon (Green et

al., 2013). Coblentz et al. (2015) demonstrated that low-energy forages (eastern grass haylage, chopped wheat straw, or chopped corn fodder) offered to Holstein heifers for ad libitum intake as diluting agents reduced caloric density and DMI, with heifers sorting the straw diet and, more severely sorting the chopped corn fodder diet. As heifers transition from post-weaning housing and feed to a more balanced ration, Miller-Cushon et al. (2015) observed that the method of transitioning heifers to a novel TMR influenced sorting behavior. Heifers will sort against long particles and thus receive a lower NDF diet than formulated (Miller-Cushon et al., 2015). This is an issue with feeding a TMR to lactating cows as well, and makes diet formulation, mixing and delivery very crucial to a successful dairy operation (Schingoethe, 2017).

A method used in lieu of limit feeding is compensatory growth and it is one such strategy that occurs when marginally fed animals are re-alimented on a higher level of nutrition (Park et al., 1987). The strategy of limit-feeding utilizes rations greater in concentrates and lesser in forages, this allows for a more energy dense diet that provides vital nutrients and decreases nutrient waste (Zanton and Heinrichs, 2008). A downside to limit-feeding is that heifers may exhibit hunger, agitation and vocalization (Hoffman et al., 2007). The main concern when feeding heifers for increased ADG is to ensure they are efficiently using the nutrients provided, which could potentially minimize rearing cost (Moody et al., 2007). The practice of combining nutrient dense feeds with forages that are low in nutrients and high in fiber is considered dietary dilution (Greter et al., 2008). The main forages used for dietary dilution include those of low value like straw and corn stalks (Greter et al., 2008; Kitts et al., 2011). This is an alternative to the strict limit-feeding of a high concentrate low forage ration, it allows for the heifer to exhibit normal

foraging behavior of a low nutritive forage in combination with a limit-fed ration (Kitts et al., 2011).

In order for producers to utilize various feeding strategies they must first consider the overall effects their dietary regimen will have on their replacement heifers. To decrease rearing time in order for heifers to calve at an earlier age, however in order to do so producers must first alter the rate of growth, modify the nutrients provided in the ration, and possibly incorporate use of nutrient partitioning agents (Hoffman and Funk, 1992). To understand nutrient partitioning in growing heifers and how it affects development many researchers have undergone studies that manipulate the dietary energy intake of growing heifers (Peri et al., 1993; Davis Rincker et al., 2008). As the heifer ages the consumed nutrients are disproportionately partitioned to maintenance instead of growth, it is imperative to therefore shift nutrient utilization away from energy storing and toward physiological functions associated with maintenance (Moody et al., 2007; Zanton and Heinrichs, 2008; Zanton and Heinrichs, 2009).

Common heifer diets

A typical heifer diet consists of a high forage ration which is not as efficiently digested due to large content of poorly digestible fiber, it has been found to be more feasible to use high concentrate diets in a limit-fed ration (Moody et al., 2007; Zanton and Heinrichs, 2010). The better quality forages on the farm are usually reserved for the more efficient mature dairy cattle (Zanton and Heinrichs, 2009). As the heifer ages the consumed nutrients are disproportionately partitioned to maintenance instead of growth, it is imperative to therefore shift nutrient utilization away from energy storing and toward physiological functions associated with maintenance (Moody et al., 2007; Zanton and

Heinrichs, 2008; Zanton and Heinrichs, 2009). Whereas, the mature dairy cow will utilize the true protein and non-protein nitrogen (NPN) provided by the highly rumen degradable protein (RDP) sources in the diet for milk production (Satter and Roffler, 1974; Rotz et al., 1999). Cows consume dietary protein to supply nitrogen (N) for microbial growth and amino acids (AA) for milk production and maintenance, and these requirements will vary depending upon the amount of milk produced and its composition (Clark et al., 1992; Davidson et al., 2003). However, only 25-30% of the nitrogen consumed is transferred into the milk, the rest is excreted (Wilkerson et al., 1997). This conversion is still higher than that of growing animals in which a very low efficiency (0-35%) has been found for the conversion from ingested N to body tissues (Lobley, 1992). The concentration and combination of AA supplied for production and maintenance depend upon the rumen undegradable protein (RUP) and the microbial supply in the rumen (Satter and Roffler, 1974; Davidson et al., 2003). The overall feed efficiency of growing dairy heifers is low and this is a result of a large proportion of the feed required for maintenance, such that the absolute amount of energy required for maintenance is several times greater than it is for growth (NRC, 2001; Zanton and Heinrichs, 2007).

The most common sources of concentrates in heifer diets include soybean and corn based products as the primary ingredient. Alternative feed sources for heifers include byproducts from the ethanol, biodiesel or vegetable oil industry. These include dried distillers grains, wet distillers grains, reduced fat dried distillers grains, and canola meal (Anderson et al., 2009; Anderson et al., 2015a, b; Schroer et al., 2014; Manthey et al., 2016). Due to the variability in grain prices, byproducts from ethanol may not always be the most viable option for producers. If producers are able to reduce the cost of heifer

raising with the new oilseeds developing in the Midwestern United States, it could be a great way to increase the agronmic value of these feedstocks. Additionally, distillers grains are considered good sources of RUP, whereas oilseed meals often have a greater proportion of RDP. Therefore, feeding a combination of the two alternative ingredients may support a more balanced approach to meeting dairy cattle protein requirements (Mulrooney et al., 2009).

Alternative Feeds

Hydroponic products in cattle rations

The method of sprouting cereal grains for human consumption has been around for centuries (Resh, 2001). Feeding sprouted grains to livestock was not examined until the early 1920s and 1930s when W. F. Gericke developed a procedure to grow plants within a solution on a large scale (Myers, 1974). This technology is now gaining renewed interest among the livestock industry. The changes in weather such as drought and decreased availability of arable land make it difficult for producers to grow high-quality forage (Nickerson et al., 2011; Griffin et al., 2014; Hafla et al., 2014). Also, the fluctuations in grain prices (increase in corn cost) has producers examining alternative feeding strategies, such as decreasing grain supplementation, to meet dairy cow needs (Hafla et al., 2014). Many companies have manufactured units to grow hydroponic sprouted grains, these systems allow for the fresh production of forages from barley, oats, wheat, and other cereal grains (Rodriguez-Muela et al., 2004).

In the past, hydroponically sprouted feeds have been produced from grains that have increased germination rates and short growth periods (barley) in a special chamber that controls environmental conditions (Sneath and McIntosh, 2003). The most common

system in today's industry utilizes trays or troughs, housed in a shed or building that is climate controlled (Sneath and McIntosh, 2003). These types of grow systems have increased labor costs, due to the need for seeding and harvesting of trays daily, often by hand (Tranel, 2013). Due to the labor intensity of these systems, interest in this technology has remained low in the dairy industry. However, an automated system may increase interest (Soder et al., 2018).

Hydroponically Grown Barley Sprouts

Several research studies suggest feeding sprouted grains increases performance in livestock not already receiving adequate nutrients (Thomas and Reddy, 1962; Sneath and McIntosh, 2003). Studies feeding hydroponic sprouted grains have found that dry matter intake may be reduced in feedlot and dairy cattle due to the high moisture content (Thomas and Reddy, 1962; Peer and Lesson, 1985). Tudor et al. (2003) found improvement in growth performance in a study using beef steers fed a restricted diet of low quality hay and supplemented with hydroponic barley sprouts. Rodriguez-Muela et al. (2004) also found that feeding grazing lactating cows hydroponic sprouted barley maintained cow body weight and increased calf weights. In contrast, Soder et al., (2018) found no differences in milk production, somatic cell count, and body weights when certified-organic cows were fed sprouted barley.

Increased use of byproducts from biodiesel production

Research has focused on byproducts from the ethanol industry mainly, with the variety of dried distillers grains (DDGS) now produced (low-fat, reduced-fat, high-protein, modified DDGS) there are many options on which type to include in the ration (Anderson et al., 2009; Anderson et al., 2015a; Manthey et al., 2016). Feeding DDGS to

dairy heifers has been found to maintain growth performance and potentially decreased age at onset of puberty (Schroer et al., 2014; Anderson et al., 2015a, b). These past studies and the increasing demand for renewable feedstocks for biofuel production has led to increased interest in Brassica crops. Byproducts from brassica crops include high quality protein meals from the biofuel industry such as camelina meal and carinata meal (Moser, 2010; Waraich et al., 2013; Marillia et al., 2014).

To date, there is very limited published research on the effects of feeding brassica oilseed meals to beef and dairy cattle. Only a few studies have examined the effects of camelina meal in cattle (Moriel et al., 2011; Cappellozza et al., 2012; Grings et al., 2014; Lawrence et al., 2016). Studies have shown camelina meal did not affect growth, but did alter metabolic levels of thyroid hormones. Feeding camelina to lactating dairy cows decreased DMI and altered fatty acid composition of milk (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau et al., 2011). Carinata meal (CRM) is more agronomically beneficial than camelina, the fatty acid profile of the extracted oil is more favorable for its use as a feedstock for biofuels (Cardone et al., 2003; Moser, 2010).

Brassica Carinata

Carinata: non-food oilseed

The Brassica family of cruciferous plants contains common food crops such as cauliflower, cabbage, kale, mustards, radish, turnips, brussel sprouts, rapeseed and canola (Moser, 2010; Waraich et al., 2013). As well as more underutilized crops such as *B. carinata* and *Camelina sativa*, which are not grown for human consumption (Cardone et al., 2003; Tiwari and Kumar, 2013). The common names for *B. carinata* include carinata and Ethiopian mustard. These nonfood oilseeds represent a very small percentage of the Brassica species grown worldwide, the major oilseed is *B. napus* or rapeseed and its

cultivars such as canola meal (Velasco and Fernandez-Martinez, 2009; Milazzo et al., 2013).

Canola oil is used mostly for human consumption, there has been renewed interest in finding alternative oilseeds for renewable feed-stocks for the production of biofuel (Marillia et al., 2014). Especially feed-stocks that are able to grow on less productive farmland with low inputs (Cardone et al., 2003; Marillia et al., 2014). Carinata can be grown in less than optimum crop land such as sandy or clay-type soils and requires less inputs (water, fertilizer) than canola (Cardone et al., 2003). This makes carinata a promising cash crop for producers that would like to utilize fallow crop land or mixed cropping systems (Moser, 2010). In the Midwest it is sought after as a rotational crop in areas that commonly grow wheat, which would aid in breaking the weed and pest cycle in these areas (Agrisoma Biosciences Inc., 2015; Atyeo, 2015). Carinata has successfully been introduced to North Dakota, South Dakota, Montana, and southern states such as Mississippi and Florida (Agrisoma Biosciences Inc., 2015; Atyeo, 2015). Another benefit for producers is the ability to grow a protein source for livestock while benefiting from the extraction of oil for biofuels.

In Canada, carinata has been developing over many years, the objectives of the breeding and transformation techniques include higher oil yield and earlier maturation of the crop in relation to canola (Marillia et al., 2014). The added agronomic benefits of carinata include cold weather tolerance, low input crop, resistant to aphids, flea beetles and blackleg disease (Drenth et al., 2014; Marillia et al., 2014; Zhao et al., 2015). In comparison to canola, carinata yields more grain under worse growing conditions (low precipitation, greater ambient temperatures (Xin and Yu, 2013).

Carinata contains about 38-40% oil on a whole seed basis, a majority of the oil is made of very long chain fatty acids (VLCFA), the main focus has been on erucic acid (C22:1) and nervonic acid (C24:1) (Ban et al., 2018; Cardone et al., 2003; Marillia et al., 2014). Long chain fatty acids, linoleic and linolenic acid (C18:2 and C18:3) are also found in carinata, the concentration of C18 fatty acids could potentially prevent carinata biofuel from being used in Europe due to specific standards set for the level of linolenic acid (Milazzo et al., 2013; Rodriguez-Hernandez, 2018). According to Zhao, et al. (2015) carinata oil is composed of 48 % erucic acid, 20 % oleic aid, 11 % linoleic acid, and 8 % palmitic acid. In addition to being a feedstock for biofuel and jet fuels, carinata oil can be used for biodiesel and biochemical production due to its non-food oilseed status (Marillia et al., 2014). The aviation industry has a goal to reduce carbon fuel by 50 % by the year 2020, increases the popularity of carinata oil (Gesch et al., 2015). In 2012, a flight of 100 % carinata based jet fuel was successful and reduced aerosol emissions by 50 % compared to petroleum based fuel (Marillia et al., 2014).

Carinata Meal

The oil content of *B. carinata* is mostly as very long chain fatty acids (VLCFA) or erucic acid (C22:1) (Cardone et al., 2003). The byproduct left after extraction of the oil is carinata meal (CRM) and it could potentially be a beneficial protein source for livestock (Marillia et al., 2014). Carinata meal contains some anti-nutritional factors such as residual erucic acid and glucosinolates that limit its inclusion in livestock diets (Fales et al., 1987, Tripathi and Mishra, 2007). Extraction methods may alter glucosinolate concentration, solvent extracted CRM has been found to contain less glucosinolates than cold press (Brake, 2017). Due to the potential for detrimental effects on thyroid gland

function and growth, it is not recommended to feed meals containing glucosinolates in excess of 10% inclusion in the diet, which is currently the federal regulation (AAFCO, 2014).

Carinata meal is attractive as a feedstuff for livestock because it is a good source of protein (48%), rich in sulfur-containing amino acids, and contains less fiber content compared with canola meal (Marillia et al., 2014; Yu et al., 2014). Lawrence and Anderson (2018) found that CRM is a great source of RDP and contains more RDP than canola meal. Total digestible protein of CRM is similar to that of soybean meal and it can be considered a high quality protein (Lawrence and Anderson, 2018).

Anti-nutritional compounds in carinata

The glucosinolates in carinata are secondary plant metabolites and biologically inactive molecules, when they are broken down into their degradation products by myrosinase in plant or gut microflora negative effects can occur (Chen and Andreasson, 2001; Tripathi and Mishra, 2007). Glucosinolates coexist with the myrosinases or endogenous thioglucosidases within plant tissue (Chen and Andreasson, 2001). The degradation products of glucosinolates include: isothiocyanates, thiocyanates, nitriles, thiourea, and oxazolidithione (Bones and Rossiter, 1996; Wallig et al., 2002). The decreased palatability of oilseed meals containing glucosinolates is due to bitterness and may reduce intake which could affect growth performance (Putnam et al., 1993; Tripathi and Mishra, 2007). Sinigrin and progroitrin, specifically their degradation products are what causes the bitterness and mustard taste. Progoitrin found in meals such as canola meal is a non-bitter compound, however when broken down during processing (heating,

crushing) or ingestion by myrosinase it is converted to goitrin a very bitter substance (van Doorn et al., 1998).

Thiocyanate ions alter thyroid hormones triiodothyronine (T_3), and thyroxine (T_4) synthesis by reducing iodine uptake by the thyroid gland which affects iodination of the hormones, and results in hormones that are biologically inactive (Guyton, 1986). Normal growth and development depend upon thyroid hormones, without them the somatic and mental growth suffer (Hadley and Levine, 2007). Goitrogenicity is also a concern when thyroid function is impaired, which caused by hypothyroidism (Tripathi et al., 2001; Hadley and Levine, 2007).

The type of glucosinolates in the feed are important to consider, for example carinata contains sinigrin which is a very bitter compound when degraded (Fenwick et al, 1982; Marillia et al., 2014). Carinata has even been studied for its use as a biopesticide because of the very bitter degradation products of sinigrin (Marillia et al., 2014). Still research has been underway to reduce or eliminate glucosinolates in carinata and camelina. Additionally, based on review of literature it is evident that not all glucosinolates have the same physiological effects (Rodriguez-Hernandez, 2018; Tripathi and Mishra, 2007), and impacts of specific types of glucosinolates warrant more research.

Cultivars of the oilseeds could potentially reduce the amount of glucosinolates in the crop, like the cultivation of rapeseed to canola (Cardone et al., 2003; Waraich et al., 2013; Colombini et al., 2014; Marillia et al., 2014; Atyeo, 2015). Research has examined the effects of processing on glucosinolates in the oilseed meal (Maheshwari et al., 1980; Fales et al., 1987; Tripathi and Mishra, 2007; Moser, 2010). As stated previously

different oil extraction methods can reduce glucosinolates, dehulled extracted meals have lower concentrations compared to solvent extracted meals (Tripathi and Mishra, 2007). Rodriguez-Hernandez (2018) found increased glucosinolate (sinigrin) concentration in cold pressed CRM compared to solvent extracted. Processing using microbial treatment, heat treatment, microwaving, micronization, water, and metals has also been proven to reduce glucosinolates in *brassica* species (Maheshwari et al., 1980; Fenwick et al., 1982; Tripathi et al., 2001; Atyeo, 2015).

Among more conventional ways to decrease concentration of glucosinolates is the practice of ensiling *brassica* oilseeds/foliage alone or with other forages (Fales et al., 1987; Rodriguez-Hernandez, 2018). Fales et al. (1987) found that ensiling rapeseed forage reduced concentration of glucosinolates in the silage, approximately to the extent of only one tenth of the original of the fresh, original sample. Rodriguez-Hernandez (2018) found that glucosinolates were reduced and fermentation characteristics were not affected when solvent extracted carinata meal (48.3 mg/g sinigrin) was ensiled with alfalfa haylage or corn silage. Feeding trials using ensiled brassica crops with forage are warranted and if feasible could potentially increase the economic value of carinata drastically.

Feeding carinata meal to growing cattle

Palatability may be an issue when feeding CRM, to evaluate taste preference a palatability study was conducted using CRM, DDGS, camelina meal, linseed meal, and canola meal (Rodriguez-Hernandez, 2018). Heifers preferred DDGS first, linseed meal second, CRM and canola meal third, and camelina meal fourth. Canola meal and CRM were comparable in taste preference (Rodriguez-Hernandez, 2018), cold pressed meal

was used in the study so potentially a solvent extracted meal would perform better than canola meal. In the growth trial using 10 % cold pressed in a limit fed ration, CRM heifers reduced DMI in the first 2 wk of the trial, researchers attribute this to the need for an adaptation period to meals containing glucosinolates (Rodriguez-Hernandez, 2018). required. The same response in DMI was not found in the trial feeding 10 % solvent extracted meal in a limit fed ration.

Growth performance and rumen fermentation were maintained when dairy heifers were fed 10% CRM (cold press) in a limit-fed ration with grass hay (Rodriguez-Hernandez and Anderson, 2018). However, decreased apparent total tract digestibility of all nutrients besides crude protein was found for the CRM treatment (Rodriguez-Hernandez and Anderson, 2018). In contrast, Schulmeister et al. (2019) did not find effects on DMI or apparent total tract digestion of nutrients in beef steers fed CRM. Feeding CRM to beef steers did increase propionate and altered A: P ratio, similar to the study feeding cold press CRM (Rodriguez-Hernandez, 2018). Researchers also analyzed blood metabolites and plasma cholesterol was found to be increased for CRM fed heifers, which may be beneficial for reproductive efficiency (Rodriguez-Hernandez, 2018). Although age at puberty was not affected, the proportions of heifers cycling by 270 kg was found to be greater for CRM (Rodriguez-Hernandez, 2018). When feeding solvent extracted CRM age and weight at puberty were not affected. The PUN concentration was similar to that of steers fed SBM when Schulmeister et al (2019) fed 0.3 % BW CRM. Beef cows fed CRM at 1.3 kg/d found decreased T3 in comparison to canola fed cows (Rosenthal, 2018). Rodriguez-Hernandez (2018) found that thyroid hormones were not

detrimentally affected, these results indicate CRM can be fed to dairy heifers without adversely affecting growth and development.

Rationale and significance

The overall objective of this research is to evaluate the potential for use of alternative feedstuffs, hydroponic barley sprouts and CRM, in dairy heifer and lactating cow diets. This encompasses analyzing the effects on animal performance (growth and milk production), blood metabolic profile, rumen fermentation characteristics, and total tract digestion of nutrients. The increased digestibility and palatability of hydroponic barley is hypothesized to improve feed efficiency and growth performance of growing dairy heifers, as well as improve milk production and composition in lactating cows. Research has proven that CRM is highly digestible and comparable to soybean meal (Lawrence and Anderson, 2018), thus, 10% CRM in the diet fed ad-libitum in a TMR to dairy heifers and lactating cows is hypothesized to improve growth performance and milk production.

The benefits of the current research on hydroponic barley sprouts and CRM include: increasing knowledge and confidence about feeding these alternative feedstuffs, improving food security of the dairy industry by using feeds not destined for the human market, and providing a frame work for future research. Hydroponic barley sprouts and CRM are quality feedstuffs that may be implemented in diets of dairy cattle to improve nutrient utilization, cattle performance, and sustainability of the dairy operation. In addition, results will increase economic viability of CRM and provide a low cost option to dairy producers for replacing more commonly used protein sources.

CHAPTER 2:

EFFECTS OF FEEDING HYDROPONIC BARLEY SPROUTS ON GROWING DAIRY HEIFER PERFORMANCE

Abstract

Our objective was to determine the effects of feeding hydroponically grown barley sprouts (HydroGreen Inc., Renner, SD) to dairy heifers on growth performance, metabolic profile, nutrient utilization, and rumen fermentation. A 12-wk randomized complete block design study was conducted using 20 Holstein and 4 Brown Swiss heifers [215.1 ± 25 d of age; body weight (BW) 229.7 ± 39 kg]. Treatments were: 1) control (**CON**) diet which was a total mixed ration (TMR) with grass hay, corn silage, and ground corn and soybean meal as major concentrate ingredients and 2) a TMR with 14% (DM basis) hydroponic barley sprouts (**HYD**) replacing a portion of the concentrate mix. Diets were fed for ad libitum intakes and formulated to have similar protein and caloric content (DM basis), although the CON was 66 % DM and HYD was 44 % DM. Intakes were measured using the Calan gate feeding system. Frame sizes, BW, and body condition scores (BCS) were measured on 2 d during every 2 wk. Blood samples were taken at the beginning of the study and then every 4 wk throughout on the same days as body measurements, approximately 3.5 hours post feeding (1230 h) via venipuncture of the jugular vein. Rumen fluid was collected 4 h post feeding via esophageal tube immediately after blood sampling. Heifer DMI was greater for HYD, heifer ADG tended to be greater for the CON treatment. Similar to ADG, BW, and gain: feed were greater for the CON treatment. Most frame measurements were similar among treatments. Heart girth was greater for the CON fed heifers. Glucose plasma concentration was greater for

the CON treatment and plasma triglycerides concentration was greater for the HYD heifers. Rumen fermentation characteristics were mostly similar between treatments, isovalerate and isobutyrate tended to be greater for CON. Results indicated that replacing ground corn and some soybean meal with hydroponic fresh barley sprouts maintained rumen fermentation and heifer body frame growth with slightly decreased gain: feed. The decreased gain:feed was most likely because of the overall high moisture content in the HYD TMR.

Key words: dairy heifer, hydroponic feed, growth performance

Introduction

The anticipated growth of the milking herd in the future will allow for the dairy heifer operation to be managed as its own enterprise (Britt et al., 2018; Heinrichs et al., 2017). Past objectives of heifer raising focus on minimizing inputs and maximizing profitable outputs, however, modern objectives to limit environmental impact and protect animal welfare must also be considered (Hoffman and Funk, 1992; Heinrichs et al., 2017). Hydroponically sprouting cereal grains such as barley may provide a strategy that producers can implement to achieve past and future objectives of heifer raising.

The total DM loss found when sprouting barley grain could potentially contradict any positive benefits in nutrient concentration or digestibility (Dung et al., 2010; Hafla et al., 2014; Soder et al., 2018). The increased labor and energy demands of sprouting barley are also not accounted for, and research has found that not every hydroponic grow is capable of supplementing organic dairy cows with adequate barley sprouts (Soder et al., 2018).

Hydroponic feeds such as barley sprouts are not well researched for dairy cattle. Maize sprouted hydroponically was fed to dairy heifers without adverse affects (Naik et al., 2016). Beef steers exhibited improved growth performance when supplemented with 15.4 kg hydroponic barley (Tudor et al., 2003). Due to the limited and variable growth responses of previous research it was imperative to evaluate the effects of feeding hydroponic barley sprouts on growing dairy heifer performance. The objectives of the current study included determining the effects of hydroponically grown barley sprouts on dairy heifer growth, nutrient utilization, metabolic profile, and rumen fermentation. It hypothesized that the increased palatability and digestibility of hydroponic barley would improve growth and nutrient utilization.

Materials and Methods

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 16-043A. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Experimental Design

A 12-wk randomized complete block design study was conducted using 20 Holstein and 4 Brown Swiss heifers (215.1 ± 25 d of age; body weight (BW) 229.7 ± 39 kg) with two treatment diets. Heifers were blocked in groups of 3 based on birth date and breed. Heifers were randomly assigned to treatment after assignment to block. Heifers were started on the study in groups of six at different times based on age. Prior to starting treatments, heifers were familiarized to the barns and feeding system for approximately 2 wk, followed by an experimental feeding period of 12 wk.

Treatments were: 1) control (**CON**) diet which was a total mixed ration (TMR) with grass hay, corn silage, and ground corn and soybean meal as major concentrate ingredients and 2) a TMR with 14% (DM basis) hydroponic barley sprouts (**HYD**) replacing a portion of the concentrate mix. The formulated ingredients and predicted nutrient composition of treatment diets are presented in Table 2.1. The HYD ration was formulated without use of ground corn in the concentrate mix, to determine if hydroponic barley could replace corn entirely in the ration and maintain performance. Diets were fed for ad libitum intakes and formulated to be isonitrogenous and isocaloric (DM basis), although anticipated DM % of the diets was 66 % for CON and 44 % for HYD.

Animal Care and Feeding

The feeding trial for this experiment was completed in its entirety at the South Dakota State University Dairy Research and Training Facility (SDSU DRTF). Animals were observed daily for any injury or disease problems and treated according to normal management practices at the DRTF. Heifers were housed in pens in groups of 6 heifers. Each pen had an inside roofed shelter area and an outside dirt exercise lot. The inside areas of the pens were manure pack bedded with straw, bedding was done at a minimum interval of every 2 wk to avoid consumption of straw.

Prior to preparing individual TMR, the hydroponically grown barley sprouts were delivered daily by the sponsor (HydroGreen Inc., Renner, SD). The barley mats were hand cut into approximately 2 in. cubes to facilitate mixing. Fresh water was provided ad libitum. Feeding occurred once daily at approximately 0900 h using the Calan gate feeding system (American Calan, Inc., Northwood, NH) so that individual intakes could be measured. At each feeding, ground hay, corn silage, grain mix, and hydroponic barley

sprouts were individually weighed for each heifer into a large tub, hand mixed, and then placed in the Calan boxes. Approximately every 2 wk throughout the study, bales of hay were coarsely pre-ground with a large vertical tub grinder to ease hand mixing. Refusals were weighed and recorded in the morning prior to feeding, to determine daily intakes and adjusted for 5-10% refusal rate.

Animal Measurements and Sampling

Each wk samples of the feed ingredients were taken and stored at -20°C until processing and analysis could be completed as described under laboratory analysis.

Body growth measurements including body weight (BW), withers heights, hip heights, heart girths, paunch girths and body lengths were taken on 2 consecutive days at 4 h post-feeding at the beginning of the study and then every 2 wk during the study. The measurement for body length was taken from the top point of the withers to the end of the ischium. Body condition scores (BCS) were recorded every 2 wk, by three independent observers based on a quarter point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). For analyses of glucose, plasma urea nitrogen (PUN), cholesterol, and triglyceride concentration; blood samples were taken at the beginning of the study and then every 4 wk throughout on the same days as body measurements. Blood samples were taken approximately 3.5 h post feeding (1230 h), while heifers were restrained in a cattle chute, via venipuncture of the jugular vein 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). Immediately after blood collection, samples were placed in ice and then brought in to the laboratory within 3 h for processing

and storage. Blood collection tubes were centrifuged at 1000 × g for 20 minutes at 4°C (Centrifuge: CR412 Jouan, Inc., Winchester, VA.). Plasma (K_2EDTA tubes) or serum (NaFl tubes) was then transferred using a plastic pipette into polystyrene storage tubes and frozen at -20°C until analysis could be completed. Rumen fluid was collected during wk 0, 4, 8, and 12 on 2 consecutive days right after blood sampling via an esophageal tube while the heifer was still restrained. The beginning stream of rumen fluid was discarded (50 mL), to try and minimize saliva contamination. In total 50 mL of rumen fluid was collected into a stainless steel cup. The pH of the sample was analyzed and recorded immediately (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, Il.). A 10 mL aliquot was mixed with 2 ml of 25% (w/v) meta-phosphoric acid for determination of VFA concentrations, and a 10 mL aliquot was mixed with 200 µl of 50% (v/v) sulfuric acid to determine rumen ammonia nitrogen (NH_3-N). The two samples from both sampling days were then frozen at -20° C until analysis. During wk 12 of the feeding period samples for analysis of total tract digestibility of nutrients were collected. The internal marker used was acid detergent insoluble ash (ADIA). Orts and fecal grab samples were collected over 3 d. Fecal grab sampling was scheduled so that samples would ultimately represent every 3 h over the 24 h period relative to time of feeding. Orts and fecal samples were stored at -20°C until processing and analysis could be completed.

Laboratory Analysis

To determine DM content, feed samples were dried for 24 h at 105°C every 2 wk, to check ingredient inclusion rates in the ration and determine DMI. For processing, feeds were thawed and samples from 4 consecutive wk were composited on an as-fed basis by volume. Composite samples and concentrate mix ingredients were dried in duplicate for

48 h at 55°C in a Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN). Composites of the forage were ground to a 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). Ground forages and the concentrates were reground to a 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). In order to correct analysis to 100% DM, 1 g aliquot of sample was dried for 4 h in a 105°C oven (AOAC 17th ed., method 935.29). The ash content was analyzed by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC 17th ed., method 942.05). Organic matter (OM) was then calculated as OM = (100 - % Ash). All samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC 17th ed, method 968.06), on a Rapid N cube (Elementar Analysen Systeme, GmbH, Hanau Germany). The resulting nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). Heat-stable α-amylase and sodium sulfite were used for the NDF. Before samples were analyzed for NDF they were pre-soaked in acetone if the fat concentration was greater than 5% or if they contained soy products according to procedure recommendations. Ether extracts (EE) were analyzed using petroleum ether (AOAC 17th ed., method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Non-fibrous carbohydrates were calculated as % NFC= 100 - (% Ash + % CP + %NDF + % EE) as described by the NRC (2001). Monthly feed composites were made into larger composites such as 2-month and 3-month, these were then sent to a commercial lab 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, 1998). Starch concentration was found using a modified method of glucose analysis completed on an YSI 2700 Select Biochemisty Analyzer instead of using the standard glucose oxidase-peroxidase (GOPOD; Bach Knudsen, 1997; YSI Biochemistry Analyzer, YSI Inc., Yellow Spring, OH).

For analysis of rumen fluid, it was first thawed and vortexed to completely mix contents before pipetting 2 ml into a microcentrifuge tube to be centrifuged at 10,000 × g for 20 min in a micro centrifuge (Model A-14, Jouan, Jouan Inc, P. O. Box 2176, Vinchester, VA, U.S.A). Ammonia-N concentration was analyzed using the assay described by Chaney and Marbach (1962). Volatile fatty acid concentrations were measured using an automated gas chromatograph (Model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d × 15m column (Nukol, 17926 to 01C, Supelco, Inc., Bellefonte, PA) with 2-ethylbutyrate used as an internal standard. The flow rate was 1.3 ml/min of Helium and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Metabolites (glucose, cholesterol, triglyceride and PUN) were analyzed with commercially available enzymatic or colormetric assay kits on a micro-plate spectrophotometer (Cary 50, Varian Inc.,Walnut Creek, CA.). Serum glucose was analyzed using glucose oxidase reagent as described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI) as described by Allain et al. (1974). Plasma urea nitrogen was analyzed using diacteylymonoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Triglycerides concentration was

determined colorimetrically using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) and Trinder (1969).

For digestibility analysis fecal and orts samples were composited on an as-is basis by volume for each heifer. Samples were processed (dried and ground) as described for the monthly feed composites. Fecal and orts samples were also analyzed for DM, CP, Ash, NDF and ADF as previously described for feeds. Acid detergent insoluble ash (ADIA) analysis was conducted on all feed composites, fecal samples, and orts. The method for ADIA analysis consists of analyzing the sample for ADF digestion (Robertson and Van Soest, 1981) and then determining the ash percentage using a modified procedure of the AOAC 17th ed., method 935.29. Digestibility calculations were performed according to Merchen (1988).

Statistical Analysis

Feed nutrient means and standard errors were calculated using the MEANS procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The total dietary nutrient values were calculated based on analysis of concentrate mixes and hay for each treatment.

Week 0 body measurements and blood metabolites were analyzed separately from the rest of the data set in MIXED procedures of SAS. Because it was a single time point, the model included only treatment and breed with block included as a random variable. Least square means are reported for each treatment in the tables for body measurements and were compared using Tukey's test. The wk 0 values of each body measurement or blood metabolite were then used as covariate terms for their respective parameter.

Changes over time for the growth parameters were calculated for each 2 wk interval during the feeding period. Gain-to-feed ratio was calculated as the ratio of ADG to DMI for each treatment. Changes for body weights, ADG, gain: feed, intakes, frame growth measurements, blood metabolites, and rumen fermentation parameters were analyzed as a randomized complete block design with repeated measures using the MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, breed, and all interactions. Minimal treatment by breed interactions were observed, so results for breed are not reported. As mentioned, wk 0 body measures and blood metabolites were used as covariates for their respective parameter within the model. Repeated measures were by week using heifer(block) as the subject. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry was chosen as the covariance structure due to having the least absolute Akaike's values. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Least square means are reported for each treatment in the tables and were compared using Tukey's test.

The MIXED procedures of SAS were also used for analysis of data for the total-tract digestibility of nutrients. As it was a single time point, the model included only treatment with block as a random variable. Least square means are again reported for each treatment in the tables and means were compared using Tukey's test.

Results and Discussion

Feed Composition

The feed ingredients used in treatment diets is presented in Table 2.2 and based on laboratory analysis. The nutrient composition of the diets fed based on laboratory analysis is presented in Table 2.3. The actual diets differed greatly in DM concentration, 69.0 % DM (SE = 1.65), and 47.4 % DM (SE = 0.60), for CON and HYD, respectively. This was slightly different than diet formulations, however, due to the increased moisture from barley sprouts (18.0 % DM) we expected the HYD ration to have a lesser DM %. The DM content of a diet impacts DMI in lactating cows. A study that used water addition to create a wet diet (47.9 % DM) versus a dry (57.6%) diet found that cows sorted for small particles and against long particles more extensively than the dry diet, altering the nutrient composition consumed (Miller-Cushon and DeVries, 2009). The wet diet is similar to the DM content of the HYD diet and water was not added to the TMR in the current study, the inclusion of hydroponic sprouts in combination with corn silage decreased DM % of the diet. Feeding barley sprouts must be done with caution, and DM % of the diet monitored closely, especially if fed with feeds with greater moisture content (corn silage, haylage, bailage). Quigley et al. (1986) found that DM %, diet ADF and NDF, and bulk density of the diet affected heifer DMI. Crude protein of the treatment diets fed, were similar to the formulations and adequate for growth. The ideal amount of CP is in excess of 13% to achieve maximum microbial fermentation (Tamminga, 1992). Fiber and EE were also very similar to the predicted nutrient composition. The energy content of the treatment diets was similar to diet formulations and did not differ between treatments.

Heifer Growth Performance

Heifer DMI presented in Table 2.4 was found to be greater ($P < 0.01$) for HYD (7.5, and 8.0 kg/d for CON and HYD, respectively; SEM = 0.42). The increased DMI is attributed to the lesser diet DM in the HYD treatment (Quigley et al., 1986). Heifer ADG tended ($P = 0.07$) to be greater for the CON treatment (1.2, and 1.0 kg/d; SEM = 0.06), and a tendency was found for a week interaction ($P = 0.09$). The ADG of the current study is similar to that of Ch. 4 in which heifers were fed a TMR containing corn silage for ad libitum intakes. Although diets were formulated for ADG of 0.8 kg/d, ad libitum fed heifers have been found to have increased ADG compared to diet formulations (Rodriguez-Hernandez, 2018; Anderson et al., 2015). Body weight (289.7, and 282.4 kg; SEM = 2.02) and gain: feed (0.16, and 0.13; SEM = 0.01) were greater ($P < 0.01$) for the CON treatment. The reduced gain: feed in the HYD fed heifers may be due to the decreased DM % of the ration (Table 2.3). Frame measurements (Table 2.5) were mostly similar between treatments; with the exception of heart girth which was found to be greater ($P < 0.01$) for the CON heifers (146.0 cm, and 145.0 cm; SEM = 0.62). Body condition score was also similar between treatments and indicates heifers were in a positive energy balance as BCS = 3.

Rumen Fermentation Characteristics

Rumen sample analysis from every 4 wk is presented in Table 2.6. Rumen ammonia–N (21.0, and 24.5 mg/dL; SEM = 2.15), pH (6.75, and 6.70; SEM = 0.06), total volatile fatty acids (95.0, and 98.0 mM; SEM = 2.32) and acetate to propionate ratio (3.4, and 3.3; SEM = 0.13) were similar. Rumen pH was not different among treatments, the values however, are what could be expected in a high forage diet (Zanton and Heinrichs,

2008). The pH ranges from 6.7 to 6.8 and this is close to the values reported by Zanton and Heinrichs (2008) when high forage rations were fed at different levels of DMI. The increased pH in the study could also be attributed to sampling method, esophageal tubing to obtain rumen samples has the likelihood of saliva contamination. The concentration of ammonia–N is in abundance of the amount needed for efficient utilization of nitrogen (5mg/dL) (Satter and Roffler, 1974). The increased amount of ammonia in both treatments was not great enough to increase plasma urea nitrogen (PUN) which was demonstrated by Gabler and Heinrichs, (2003a). In comparison, Surber and Bowman (1998) observed a greater ruminal ammonia-N concentration for the steers fed a barley-based diet than for corn fed diet. In disagreement with the current research and published literature Overton et al. (1995) observed a linear reduction in ammonia-N concentration when corn was replaced by 25% of barley. The total concentration of volatile fatty acids (VFA) did not differ between treatments and was greater than the concentration (71.1-77.6 mg/dL) reported in a study that increased dietary CP using common protein sources from 11.9 to 21 % (Gabler and Heinrichs, 2003b). In contrast, isovalerate and isobutyrate tend to be reduced ($P < 0.10$) in the HYD fed heifers.

Blood Metabolites

Metabolite analysis is presented in Table 2.7, heifers fed the CON treatment had greater ($P = 0.04$) glucose concentrations. Heifers fed a high forage or high concentrate diet for a high or low level of gain also exhibited increased glucose concentration for the high concentrate fed heifers, which was attributed to increase in propionic acid provided from increased starch (Allen and Bradford, 2012; Allen et al., 2017). Corn and barley are the widely used grain sources in dairy cattle diets that vary in the starch content and

degradability in the rumen (Huntington, 1997; Herrera-Saldana et al., 1990). Thus, the CON treatment contained ground corn in the diet compared to the HYD treatment that did not include ground corn. In lactating cows, McCarthy et al. (1989) proposed that the shift in the site of starch digestion of corn to the small intestine increased the glucose availability for lactose synthesis, however, in growing heifers this excess in glucose would remain in the plasma as lactose is not being produced. Plasma concentration of triglycerides was greater ($P = 0.01$) for the HYD treatment. This finding is unexpected, since both treatments have similar EE % in the ration. The triglyceride concentration in the current study is also greater than anticipated. Results are similar to those reported in Ch. 4, triglycerides were also greater for the CRM fed heifers compared to the CON. However, heifers in that study were at a greater BW than the current trial and should be producing and storing triglycerides for adipose tissue. Cholesterol and plasma urea nitrogen (PUN) were similar between treatments. It was interesting to find that PUN was not increased when ammonia-N was at elevated concentrations. The study by Gabler and Heinrichs (2003a) found a similar relationship with ammonia-N and PUN.

Apparent Total Tract Digestion of Nutrients

Digestibility of nutrients evaluated did not differ between treatments (Table 2.8). The lack of response in nutrient digestion may be due to the decreased digestibility of a high forage ration which is not as efficiently digested due to large content of poorly digestible fiber (i.e. grass hay; Moody et al., 2007). In contrast, total tract digestion of nutrients in the current study is slightly decreased compared to Anderson et al. (2015a). This is due to feeding strategies, in the current study we fed for ad libitum intake and in most recent studies conducted with alternative feedstuffs limit feeding was implemented

(Anderson et al., 2015a; Lawrence et al., 2016; Rodriguez-Hernandez and Anderson, 2018).

Conclusion

Results indicate that replacing corn and some soybean meal with hydroponic barley sprouts maintained heifer frame growth without affecting blood metabolic profile or rumen fermentation. The decreased gain: feed was most likely because of the high moisture content in the HYD TMR. Apparent total tract digestion of nutrients was not affected by feeding hydroponic barley. A limitation of this study is the lack of particle size distribution of the diets fed, although no formal observations were made heifers tended to sort for the barley sprouts. The proper incorporation of barley sprouts has yet to be identified, cutting the sprouts by hand is not feasible for dairy producers and a method for chopping needs to be investigated. In addition, further research into inclusion amounts in a dairy heifer ration should be conducted to fully understand optimal level of hydroponic feeds in dairy cattle diets.

Table 2.1. Ingredient composition and formulated[1] nutrient composition of the control (CON) and hydroponic barley (HYD) diets fed to growing dairy heifers for 12 weeks

	Diet	
Ingredients, % of DM	CON	HYD
Grass Hay	50.0	50.0
Corn Silage	17.0	17.0
Hydroponic Barley Sprouts	-	14.0
Ground Corn	11.0	-
Soybean Meal	11.0	8.0
DDGS	8.0	8.0
Mineral and Vitamin premix[2]	1.0	1.0
Salt	1.0	1.0
Limestone	0.5	0.5
Energy Booster (Rumen inert fat)	0.5	0.5
Nutrients, % of DM[3]		
DM, % of diet	66.1	43.7
CP	14.0	13.9
RDP	9.1	9.3
RUP	5.0	4.7
NDF	43.8	46.0
ADF	24.9	26.2
EE	3.1	3.0
NFC	33.7	32.3
ME, Mcal/kg DM	2.3	2.2
NEg, Mcal/kg DM	0.8	0.8

[1] Based on formulation predictions of NRC (2001) when initial analyses values for samples were entered into the program.

[2] Contained: 3.2 g/kg of lasolocid sodium, 18.9% Ca, 24.3% NaCl, 1.6% Mg, 0.5% K, 3,880 mg/kg Zn, 880 mg/kg Cu, 50 mg/kg I, 25 mg/kg Se, 550,000 IU/kg Vitamin A, 110,000 IU/kg Vitamin D3, and 4180 IU/Kg Vitamin E (HeiferSmart No Phos B2909 Medicated, Purina Animal Nutrition, LLC., Shoreview, MN).

[3] % of DM, unless otherwise indicated.

Table 2.2. Nutrient composition of the forages, control and hydroponic diet grain mixes, and hydroponic barley sprouts used in treatment diets fed to dairy heifers for 12 weeks

Item[1]	Ingredients				
	Grass Hay	Corn Silage	Control Grain Mix	Hydroponic Grain Mix	Hydroponic Barley Sprouts
DM, %	86.9	34.2	87.9	89.3	17.1
CP	7.2	8.3	27.4	35.1	16.0
ADF	36.9	24.0	4.7	6.0	12.2
NDF	67.6	41.2	13.2	16.1	28.4
Starch	0.5	34.1	25.2	3.2	25.2
EE[2]	1.1	2.8	4.1	5.4	2.6

[1]% of DM unless otherwise indicated.
[2]Ether extract, analyzed with petroleum ether.

Table 2.3. Nutrient composition based on laboratory analysis for the control (CON) and hydroponic barley (HYD) treatments fed to growing dairy heifers for 12 weeks

Item[1]	Treatment			
	CON		HYD	
	Mean	SE	Mean	SE
DM[2], %	69.0	1.65	47.4	0.60
CP[2]	14.1	0.20	13.6	0.15
NDF[2]	45.2	0.10	47.7	0.33
ADF[2]	24.1	0.30	25.3	0.41
EE[2,3] (Petroleum)	2.4	0.04	2.4	0.01
RDP[5]	8.7	-	8.3	-
RUP[5]	5.3	-	4.8	-
Forage NDF[5]	40.0	-	43.7	-
Nonforage NDF[5]	5.2	-	4.0	-
Starch[2]	14.3	0.61	10.2	1.40
Ca[2]	0.70	0.013	0.65	0.008
P[2]	0.28	0.005	0.27	0.006
Mg[2]	0.24	0.001	0.23	0.001
K[2]	1.30	0.006	1.17	0.011
S[2]	0.19	0.010	0.19	0.002
Na[2]	0.60	0.014	0.58	0.045
Cl[2]	0.40	0.008	1.22	0.028
ME[5], Mcal/kg DM	2.40	-	2.31	-
NE_g[5], Mcal/kg DM	0.90	-	0.82	-

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

Table 2.4. Dry matter intakes, BW, and gain: feed ratios for dairy heifers fed the control (CON) and hydroponic barley (HYD) diets for 12 weeks

	Treatment			*P values*		
Item	CON	HYD	SEM	Treatment	Week	Treatment ×Week
Age, initial, d	229.7 ± 41.0	229.7 ± 45.0				
BW, kg						
Mean	289.7	282.4	2.02	<0.01	<0.01	0.02
Initial	225.0	237.0	2.24	0.44		
Final	330.3	315.0	3.02	<0.01		
ADG[1], kg/d	1.2	1.0	0.06	0.07	0.09	0.22
DMI, kg	7.5	8.0	0.42	<0.01	<0.01	0.73
Gain: Feed	0.16	0.13	0.01	<0.01	<0.01	0.01

[1] Calculated based on BW change per 2-wk intervals.

Table 2.5. Frame size measurements for dairy heifers fed the control (CON) and hydroponic barley (HYD) diets for 12 weeks

Item	Treatment			*P values*		
	CON	HYD	SEM	Treatment	Week	Treatment ×Week
Withers Height, cm						
Mean	121.7	121.9	0.54	0.51	<0.01	0.61
Initial	114.0	116.1	1.35	0.27		
Final	126.2	125.7	0.74	0.47		
Change[1], cm/d	0.14	0.12	0.014	0.24	<0.01	0.80
Hip Height, cm						
Mean	125.7	125.1	0.50	0.14	<0.01	0.95
Initial	118.2	120.6	1.20	0.16		
Final	130.0	129.0	0.73	0.25		
Change[1], cm/d	0.13	0.11	0.01	0.52	0.14	0.96
Body Length, cm						
Mean	119.0	119.3	0.64	0.47	<0.01	0.94
Initial	113.5	114.5	1.30	0.61		
Final	122.8	123.8	0.97	0.41		
Change[1], cm/d	0.11	0.11	0.02	0.82	0.17	0.96
Heart Girth, cm						
Mean	146.0	145.0	0.62	<0.01	<0.01	0.45
Initial	135.9	137.0	1.74	0.65		
Final	153.0	150.5	0.87	0.01		
Change[1], cm/d	0.20	0.16	0.02	0.20	<0.01	0.40
Paunch Girth, cm						
Mean	186.0	187.0	1.71	0.30	<0.01	0.32
Initial	171.0	173.2	2.91	0.60		
Final	192.0	190.0	2.11	0.32		
Change[1], cm/d	0.23	0.19	0.05	0.60	<0.01	0.10
Hip Width, cm						
Mean	37.3	37.1	0.57	0.83	<0.01	0.99
Initial	32.8	34.1	0.68	0.20		
Final	40.1	40.1	0.93	0.99		
Change[1], cm/d	0.10	0.10	0.03	0.90	0.79	0.97
BCS						
Mean	3.11	3.10	0.025	0.28	0.15	0.32
Initial	2.94	3.00	0.016	0.02		
Final	3.11	3.05	0.040	0.26		
Change[1], cm/d	-0.003	0.002	0.0027	0.18	0.63	0.40

[1] Calculated based on change per 2-wk intervals.

Table 2.6. Rumen fermentation characteristics for dairy heifers fed the control diet (CON) and hydroponic barley (HYD) diets for 12 weeks

	Treatment			*P value*
Item	CON	HYD	SEM	Treatment
pH	6.7	6.7	0.06	0.33
Ammonia-N, mg/dL	21.0	24.5	2.15	0.25
Total VFA, mM	95.0	98.0	2.32	0.40
VFA, mM/100mM				
Acetate	66.1	66.1	0.85	0.99
Propionate	19.7	19.9	0.56	0.84
Isobutyrate	1.6	1.3	0.12	0.06
Butyrate	10.3	10.8	0.30	0.26
Isovalerate	1.1	1.0	0.05	0.08
Valerate	0.9	1.0	0.06	0.55
Acetate: Propionate	3.4	3.3	0.13	0.83

Table 2.7. Plasma metabolites for dairy heifers fed the control diet (CON) and hydroponic barley (HYD) diets for 12 weeks

	Treatment			*P values*		
Item	CON	HYD	SEM	Treatment	Week	Treatment ×Week
Glucose, mg/dL	81.3	77.6	1.21	0.04	<0.01	0.30
PUN[1], mg/dL	14.5	13.8	0.48	0.28	<0.01	0.20
Cholesterol, mg/dL	73.1	73.0	4.60	0.96	<0.01	0.43
Triglycerides, mg/dL	21.2	25.7	1.15	0.01	0.09	0.83

[1]Plasma Urea Nitrogen

Table 2.8. Total tract digestion of nutrients for dairy heifers fed the control diet (CON) and hydroponic barley (HYD) diets for 12 weeks

	Treatment			*P value*
Item, % digested	CON	HYD	SEM	Treatment
DM	66.7	65.3	1.43	0.41
NDF	54.0	54.2	1.78	0.83
ADF	53.2	53.4	1.53	0.90

Figure 2.1. Dry matter intake (DMI) of dairy heifers fed the control diet (CON) and hydroponic barley (HYD) diets for 12 weeks. Error bars represent SEM = 0.42.

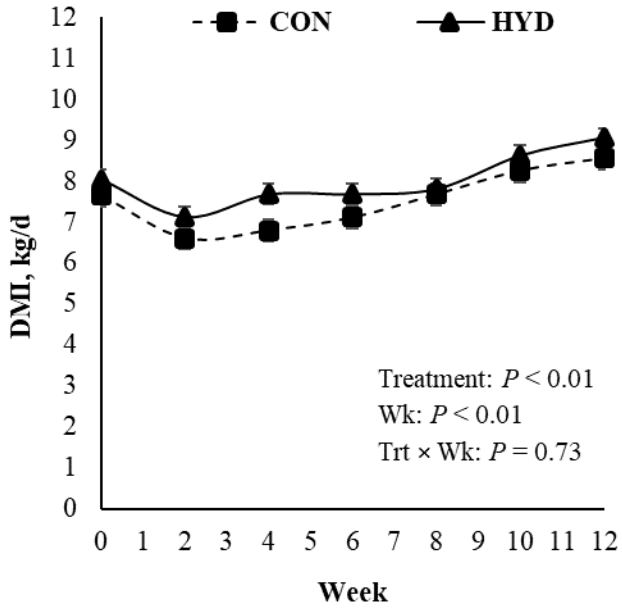
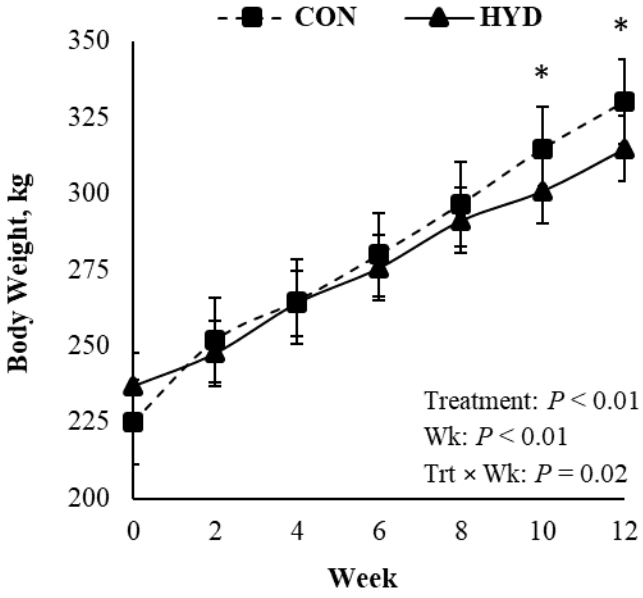


Figure 2.2. Body weights over the course of the study for dairy heifers fed the control diet (CON) and hydroponic barley (HYD) diets for 12 weeks. Error bars represent SEM = 2.02. * Indicates values differ by $P < 0.05$ with Tukey's test.



CHAPTER 3:

EFFECTS OF FEEDING HYDROPONIC BARLEY SPROUTS TO LACTATING DAIRY COWS ON MILK PRODUCTION AND COMPOSITION

Abstract

The objective of this study was to determine the effects on milk production, milk composition, metabolic profile, rumen fermentation and apparent total tract digestion of nutrients in cows fed hydroponically grown barley sprouts (HydroGreen Inc., Renner, SD). Twenty mid-lactation Holsteins (DIM 205 ± 47.4) were used in a 6-wk randomized complete block design study. Treatments included: 1) control diet with ground corn and soybean meal as major concentrate ingredients (**CON**) and 2) 8% (DM basis) as hydroponic barley sprouts replacing some corn and soybean meal (**HYD**). Both diets were individually fed as total mixed rations using Calan gates and were similar in crude protein and caloric content. Cows were milked 2×/d in a double 8 parallel parlor at 0500 and 1700. At the beginning of the study and every 2 wk on two consecutive days at approximately 4 h post feeding blood samples from the coccygeal vein were collected for analysis of metabolites related to energy partitioning and protein utilization. Immediately following blood sampling rumen fluid was collected via esophageal tube. Body condition scores (BCS) and body weight (BW) were recorded at the same time prior to sample collection. Milk samples were taken on the same days at each individual milking. A blind triangle taste test was done on 2 d in wk 6 with 25 volunteers. Dry matter intakes and BW were similar between treatments. Body condition score was greater for CON fed cows. Milk production and feed efficiency had treatment by wk interactions. Milk protein and fat yields were similar between treatments. In addition, milk fatty acid composition was

not different. Triangle test participants were unable to discern a taste difference between milk from CON vs. HYD on d 1 (Chi-squared = 0.55; P = 0.46) and d 2 (Chi-squared = 1.67; P = 0.20). Concentration of plasma cholesterol was found to be greater for cows fed HYD, all other blood metabolites were similar between treatments. Rumen VFA and ammonia-N were similar between treatments with the exception of isovalerate which was greater for HYD and isobutyrate which also tended to be greater than CON. Organic matter and DM apparent total tract nutrient digestion tended to be greater for cows fed HYD. Results demonstrate that hydroponic barley sprouts can replace a portion of the corn and soybean meal and maintain rumen fermentation and lactation performance and potentially improve nutrient utilization through increased digestion.

Key words: dairy cow, hydroponic feed, milk production

Introduction

Hydroponically grown feeds have not been well researched for dairy cattle, despite the potential environmental and animal performance benefits. Bench top digestibility studies have been conducted on hydroponically grown barley and it was found that nutrient digestibility may be increased (Hafla et al., 2014), or show no improvement when compared to the original grain (Dung et al., 2010a). Testimonials from dairy producers have been anecdotal and consist of improved dry matter intake, animal health, milk yield and quality (Anderson, 2009; Benson and Burrichter, 2014; Sergeant, 2012).

The objectives of this research study were to evaluate the effects of hydroponic barley sprouts on milk production, milk composition, feed efficiency, metabolic profile,

and rumen fermentation characteristics. It is hypothesized that increased palatability and digestibility of hydroponic feed would improve mid-lactation dairy cow performance. Another objective was to assess milk quality through sensory analysis using benchtop pasteurization and blind triangle taste testing. The overall objective of this was to determine the effects on mid-lactation dairy cows in a conventional system when hydroponic barley sprouts replaced a proportion of grain in the diet.

Materials and Methods

Experimental Design

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 16-017A. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Twenty mid-lactation Holsteins (DIM 205 ± 47.4) were used in a 6-wk randomized complete block design study. Cows were blocked in pairs based on parity, DIM, and milk production and randomly assigned to treatment. The feeding trial was conducted from May 2016 to July 2016 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). Prior to feeding the treatment diets, there was a 14 d adaptation period for cows to adjust to the Calan gate feeding system (American Calan, Inc., Northwood, NH), followed by an experimental feeding period of 6 wk. Treatments included: 1) control diet with ground corn and soybean meal as major concentrate ingredients (**CON**) and 2) 8% (DM basis) as hydroponic barley sprouts replacing some corn and soybean meal (**HYD**). Diets were formulated to meet the requirements for a mature, lactating Holstein cow, at 680 kg body weight (BW), 200

DIM, and 36 kg of milk production, according to the 2001 Dairy NRC. Both treatment diets were formulated to contain similar forage concentrations and to be isonitrogenous with 17% CP and isocaloric. Table 3.1 presents the ingredient formulations of the diets on a DM basis, Table 3.2 presents the diet ingredient formulation on an as-fed percentage basis, and Table 3.3 is the nutrient composition the diets were formulated for on a DM basis.

Animal Care and Feeding

Animals were observed daily for any injury or disease problems and treated according to normal farm management practices. Over the course of the study cows were housed in a free-stall barn and diets were fed as a total mixed ration (TMR) using the Calan Data Ranger (American Calan, Inc., Northwood, NH) so that individual intakes could be measured. Hydroponically grown barley sprouts were delivered daily by the sponsor (HydroGreen Inc., Renner, SD) and hand cut into approximately 2 in. cubes to facilitate mixing. Feeding occurred once daily at approximately 0900 h, orts were weighed and recorded each morning prior to feeding, to determine individual cow daily intakes. Forages were premixed in a large TMR mixer wagon, then forage mix, the concentrate mix and hydroponic barley sprouts were added into the Calan Data Ranger, mixed, and individual ration weights were recorded for each cow. Ration mixes were adjusted weekly based on DM analysis of feed ingredients. Feed was offered for ad libitum consumption (10% refusal). Cows were allowed access to feed and fresh water at all times, except during milking. Milking occurred 2 times per day at 0500 and 1700 h in a double 8 parallel parlor and milk production was recorded at each milking and averaged by day.

Animal Measurements and Sampling

At the start of the study and every two weeks on two consecutive days throughout the experiment cows were weighed and body condition scored by three individuals on a scale of 1 to 5 with 1 being emaciated and 5 being obese (Wildman et al., 1982). On two consecutive days at approximately 4 h post feeding, during wk 0, 2, 4, and 6 of the feeding period, blood samples from the coccygeal vein were collected for analysis of metabolites related to energy partitioning and protein utilization. Blood samples were collected 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 (K2EDTA) for all other analyses (Cat. #:366643). Immediately after blood collection, samples were placed in ice and then brought in to the laboratory within 3 h for processing and storage. Blood collection tubes were centrifuged at 1000 × g for 20 minutes at 4°C (CR412 centrifuge, Jouan, Inc., Winchester, VA). Plasma or serum was then transferred and frozen at -20°C until metabolite analysis.

Rumen fluid was collected just prior to blood sample collection via esophageal tubing for analysis of volatile fatty acids and ammonia-N. The beginning stream of rumen fluid was discarded in order to minimize saliva contamination. Approximately 100 mL of rumen fluid was collected into a stainless steel cup. The pH of the sample was recorded immediately (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL). Then a 10 mL aliquot was collected and mixed with 2 mL of 25% (w/v) meta-phosphoric acid for later determination of VFA concentrations, and a 10 mL aliquot was collected and mixed with 200 µL of 50% (v/v) sulfuric acid for later analysis of rumen ammonia nitrogen (NH3-N). The two samples from both sampling days were stored at -20° C until analysis.

On two consecutive days in wk 0, 2, 4, and 6 of the study, milk samples were taken at each milking for compositional analysis (fat, protein, lactose, milk urea nitrogen, total solids, and somatic cell counts). During wk 4 and 6 extra milk samples were collected for fatty acid analysis and anti-oxidation potential analysis. Also during wk 6, milk was obtained on 2 days from each treatment group and pasteurized using bench-top methods for a blind triangle test taste conducted with 25 volunteers who were employees of SDSU. At the same time analyses of ferric reducing antioxidant power (FRAP) was conducted according to Amamcharla and Metzger, 2014.

Each week samples of the forages, grain mixes and hydroponically grown barley sprouts were taken and stored at -20°C until processing and analysis could be completed as described under laboratory analysis. During wk 6 of the feeding period samples for analysis of total tract digestibility of nutrients were collected. Orts and fecal grab samples were collected over 3 d. Fecal grab sample collections were scheduled so that samples would ultimately represent every 3 h over the 24 h period relative to time of feeding. Orts and fecal samples were stored at -20°C until processing and analysis could be completed. Total tract digestibility of nutrients was then calculated using the internal marker acid detergent insoluble ash (ADIA) according to equation provided by Merchen, 1988. Nutrients in fecal samples were analyzed using similar procedures as used for analysis of feed samples.

Laboratory analysis

To determine DM content, feed samples were dried for 24 h at 105°C every 2 wk, to check ingredient inclusion rates in the ration and determine DMI. For processing, feeds were thawed and samples from three consecutive weeks were composited on an as-fed

basis by volume. Composite samples were dried in duplicate for 48 h at 55°C in a Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN). Composites of the forage were ground to a 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). Ground forages and the concentrates were reground to a 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To correct analysis to 100% DM, 1 g aliquot of sample was dried for 4 h in a 105°C oven (AOAC, 1998; method 935.29). Ash content was analyzed by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC, 1998; method 942.05). Organic matter (OM) was then calculated as OM = (100 - % Ash). All samples were analyzed for nitrogen (N) content via Dumas combustion analysis (AOAC, 2002; method 968.06), on a Rapid N cube (Elementar Analysensysteme, GmbH, Hanau Germany). The resulting nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). Heat-stable alpha-amylase and sodium sulfite were used for the NDF. Before samples were analyzed for NDF they were pre-soaked in acetone if the fat concentration was greater than 5% or if they contained soy products according to procedure recommendations. Ether extracts (EE) were analyzed using petroleum ether (AOAC, 1998; method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Non-fibrous carbohydrates were calculated as % NFC= 100 - (% Ash + % CP + %NDF + % EE) as described by the NRC (2001). Feed composites were then sent to a commercial lab 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, 1998). Starch concentration was found using a modified method of glucose analysis completed on an YSI 2700 Select Biochemistry Analyzer (YSI Biochemistry Analyzer, YSI Inc., Yellow Spring, OH).

Samples of the TMR and orts collected weekly were composited by treatment and used for analysis of particle size and nutrient composition. Samples were analyzed on the day of collection. For example, on Monday morning samples of the TMR were collected and evaluated after feeding was completed. Orts samples were collected on Tuesday morning to compare to the TMR fed the previous day. Particle size was evaluated using the 3-screen Penn State Particle Separator (PSPS; Heinrichs, 2013). The particles were separated into 4 categories using the PSPS: long (>19 mm), medium (8-19 mm), short (1.18-8 mm), and fine (<1.18 mm).

Milk samples collected at both milking times on 2 d during wk 0, 2, 4, and 6 were sent to Heart of America DHIA Laboratory (Manhattan, KS) for component analysis. Milk composition analysis was conducted according to AOAC (1995). Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments). Energy-corrected milk was determined using the equation: [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] (Orth, 1992). Also, composites from 2 d of wk 4 and 6 milk samples were prepared for analysis of milk fatty acid composition. Fatty acid profiles were analyzed via direct

butylation method as originally described by Sukhija and Palmquist (1988) with adaptations described by (Abdelqader et al., 2009). Prepared fatty acid samples were analyzed via gas chromatography (Hewlett Packard 6890, Palo Alto, CA) as also described by Abdelqader et al. (2009). The FRAP assay was conducted according to Amamcharla and Metzger, 2014, which is a modification of the original assay (Benzie and Strain, 1996). Modifications include changing the milk to reagent ratio and use of a syringe filter to remove milk proteins.

For analysis of rumen fluid, it was first thawed and vortexed to completely mix contents before pipetting 2 ml into a microcentrifuge tube to be centrifuged at 10,000 × g for 20 min in a micro centrifuge (Model A-14, Jouan Inc., Vinchester, VA). Samples acidified with sulfuric acid were used analyzed for Ammonia-N concentration using the assay described by Chaney and Marbach (1962). Volatile fatty acid concentrations were measured in samples acidified with meta-phosphoric acid using 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) with 2-ethylbutyrate used as an internal standard. The flow rate was 1.3 ml/min of helium and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Blood metabolites (glucose, cholesterol, triglyceride and PUN) were analyzed with commercially available enzymatic or colorimetric assay kits on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA.). Serum glucose was analyzed using glucose oxidase reagent as described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI) as described by

Allain et al. (1974). Plasma urea nitrogen (PUN) was analyzed using diacteylymonoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Triglyceride concentration was determined using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) and Trinder (1969).

For digestibility analysis, fecal and orts samples were composited on an as-is basis by volume for each cow. Samples were processed (dried and ground) as described for the feed composites. Fecal and orts samples were also analyzed for DM, Ash, CP, NDF and ADF as previously described for feeds. Acid detergent insoluble ash (ADIA) analyses was conducted on all feed composites, fecal samples, and orts. The method for ADIA analysis consists of analyzing the sample for ADF digestion (Robertson and Van Soest, 1981) and then determining the ash percentage using a modified procedure of the AOAC (1998), method 935.29.

Statistical Analysis

Feed nutrient means and standard errors were calculated using the MEANS procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The total dietary nutrient values were calculated based on analysis of concentrate mixes and forages for each treatment.

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Lactation performance data were analyzed as a randomized complete block design with week as the repeated measure and cow (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, parity and the interactions of all terms. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures

tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. The blind triangle taste test was analyzed using a Chi-squared test for given probabilities in R. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

The MIXED procedures of SAS were also used for analysis of data for the total-tract digestibility of nutrients. As it was a single time point, the model included only treatment with block as a random variable. Least square means are again reported for each treatment in the tables and means were compared using Tukey's test.

Results and Discussion

Feed Composition

The nutrient compositions of concentrate mixes, forages (alfalfa hay and corn silage) and hydroponically grown barley sprouts are presented in Table 3.4. The nutrient composition of the diets fed based on laboratory analysis is presented in Table 3.5. The actual diets fed had different DM concentration at 57.8% (SE = 0.46), and 47.5% (SE = 0.50), for CON and HYD, respectively. This was similar to diet formulations and expected due to the increased moisture from barley sprouts (17.3% DM). Diets were formulated to be 17% CP, but only contained 16.5 and 16.6% CP, which should not have affected animal performance. The optimum dietary concentration of CP has been found to be 17 %, the ideal CP % for milk and protein yield has been found to be between 16.7 and 17.1 % CP (Olmos Colmenero and Broderick, 2006). The fiber composition (NDF and ADF) of the treatment diets matched closely with what was formulated. The energy

content of the treatment diets was similar to diet formulations and did not differ between treatments.

The particle size distribution of TMR and orts or refusal are presented in Table 3.6. The relative particle size of the TMR differed between treatment diets mainly in the distribution of long particles (>19 mm) and fine (<1.18 mm) particles. The CON TMR had less (7.0) retained on the >19 mm sieve than the HYD TMR (21.4) and this is attributed to the larger (2 in. cubes) of the hydroponic barley sprouts added to the diet. The distribution of fine particles was greater in the CON treatment; this was expected since hydroponic sprouts replaced a portion of the ground corn and soybean meal in the HYD treatment. Nutrient utilization in dairy cows is highly influenced by the physical characteristics and the chemical composition of the ration (Mertens, 1997). The treatment TMR are within ranges recommended by the Penn State Extension for lactating cows (Heinrichs, 2013). Particle size is important because it stimulates mastication, saliva buffering of the rumen, and rumination. When particle size is not adequate it may cause a decrease in ruminal pH and alter fermentation patterns and influence a decrease in acetate: propionate ratio (A: P) (Mertens, 1997). Evaluation of particle size of orts or refusals was used to determine if sorting was occurring over the course of the day. The HYD treatment had a greater long distribution of particles in refusal samples, and a lesser amount of fine particles compared to the CON. Observations were not officially recorded but the HYD treatment tended to sort for the hydroponic sprouts immediately after feeding. However, due to the similar distribution of HYD ORTS to the original TMR, sorting was found to not influence particle size intake.

Animal Performance

Dry matter intake (23.0, and 23.0 kg/d, for CON and HYD, respectively; SEM = 0.54) and BW (690.0, and 680.5 kg; SEM = 3.72) are presented in Table 3.7 and were similar between treatments. Body condition scores were also similar, and indicative (BCS = 3) of cows maintaining condition over the course of the study. Similar BW and BCS were observed between treatments by Soder et al. (2018) when sprouted barley was supplemented to grazing organic dairy cows.

Milk production (Table 3.7) was also similar between treatments, however; there was a significant treatment by week interaction ($P = 0.01$) for milk production (Figure 3.1). This is in agreement with Soder et al. (2018), researchers found no difference in milk yield. However, as dry matter intakes were similar between treatments, feed efficiency (1.33 and 1.40; SEM = 0.046; Figure 3.3) also had a treatment by week interaction in a similar pattern over time as milk production. Milk component percentages and yields were also similar between treatments, which is not in agreement with Soder et al. (2018).

The results of the FRAP assay are also presented in Table 3.7, differences between treatments were not found. A high FRAP value is considered desirable in the industry to limit oxidative deterioration (Amamcharla and Metzger, 2014). Since FRAP values decrease with storage time, analysis was performed on fresh raw milk (Amamcharla and Metzger, 2014). More commonly oxidative stability of milk is examined through lipid oxidation, protein oxidation and sensory analysis (Smet et al., 2008). However, the modified FRAP assay is known as a time saving, easy to perform, and cost-effective method to identify milk that may be susceptible to oxidation

(Amamcharla and Metzger, 2014). Negative aspects of the FRAP assay include use of the syringe filter to remove milk proteins. When analysis was performed it was apparent that the force required for the filtration using the syringe made the assay much more difficult and time consuming. The increased time required to use the syringe filter could have potentially increased oxidation of the samples as they were exposed to air for a greater period of time prior to reading on the spectrophotometer.

Sensory analysis was conducted by 4 individuals trained in dairy products judging, and treatments were similar in flavor, with no off flavors present. The twenty-five blind triangle test participants were unable to discern a flavor difference between milk from CON vs. HYD on day 1 (Chi-squared = 0.55; $P = 0.46$) and day 2 (Chi-squared = 1.67; $P = 0.20$) of samples collected during week 6 of the feeding period.

The average temperature for the Brookings, SD area over the course of the study is presented in Figure 3.4. The maximum and minimum temperature per day was recorded, to determine if it could possibly have affected milk production, DMI, and explain the treatment by week interaction that was found for both parameters. Heat stress of cattle may be affected by environmental climate, climatic effects on the cow, or changes in production or physiology (West, 2003). Usually periods of heat stress reduce DMI and milk yield, however, in the current study HYD fed cows increased DMI and decreased production around wk 3. During that time average temperature was near 32°C. When environmental temperature reaches 31°C, rectal temperature was found to be increased and feed intake decreased in a study using a temperature controlled chamber (Wayman et al., 1962). Thus, when DMI increased in the HYD treatment it was difficult to attribute this to temperature alone. A possible explanation could be that eating of the

HYD ration which contained the barley sprouts and a lesser % DM, the cows were able to stay cooler due to the digestibility of barley sprouts (Abel-Caines, 2013). However, the relationship between DMI and dietary moisture has not been found to be conclusive (NRC, 2001).

Milk fatty acid profiles presented in Table 3.8 were similar between treatments, with the one exception of C16:0, which was found to be greater for the CON fed cows. In contrast, Soder et al. (2018) found that cows supplemented with sprouts tended to have lower omega 6: omega 3 fatty acid ratio. The concentration of short-chain fatty acids (SCFA) and long-chain fatty acids (LCFA) was similar between treatments. Also, milk fat composition of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) were not affected by treatment.

Rumen Fermentation Characteristics

Rumen fermentation characteristics are presented in Table 3.9. Rumen pH (6.53, and 6.60; SEM = 0.11) were similar between treatments. The pH values are similar to those reported by Hafla et al. (2014) when barley grain or sprouted barley was supplemented into a continuous-culture fermentation system. The pH consistency between treatments may also be attributed to sampling method, esophageal tubing to obtain rumen samples has the likelihood of saliva contamination. However, use of rumen-fistulated cows was beyond the scope of this initial study so, esophageal tubing was the best option and most minimally invasive way to collect rumen samples.

The ammonia-N concentration was similar between treatments. For both treatments, concentrations of ammonia–N are greater than the amount needed for efficient utilization of nitrogen (5mg/dL) (Satter and Roffler, 1974). The increased

ammonia–N concentration may have been affected by sampling method, approximately 4 h post feeding is when samples were collected via esophageal tube, which could also potentially be when concentrations are at their peak (Owens and Zinn, 1988). The greater percentage of ruminal protein degradation of alfalfa hay and hydroponically grown barley was likely the cause of the increased ammonia–N concentrations. However, ammonia–N concentration in the current study is comparable to Hafla et al. (2014) when a haylage based diet was supplemented with sprouted barley (14.4 mg/dL ammonia–N).

The total concentration of VFA (Table 3.9) did not differ (95.6, and 95.0 mM; SEM = 1.81) between treatments and was greater than the concentrations reported by Hafla et al. (2014). In another study examining barley, Dung et al. (2010b) found that total VFA concentration did not differ when sheep fed oaten chaff were supplemented with barley grain or sprouted barley. Total VFA concentrations in the current study were comparable to concentrations (97.6 mM) observed by Khorasani et al. (2001) when a barley-corn based diet was fed. Acetate: propionate ratios (A: P) were similar between treatments and were greater than the ratios observed by other studies examining barley (Chibisa et al., 2015; Hafla et al., 2014; Khorasani et al., 2001). However, A: P in the current study were less than that found by Feng et al. (1995) when beef steers were supplemented with barley cultivars in replacement of corn. The proportions of all other VFAs were similar between treatments, with the exception of isobutyrate and isovalerate. Isovalerate concentration (1.21, and 1.24 mM) was greater for the HYD fed cows, and concentration of isobutyrate (0.9, and 1.0 mM) also had a tendency to be greater. In contrast, the concentration of isovalerate and isobutyrate were reduced for the HYD fed heifers in Chapter 2. However, these are considered minor VFA and these differences do

not convey changes of biological significance. Rumen fermentation profile did not differ between treatments and this may be due to similar amounts of structural carbohydrates in the rumen which are degraded by cellulolytic bacteria to produce acetate and nonstructural carbohydrates which amylolytic bacteria convert to propionate (Enjalbert et al., 1999).

Blood Metabolites

Blood metabolite concentrations are presented in Table 3.10. Treatment by week interactions for any of the blood metabolite concentrations measured were not found. Glucose, plasma urea nitrogen, and triglyceride concentrations were similar between treatments. Sun and Oba, (2014) fed a barley based diet to lactating cows and reported similar glucose concentration to that of the current study. However, serum glucose concentrations in the current study were less than that reported by Chibisa et al. (2015) when lactating cows were fed a barley based diet with or without sugar supplementation (dried whey). Plasma urea nitrogen and triglyceride concentrations have not been tested in the other research we are aware of related to feeding hydroponic barley sprouts. The similar triglyceride concentrations between treatments indicate cows were in a similar energy status. Similar PUN, ammonia-N and MUN concentration indicate there were no difference in protein utilization between treatments, as both are highly correlated and indicative of the energy to protein ratio in healthy cattle (Hammond, 1996).

Plasma cholesterol had a tendency to be greater ($P = 0.09$) for the HYD treatment. This was unexpected, due to the similar EE content of the treatment diets. Many studies do not determine cholesterol concentration unless the study is designed to provide additional or supplementary fat. Blood cholesterol concentrations are subject to increase

when high fat diets are fed, specifically linoleic acid, which is a precursor for arachidonic acid and cholesterol (Palmquist, 1994). Cholesterol is precursor to several reproductive hormones and increasing concentration of circulating cholesterol is generally viewed as favorable in lactating cows.

Apparent Total Tract Digestion of Nutrients

Apparent total tract nutrient digestibilities of nutrients are presented in Table 3.11. Crude protein and fiber digestibilities were similar between treatments. Digestibility of DM and OM tended ($P = 0.07$) to be greater for the HYD treatment. These findings are also supported by Hafla et al. (2014). The researchers found that sprouted barley in a continuous culture fermentation system tended to increase DM digestibility. Sprouted barley grown in a rice straw medium supplemented to lambs also increased digestibility of DM, OM, CP, EE, and cellulose (Fayed, 2011). In contrast, Dung et al. (2010b) found no differences in digestibility of DM and OM of sprouted barley versus cracked barley grain. The increased DM digestibility of Hafla et al. (2014) could potentially be attributed to a release of soluble carbohydrates and nitrogen from the sprouted barley, which may have encouraged greater degradation of the low-quality forages, bacterial growth, and microbial colonization (Pond et al., 1984). Authors argued that the haylage and herbage forages used were of high-quality protein (18 and 26% CP) and that the slight increase in DM digestibility may be due to an increased water-soluble carbohydrate fraction in sprouted barley supplemented diets (Hafla et al., 2014). Since the corn silage in the current study could be considered low in protein (8.4% CP), we believe the increase in DM and OM digestibility was due to the increased soluble carbohydrates provided by hydroponically grown barley sprouts. Feed efficiency did not differ between treatments,

indicating that even though DM and OM digestion increased, the effect did not cause a response in FE.

Conclusion

Results indicate that hydroponically grown barley sprouts can replace a portion of the corn and some soybean meal in diets of mid-lactation cows and maintain production performance. Rumen fermentation characteristics and blood metabolic profile were not affected by feeding hydroponic barley sprouts. Total tract digestibility of DM and OM was increased. There has been limited scientific research or feeding studies with hydroponic feed and most industry evidence is anecdotal. This is one of the first formal studies we are aware of in conventionally fed dairy cows. More research is warranted to further develop strategies for optimal incorporation of hydroponic feeds into dairy cattle rations. As this was an initial preliminary study, diets were conservative on inclusion amount of the hydroponic feeds. It is suggested that more research is needed on the optimum inclusion rates of the test feed. Additionally, there could be negative interactions with other feeds with high water content in the rations and total water or moisture content in the ration that should be examined further. With limited performance benefits dairy producers will have to consider the increased economics of producing the barley sprouts, when considering if it should be utilized in lactation diets.

Table 3.1. Formulations of the control (CON) and hydroponic barley (HYD) diets fed during the lactation study[1]

	Diet	
Ingredients, % of DM	CON	HYD
Alfalfa Hay	28.05	28.04
Corn Silage	29.70	29.70
Ground Corn	24.50	17.60
Soybean Meal	6.31	5.21
DDGS	8.43	8.43
Hydroponic Barley Sprouts	-	8.01
Salt	0.50	0.50
Calcium Carbonate	0.60	0.60
Vitamin Premix[2]	0.09	0.09
Trace mineral Premix[3]	0.09	0.09
Magnesium Oxide	0.20	0.20
Vitamin E	0.05	0.05
Sodium Bicarbonate	0.50	0.50
Energy Booster (Rumen inert fat)	1.00	1.00

[1] Based on formulation predictions of NRC (2001) when initial analyses values for samples were entered into the program.

[2] Contained: 25.8 % Ca (DM basis) 7, 507 IU/kg Vitamin A, 1,878 IU/kg Vitamin D, and 23,457 IU/kg Vitamin E (JPW Vitamin Premix, JPW Nutrition).

[3] Contained 11.7 % Ca (DM basis), 1.96 % S, 10,527 mg/kg Fe, 63,158 mg/kg Zn, 12,632 mg/kg Cu, 63,158 mg/kg Mn, 325 mg/kg Se, 632 mg/kg Co, and 1,053 mg/kg I (JPW Vitamin Trace Mineral Mix, JPW Nutrition).

Table 3.2. As-fed formulations of the control (CON) and hydroponic barley (HYD) diets fed during the lactation study[1]

	Diet	
Ingredients, As-Fed %	CON	HYD
Alfalfa Hay	18.68	15.76
Corn Silage	54.98	46.42
Ground Corn	15.39	9.32
Soybean Meal	3.90	2.72
DDGS	5.37	4.53
Hydroponic Barley Sprouts	-	19.82
Salt	0.28	0.23
Calcium Carbonate	0.33	0.28
Vitamin Premix[2]	0.05	0.04
Trace mineral Premix[3]	0.05	0.04
Magnesium Oxide	0.11	0.09
Vitamin E	0.03	0.02
Sodium Bicarbonate	0.28	0.23
Energy Booster (Rumen inert fat)	0.55	0.47

[1] Based on formulation predictions of NRC (2001) when initial analyses values for samples were entered into the program.

[2] Contained: 25.8 % Ca (DM basis) 7, 507 IU/kg Vitamin A, 1,878 IU/kg Vitamin D, and 23,457 IU/kg Vitamin E (JPW Vitamin Premix, JPW Nutrition).

[3] Contained 11.7 % Ca (DM basis), 1.96 % S, 10,527 mg/kg Fe, 63,158 mg/kg Zn, 12,632 mg/kg Cu, 63,158 mg/kg Mn, 325 mg/kg Se, 632 mg/kg Co, and 1,053 mg/kg I (JPW Vitamin Trace Mineral Mix, JPW Nutrition).

Table 3.3. Formulated nutrient composition[1] for the control (CON) and hydroponic barley (HYD) diets during the lactation study

	Diet	
Item[2]	CON	HYD
DM, %	55.3	46.7
CP	17.1	17.2
Fat[3]	4.6	4.4
RDP	11.3	11.6
RUP	5.8	5.6
ADF	17.8	18.4
NDF	27.7	28.8
Forage NDF	22.3	24.1
NFC[4]	45.7	44.7
Ca	0.90	0.90
P	0.40	0.40
Mg	0.34	0.34
Cl	0.61	0.66
K	1.40	1.40
Na	0.40	0.40
S	0.24	0.24
ME, Mcal/Kg DM	2.51	2.49
$NE_{L,}$ Mcal/Kg DM	1.58	1.58

[1] Based on Dairy NRC (2001) when initial analyses values or program values for feeds were entered into the program.
[2] % of DM, unless otherwise indicated.
[3] Ether extract.
[4] NFC (non-fibrous carbohydrate) = 100-(NDF + CP + EE + Ash) (NRC, 2001).

Table 3.4. Nutrient composition of the forages, control and hydroponic diet grain mixes, and hydroponic barley sprouts used in treatment diets fed during the lactation study

Item[1]	Ingredients				
	Alfalfa Hay	Corn Silage	Control Grain Mix	Hydroponic Grain Mix	Hydroponic Barley Sprouts
DM, %	88.5	31.0	89.3	89.4	17.3
CP	20.9	8.4	18.4	19.7	17.4
ADF	29.1	25.4	4.2	4.7	12.3
NDF	39.0	43.9	13.2	14.3	28.0
Starch	0.2	27.2	43.2	39.3	24.0
EE[2]	1.7	3.4	5.1	5.4	2.3
Ash	10.2	4.8	6.9	8.0	2.9
NFC[3]	28.2	39.5	56.4	52.6	49.4

[1]% of DM unless otherwise indicated.

[2]Ether extract, analyzed with petroleum ether.

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

Table 3.5. Nutrient composition based on laboratory analysis for the control (CON) and hydroponic barley (HYD) treatments fed during the lactation study

Item[1]	Treatment			
	CON		HYD	
	Mean	SE	Mean	SE
DM[2], %	57.8	0.46	47.5	0.50
OM[2]	92.6	0.14	92.6	0.25
Ash[2]	7.4	0.14	7.3	0.25
CP[2]	16.5	0.03	16.6	0.01
ADF[2]	17.6	0.93	18.4	0.10
NDF[2]	29.2	1.04	30.4	0.25
EE[2,3] (Petroleum)	3.6	0.03	3.5	0.02
NFC[2,4]	44.6	0.97	43.1	0.50
RDP[5]	10.8	-	11.1	-
RUP[5]	5.7	-	5.1	-
Forage NDF[5]	24.0	-	26.2	-
Nonforage NDF[5]	5.2	-	4.2	-
Starch[2]	26.6	0.70	24.3	1.32
Ca[2]	0.76	0.045	0.70	0.010
P[2]	0.34	0.015	0.33	0.005
Mg[2]	0.38	0.020	0.36	0.010
K[2]	1.49	0.035	1.45	0.075
S[2]	0.23	0.010	0.23	0.010
Na[2]	0.40	0.015	0.40	0.025
Cl[2]	0.53	0.010	0.54	0.020
ME[5], Mcal/kg DM	2.50	-	2.50	-
NE_L[5], Mcal/kg DM	1.60	-	1.60	-

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

Table 3.6. Particle size distribution for the control (CON) and hydroponic barley (HYD) treatments fed during the lactation study

Item[1]	Treatment			
	CON		HYD	
	Mean	SE	Mean	SE
Particle Size[2] (TMR)				
>19 mm	7.0	0.68	21.4	3.32
8-19 mm	30.2	0.52	29.5	1.73
1.18-8 mm	10.3	0.21	10.5	0.52
<1.18 mm	52.5	0.79	38.3	1.56
Particle Size[2] (ORTS)				
>19 mm	9.7	0.94	26.3	1.31
8-19 mm	32.1	1.06	31.7	0.66
1.18-8 mm	10.5	0.18	10.2	0.17
<1.18 mm	47.8	1.10	31.8	1.70

[1] As-fed.

[2] Penn State Particle Separator (PSPS).

Table 3.7. Dry matter intake, milk yield and composition, feed efficiency, and body characteristics for cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks

	Treatment			*P values*		
Item	CON	HYD	SEM	Treatment	Week	Treatment ×Week
DMI, kg/d	23.0	23.0	0.54	0.14	0.02	0.24
Milk kg/d	30.6	31.5	1.60	0.70	<0.001	0.01
Fat, %	3.71	3.63	0.23	0.55	0.90	0.22
Fat, kg/d	1.10	1.07	0.06	0.50	0.97	0.14
Protein, %	3.12	3.13	0.04	0.40	0.84	0.05
Protein, kg/d	0.94	0.94	0.03	0.64	0.32	0.53
Lactose, %	4.94	4.92	0.02	0.83	0.60	0.12
Lactose, kg/d	0.94	0.94	0.03	0.64	0.32	0.53
MUN, mg/dL	12.80	12.60	0.44	0.66	0.82	0.90
SCC, 10^5/mL	28.30	11.52	6.60	0.34	0.24	0.31
FRAP[4], µmol L^{-1}	547.64	587.63	16.80	0.15	<0.01	0.35
ECM[1], kg/d	30.90	30.42	1.15	0.80	0.70	0.32
Feed Efficiency[2]	1.33	1.40	0.046	0.43	0.21	<0.01
Feed Efficiency[3]	1.37	1.35	0.057	0.55	0.43	<0.01
Body Weight, kg	690.0	680.5	3.72	0.76	0.03	0.18
Body Condition Score[5]	3.09	3.08	0.03	0.03	0.14	0.09

[1] Energy corrected milk (ECM) = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] (Orth, 1992).
[2] Feed efficiency= Milk /DMI.
[3] Feed efficiency= ECM/DMI.
[4] Ferric reducing antioxidant power (FRAP) and only conducted in weeks 4 and 6.
[5]Body condition score is on a scale of 1 to 5 with 1 being emaciated and 5 being obese (Wildman et al, 1982).

Table 3.8. Milk fatty acid composition for cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks

	Treatment			*P-value*
Item[1], mg/100 mg FA	CON	HYD	SEM	Treatment
C4:0	3.357	3.383	0.0887	0.84
C6:0	1.838	1.830	0.0753	0.95
C8:0	1.381	1.386	0.0662	0.95
C10:0	3.399	3.399	0.194	0.97
C12:0	3.760	3.749	0.2165	0.97
C12:1	0.065	0.068	0.0166	0.89
C14:0	13.477	13.564	0.3449	0.86
C14:1	1.352	1.417	0.0872	0.60
C16:0	22.691	21.661	0.3070	0.03
C16:1 *cis*	0.238	0.250	0.0203	0.66
C18:0	13.138	12.797	0.4900	0.63
C18:1 *trans* 9	0.282	0.317	0.0197	0.22
C18:1 *trans* 10	0.949	0.998	0.0530	0.52
C18:1 *trans* 11	0.503	0.558	0.0216	0.08
C18:1 *cis* 6	0.528	0.579	0.1140	0.75
C18:1 *cis* 9	23.161	23.848	0.7034	0.50
C18:1 *cis* 11	1.453	1.502	0.0936	0.71
C18:2 *cis* 9 *cis* 12	3.764	3.937	0.1556	0.44
C18:2 *cis* 9 *trans* 11 (CLA)	0.226	0.222	0.0146	0.83
C18:2 *trans* 10 *cis* 12 (CLA)	0.045	0.039	0.0081	0.65
C18:3 *gamma*	0.069	0.071	0.0092	0.93
C18:3 *alpha*	0.536	0.567	0.0254	0.39
C20:0	1.412	1.507	0.0802	0.40
Others[2]	2.386	2.331	0.0809	0.65
SCFA[3]	30.049	30.131	0.8069	0.94
LCFA[4]	69.952	69.867	0.8069	0.94
Saturated FA	66.165	64.906	0.9915	0.38
Unsaturated FA	33.835	35.094	0.9915	0.38
MUFA[5]	28.961	29.991	0.9353	0.45
PUFA[6]	4.874	5.098	0.1655	0.35

[1] Number of carbons: number of double bonds

[2] Others: sum of C5:0, C7:0, C9:0, C11:0, C11:1, C13:0, C15:0, C15:1, C16:1 *trans*, C17:0, C17:1, C18:1 trans 6 , C19:0, C18:2 trans 9 trans 12, C20:1, C20:2

[3] Short Chain Fatty Acids, <C16:0

[4] Long Chain Fatty Acids, ≥ C16:0

[5] Monounsaturated fatty acids

[6] Polyunsaturated fatty acids

Table 3.9. Rumen fermentation characteristics for cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks

	Treatment			*P value*
Item	CON	HYD	SEM	Treatment
pH	6.5	6.6	0.11	0.78
Ammonia-N, mg/dL	12.7	13.9	1.00	0.58
Total VFA, mM	95.6	95.0	1.70	0.57
VFA, mM/100mM				
Acetate	61.1	59.4	0.94	0.67
Propionate	21.6	20.6	1.01	0.45
Isobutyrate	0.9	1.0	0.09	0.10
Butyrate	9.7	10.4	0.37	0.87
Isovalerate	1.21	1.24	0.04	<0.01
Valerate	1.4	1.4	0.07	0.80
Acetate: Propionate	3.0	3.0	0.11	0.67

Table 3.10. Plasma metabolites for cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks

Item	Treatment			*P values*		
	CON	HYD	SEM	Treatment	Week	Treatment ×Week
Glucose, mg/dL	54.9	54.7	1.07	0.80	0.02	0.42
PUN[1], mg/dL	13.0	13.4	0.44	0.79	<0.01	0.25
Cholesterol, mg/dL	120.7	133.8	5.23	0.09	0.67	0.34
Triglycerides, mg/dL	14.0	13.3	1.02	0.50	0.72	0.31

[1]Plasma Urea Nitrogen

Table 3.11. Total tract digestion of nutrients for cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks

	Treatment			*P value*
Item, % digested	CON	HYD	SEM	Treatment
DM	65.0	70.8	2.08	0.07
OM	66.6	72.3	2.03	0.07
CP	63.7	68.8	2.31	0.13
NDF	39.0	47.1	3.64	0.13
ADF	39.6	47.9	3.70	0.13

Figure 3.1. Milk yield (kg) for Holstein cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks. Error bars represent SEM = 1.79.

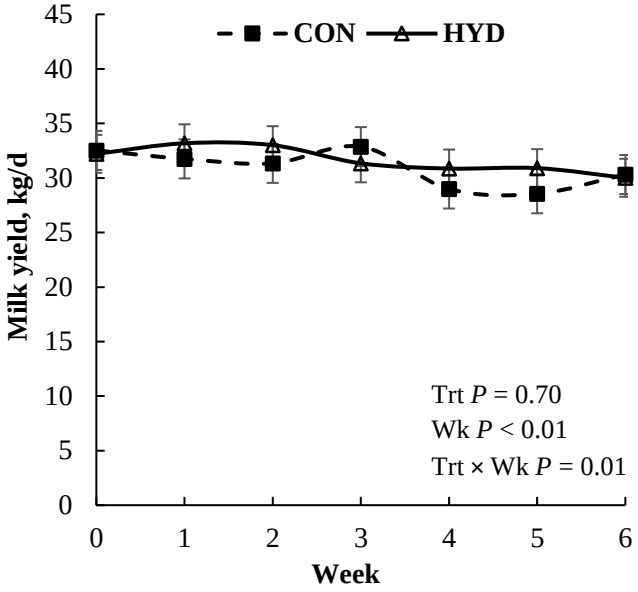


Figure 3.2. Dry matter intake (kg/d) for Holstein cows fed the control diet (CON) and hydroponic barley (HYD) diets for six weeks. Error bars represent SEM = 0.54.

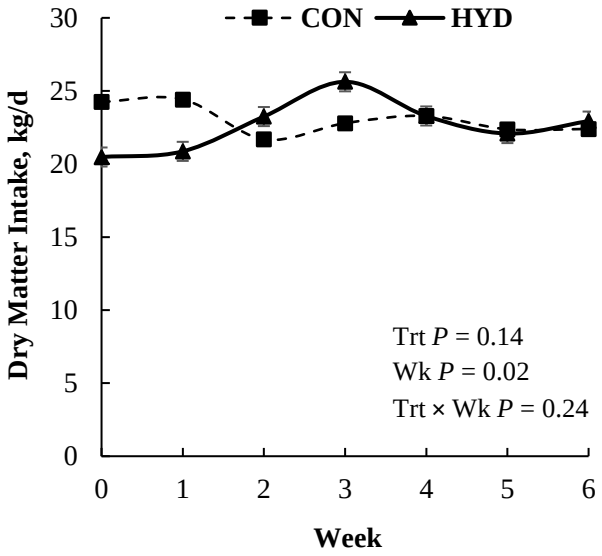


Figure 3.3. Feed efficiency (Milk/DMI) for Holstein cows fed the control diet (CON) and hydroponic barley (HYD) diet for six weeks. Error bars represent SEM = 0.05.

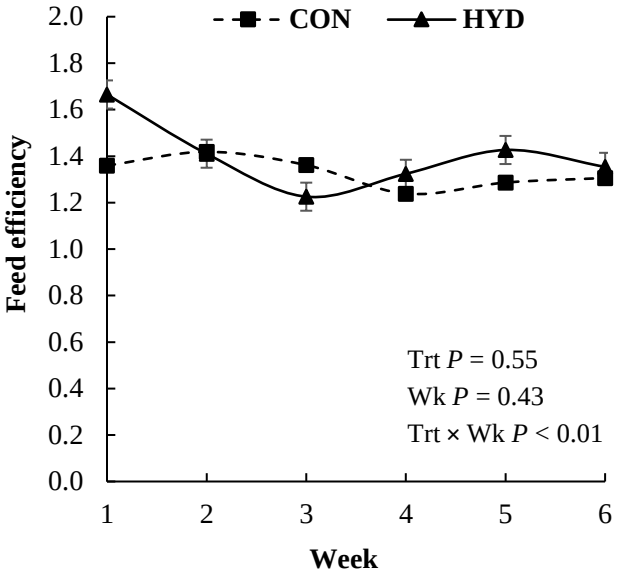
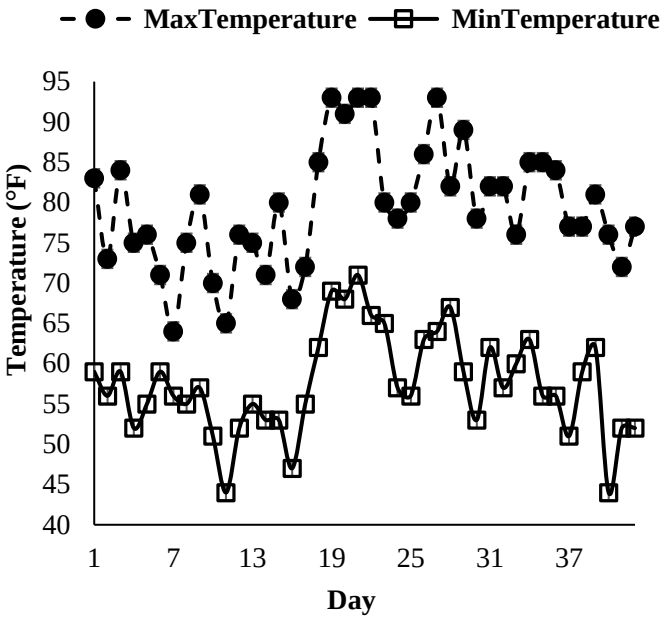


Figure 3.4. Temperature variation during the 6 wk lactation study.



CHAPTER 4:

EVALUATION OF CARINATA MEAL INCLUDED IN A TOTAL MIXED RATION FED AD LIBITUM TO DAIRY HEIFERS

Abstract

The objective of this research was to determine the effects on the growth performance, metabolic profile, rumen fermentation and nutrient utilization of dairy heifers when fed carinata meal in a total mixed ration (TMR) containing corn silage. A 12-wk randomized complete block design study was conducted using 24 Holstein heifers [242.4 ± 34 d of age; body weight (BW) 272.8 ± 45 kg]. Treatments were: 1) control (CON) a TMR with grass hay, corn silage, and soybean meal and dried distiller's grains with solubles as major concentrate ingredients and 2) a TMR with 10% (DM basis) carinata meal (CRM) replacing a portion of the soybean meal in the grain mix. Diets were fed for ad libitum intakes and formulated to be isonitrogenous and isocaloric. Rations were fed to target 10% refusal rate and intakes were measured using Calan gates. Frame sizes, BW, and body condition scores (BCS) were measured on 2 d during wk 0, 2, 4, 6, 8, 10, and 12. Heifer dry matter intake (DMI) was greater for CON compared to CRM fed heifers. Body weights, ADG, and gain: feed were not different between treatments. Frame measurements were mostly similar between treatments, with the exception of heart girth which was greater for the CON heifers and hip width which was greater for CRM. Rumen fermentation characteristics were not different, isovalerate tended to be greater for the CON treatment. Concentrations of plasma triglycerides were greater for heifers on the CRM treatment, all other blood metabolites and metabolic hormones were similar. The total tract digestion of neutral detergent fiber and acid detergent fiber was greater for

CRM fed heifers. Results indicated that replacing soybean meal with carinata meal at 10% of the ration and feeding as a TMR for ad libitum consumption maintained heifer body frame growth while decreasing DMI. Carinata meal shows potential as an alternative protein source to be included in TMR fed to dairy heifers.

Key words: carinata meal, dairy heifer, growth performance

Introduction

There is an ever-increasing global demand for vegetable-based, renewable sources of oils for food and non-food uses. A relatively novel cruciferous oilseed crop *Brassica carinata* or carinata is being introduced to the Midwest. The agronomic benefits of carinata include its ability to adapt to adverse soil conditions, in addition to this it is also drought tolerant and has a resistance to insects that commonly affect canola crops (Cardone et al., 2003). The oil content of carinata seed is mostly very-long-chain fatty acids or erucic acid (C22:1). The byproduct left after extraction of the oil is carinata meal (CRM), and it could potentially be a quality protein (30-40% CP) source for livestock.

The main concern with feeding CRM is the antinutritional compounds found in all *Brassica* species. When feeding CRM, the effects of glucosinolates on thyroid hormones (T3 and T4) pose a potential problem (Fales et al., 1987). Ruminants are more tolerant of glucosinolates; however, it is not recommended to feed meals containing glucosinolates in excess of 10% inclusion in the diet, which is currently the federal regulation (AAFCO, 2014). Previous in-situ and in-vitro research has established that solvent extracted CRM is highly digestible and comparable to soybean meal, but processing method could affect how the meal is utilized (Lawrence and Anderson, 2018; Lawrence et al., 2019). Heifers

fed 10 % cold pressed CRM in a limit-fed ration with grass hay maintained growth performance and rumen fermentation was not affected (Rodriguez-Hernandez and Anderson, 2018). Researchers found that cholesterol plasma concentrations were increased and T3 tended to be less (Rodriguez-Hernandez, 2018). In contrast, in a follow up study, research has found that feeding 10 % solvent extracted CRM in a limit fed ration with hay did not alter growth performance, rumen function, blood metabolic profile, thyroid hormone concentration, or onset of puberty (Rodriguez-Hernandez, 2018).

Thus, it was important to determine how CRM affected dairy heifers when combined in a TMR with corn silage. Especially since the TMR would contain more moisture than previous trials feeding CRM, and the glucosinolates (sinigrin) within CRM are degraded in the presence of myrosinase which is released during mastication and enzyme reactions may vary due to water and pH of the feeds (Duncan and Milne, 1993; Martinez-Ballesta and Carbajal, 2015; Peng et al., 2014). As producers typically feed a high forage TMR to growing dairy heifers it was imperative that this new feedstuff be evaluated when heifers were offered the TMR ad-libitum, instead of in limit-fed diets as used in previous research. The overall objective of this research was to determine the effects of feeding 10% solvent extracted CRM in ad libitum fed TMR to growing heifers on growth performance, metabolic profile, total tract digestion of nutrients and rumen fermentation. Based on previous research we hypothesize that feeding CRM in a TMR will maintain or improve growth performance compared to the control diet.

Materials and Methods

Experimental Design

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 17-063E. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

A 12-wk randomized complete block design study was conducted using 24 Holstein heifers (242.4 ± 34 d of age; BW 272.8 ± 45 kg) with two treatment diets. Heifers were blocked in pairs based on birth date and then randomly assigned to treatments within blocks. An adaptation period of 2 wk for training to the Calan doors was followed by experimental feeding for 12 wk. Treatments were: 1) control (**CON**) diet which was a total mixed ration (TMR) with grass hay, corn silage, and soybean meal with DDGS as major concentrate ingredients and 2) a TMR with 10% (DM basis) carinata meal (**CRM**) replacing a portion of the soybean meal. The formulated ingredients and predicted nutrient composition of treatment diets are presented in Table 4.1. Diets were fed for ad libitum intakes and formulated to be isonitrogenous and isocaloric on a DM basis.

Animal Care and Feeding

The feeding trial was conducted from August 2017 to December 2017 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). Animals were observed daily for any injury or disease problems and treated according to normal management practices at the DRTF. Heifers were housed in pens in groups of 6 heifers. Each pen had an inside roofed shelter area and an outside dirt exercise lot. The

inside areas of the pens were manure pack bedded with straw, bedding was done at a minimum interval of every 2 wk to avoid consumption of straw.

Prior to feeding the individual TMR, components including ground hay, corn silage, and treatment grain mixes were individually weighed for each heifer into a large tub and hand mixed thoroughly. Fresh water was provided ad libitum. Feeding occurred once daily at approximately 0900 h using the Calan gate feeding system (American Calan, Inc., Northwood, NH) so that individual intakes could be measured. Approximately every 2 wk throughout the study, bales of hay were coarsely pre-ground with a large vertical tub grinder (Haybuster 1130, DuraTech Industries International Inc., Jamestown, ND) to facilitate hand mixing. Refusals were weighed and recorded in the morning prior to feeding, to determine daily intakes and adjusted for 5-10% refusal rate.

Animal Measurements and Sampling

Each wk samples of the feed ingredients were taken and stored at -20°C until processing and analysis could be completed as described under laboratory analysis.

On 2 consecutive days at 4 h post-feeding at the beginning of the study and then every 2 wk during the study, body growth measurements including BW, withers height, hip height, heart girth, paunch girth and body length were recorded. Body length measurements were taken from the top point of the withers to the end of the ischium. Body condition scores (BCS) were recorded every 2 wk, by three independent observers based on a quarter point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). For analysis of glucose, plasma urea nitrogen (PUN), cholesterol, triglycerides, and thyroid hormone (T3 and T4) concentrations; blood samples were taken at the

beginning of the study and then every 4 wk throughout on the same days as body measurements.

Blood samples were taken approximately 3.5 hours post feeding (1230 h), while heifers were restrained in a cattle chute, via venipuncture of the jugular vein 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). Immediately after blood collection, samples were placed in ice and then brought in to the laboratory within 3 h for processing and storage. Blood collection tubes were centrifuged at 1000 × g for 20 minutes at 4°C (Centrifuge: CR412 Jouan, Inc., Winchester, VA.). Plasma (K_2EDTA tubes) or serum (NaFl tubes) was then transferred using a plastic pipette into polystyrene storage tubes and frozen at -20°C until analysis could be completed.

Rumen fluid was collected during wk 0, 4, 8, and 12 on 2 consecutive d after blood sampling via an esophageal tube while heifers were still restrained in the cattle chute. The beginning stream of rumen fluid was discarded, to try and minimize saliva contamination. In total 50 mL of rumen fluid was collected into a stainless steel cup. The pH of the sample was analyzed and recorded immediately (Waterproof pH Tester 30, Oakton Instruments, Vernon Hills, Il.). A 10 mL aliquot was mixed with 2 ml of 25% (w/v) meta-phosphoric acid for determination of VFA concentrations, and a 10 mL aliquot was mixed with 200 µl of 50% (v/v) sulfuric acid to determine rumen ammonia nitrogen (NH_3-N). The two samples from both sampling days were then frozen at -20° C until analysis. During wk 12 of the feeding period samples for analysis of total tract digestibility of nutrients were collected. The internal marker used was acid detergent

insoluble ash (ADIA). Orts and fecal grab samples were collected over 3 d. Fecal grab sampling was scheduled so that samples would ultimately represent every 3 h over the 24 h period relative to time of feeding. Orts and fecal samples were stored at -20°C until processing and analysis could be completed.

Laboratory Analysis

To determine DM content, feed samples were dried for 24 h at 105°C every 2 wk, to check ingredient inclusion rates in the ration and determine DMI. For processing, feeds were thawed and samples from 4 consecutive wk were composited on an as-fed basis by volume. Composite samples and concentrate mix ingredients were dried in duplicate for 48 h at 55°C in a Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN). Composites of the forage were ground to a 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). Ground forages and the concentrates were reground to a 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). In order to correct analysis to 100% DM, 1 g aliquot of sample was dried for 4 h in a 105°C oven (AOAC 17th ed., method 935.29). The ash content was analyzed by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC 17th ed., method 942.05). Organic matter (OM) was then calculated as OM = (100 - % Ash). All samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC 17th ed, method 968.06), on a Rapid N cube (Elementar Analysen Systeme, GmbH, Hanau Germany). The resulting nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). Heat-stable alpha-amylase and sodium sulfite were

used for the NDF. Before samples were analyzed for NDF they were pre-soaked in acetone if the fat concentration was greater than 5% or if they contained soy products according to procedure recommendations. Ether extracts (EE) were analyzed using petroleum ether (AOAC 17th ed., method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Non-fibrous carbohydrates were calculated as % NFC= 100 - (% Ash + % CP + %NDF + % EE) as described by the NRC (2001). Monthly feed composites were made into larger 3-month composites, these were then sent to a commercial lab for mineral 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, 1998).

Glucosinolate analysis and quantitation was performed under the supervision of Mark Berhow at the USDA Agricultural Research Station (Peoria, IL). Analysis methods performed on the carinata meal were similar to those described by Berhow et al. (2013). Quantitation was completed using a modified method for HPLC developed by Betz and Fox (1994). The preparation of sinigrin standards (Sigma-Aldrich Co., St. Louis, MO) was done on a molar concentration basis to determine standard curve and lower detection limits. Dried ground samples were extracted with methanol and analyzed using liquid chromatic mass spectrometry to evaluate glucosinolate composition and reversed-phase HPLC at 237 nm was used to determine concentrations of individual glucosinolates.

For analysis of rumen fluid, it was first thawed and vortexed to completely mix contents before pipetting 2 ml into a microcentrifuge tube to be centrifuged at 10,000 × g for 20 min in a micro centrifuge (Model A-14, Jouan, Jouan Inc, P. O. Box 2176, Vinchester, VA, U.S.A). Ammonia-N concentration was analyzed using the assay

described by Chaney and Marbach (1962). Volatile fatty acid concentrations were measured using an automated gas chromatograph (Model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d × 15m column (Nukol, 17926 to 01C, Supelco, Inc., Bellefonte, PA) with 2-ethylbutyrate used as an internal standard. The flow rate was 1.3 ml/min of Helium and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Metabolites (glucose, cholesterol, triglyceride and PUN) were analyzed with commercially available enzymatic or colormetric assay kits on a micro-plate spectrophotometer (Cary 50, Varian Inc.,Walnut Creek, CA.). Serum glucose was analyzed using glucose oxidase reagent as described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI) as described by Allain et al. (1974). Plasma urea nitrogen was analyzed using diacteylymonoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Triglyceride concentration was determined colorimetrically using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) and Trinder (1969).

Thyroid hormone concentrations, total T3 and total T4 were analyzed in duplicate according to the manufacturer's protocol using solid phase RIA and Coat-A-Count kits (MP Biomedicals, Orangeburg, NY). The sensitivity, intra- and interassay coefficients of variation were respectively, 2.95 ng/dL, 8.7% and 4.1% for T3, and 1.74 μg/dL, 14.8 and 5.9% for T4.

For digestibility analysis fecal and orts samples were composited on an as-is basis by volume for each heifer. Samples were processed (dried and ground) as described for the monthly feed composites. Fecal and orts samples were also analyzed for DM, Ash, NDF and ADF as previously described for feeds. Acid detergent insoluble ash (ADIA) analyses was conducted on all feed composites, fecal samples, and any orts. The method for ADIA analysis consists of analyzing the sample for ADF digestion (Robertson and Van Soest, 1981) and then determining the ash percentage using a modified procedure of the AOAC 17th ed., method 935.29. Digestibility calculations were performed according to Merchen (1988).

Statistical Analysis

Feed nutrient means and standard errors were calculated using the MEANS procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The total dietary nutrient values were calculated based on analysis of concentrate mixes and hay for each treatment.

The initial (wk 0) body measurements, blood metabolites, rumen parameters, and thyroid hormones were analyzed separately from the rest of the data set in MIXED procedures of SAS. Because it was a single time point, the model included only treatment and heifer (block) included as a random variable. The wk 0 values of each body measurement or blood metabolite were then used as covariate terms for their respective parameter and the least square means reported.

Changes over time for the growth parameters were calculated for each 2 wk interval during the feeding period. Gain-to-feed ratio was calculated as the ratio of ADG to DMI for each treatment. Changes for BW, ADG, gain: feed, intakes, frame growth

measurements, blood metabolites and hormones, and rumen fermentation parameters were analyzed as a randomized complete block design with repeated measures using the MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, and treatment × week interaction. As mentioned, wk 0 values were used as covariates for their respective parameter within the model. Repeated measures were by week using heifer(block) as the subject. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry was chosen as the covariance structure due to having the least absolute Akaike's values. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Least square means are reported for each treatment in the tables and were compared using Tukey's test.

The MIXED procedures of SAS were also used for analysis of data for the total-tract digestibility of nutrients. As it was a single time point, the model included only treatment with heifer (block) as a random variable. Least square means are again reported for each treatment in the tables and means were compared using Tukey's test.

Results and Discussion

Feed Composition

The nutrient composition based on laboratory analysis of the forages and concentrate mixes used in the treatment diets is presented in Table 4.2. In Table 4.3 the nutrient composition of the CON and CRM TMR fed during the 12 wk feeding period is presented. Crude protein of the treatment diets fed, 17.1 % (SE = 1.13), and 16.7 % (SE =

1.21), for CON and CRM, respectively were slightly greater than the diet formulation. The ideal amount of CP is in excess of 13% to achieve maximum microbial fermentation (Tamminga, 1992). The CP recommendation was supported by Gabler and Heinrichs (2003b), who fed Holstein heifers between 153 and 196 kg of BW diets containing 11.9 to 20.1% CP at 2.0% of BW. A better synergistic relationship between dietary protein and energy was found at concentrations of 16.7 % CP and 2.6 Mcal of ME/kg of DM, as in the CRM diet. Heifers in the current study are similar in BW to those used by Gabler and Heinrichs (2003b), at the beginning of the trial; therefore, heifers on our study may have benefited from a greater amount of CP fed. Fiber in terms of NDF was formulated to be slightly greater than values found through laboratory analysis, and ADF was slightly less in actual treatment diets fed. The EE was also less than the predicted nutrient composition, but energy content including ME and Neg (Mcal/kg) were consistent with diet formulations and did not differ between treatments.

Heifer Growth Performance

Over the course of the study, one heifer was dropped for reasons unrelated to treatments. The dropped heifer had difficulty adapting to the Calan door feeding system. Without any replacement heifers of similar size and age available, the CRM treatment had a total of 11 heifers and the CON treatment had a total of 12 heifers.

Heifer DMI, ADG, BW, and gain:feed is presented in Table 4.4. Dry matter intake was found to be greater ($P < 0.01$) for the CON fed heifers (Figure 4.1). A treatment × week interaction was found for DMI, during wk 5 and for the remainder of the feeding period CRM fed heifers had a decreased DMI compared to CON. This is in agreement with the treatment × week interaction reported in previous research that fed 10

% cold press CRM in a limit-fed diet with hay to dairy heifers (Rodriguez-Hernandez and Anderson, 2018). In contrast, limit-fed heifers consuming 10 % solvent extracted CRM similar to the current study, did not elicit a treatment or treatment × week interaction when compared to diets containing 10 % canola meal or a control diet containing DDGS (Rodriguez-Hernandez, 2018). The DMI of heifers fed 10 % CRM in previous research is less than that of the current study and this is due to the limit-feeding strategy implemented (Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). For beef steers fed 1.39 kg/d solvent extracted carinata meal with ad libitum hay, intake was approximately 6.83 kg/d which was also less than the 8.4 kg/d DMI in the current study for CRM fed heifers (Schulmeister et al., 2019). As a brassica crop carinata meal has the potential for tasting bitter due to hydrolysis of the glucosinolate sinigrin into its degradation products isothiocyanate, allyl cyanide, and allyl thiocyanate (Marillia et al., 2014; Tripathi and Mishra, 2007). Researchers found a DMI more similar to the current study at 7.35 to 7.61 kg/d when feeding carinata meal in a TMR to recently weaned calves (Guidotti, 2018). Due to the initial age of the heifers on trial and the feeding of an ad-libitum TMR, we contribute the greater DMI compared to current literature to a more palatable diet containing corn silage and the difference in nutrient requirements of dairy versus beef heifers.

Average daily gain, BW, and gain: feed were similar ($P > 0.05$) between treatments. The decreased DMI of CRM fed heifers did not affect overall gain or growth. There was a week effect for all growth parameters due to heifers growing adequately during the feeding trial. Although diets were formulated for 0.8 kg/d ADG, both treatments had increased ADG of 1.1, and 1.0 kg/d (SE = 0.05); for CON and CRM,

respectively. The ADG increase compared to NRC formulations was also exhibited in other studies examining 10 % CRM (Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). This is not attributed to CRM, as it is more likely that the NRC (2001) overestimates heifer energy requirements or underestimates the energy provided by DDGS which was used at similar inclusion rates in both treatment diets (Anderson et al., 2015; Manthey et al., 2016; Rodriguez-Hernandez, 2018). The ADG found in the current study is in agreement with that of previous research focused on feeding CRM to dairy heifers (Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). In contrast, beef heifers fed CRM at 0.3 % of BW (as-fed) had a greater ADG than the control treatment not receiving supplementation (Schulmeister et al., 2016).

Frame measurements were comparable to other studies conducted at the SDSU DRTF for growing Holstein heifers (Manthey et al., 2016; Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). Most skeletal measurements (Table 4.5) were also similar between treatments, except heart girth which was found to be greater ($P < 0.01$) for CON. Rodriguez-Hernandez (2018) also found decreased heart girth for CRM fed heifers, in both the current and past studies the difference is numerically small and not biologically significant. In addition, CRM heifers had greater ($P < 0.01$) hip width, however, averages are similar to previous studies (Rodriguez-Hernandez, 2018).

Rumen Fermentation Characteristics

The total VFA, pH, and ammonia-N concentrations were similar ($P > 0.05$) between treatments (Table 4.6). Most VFA concentrations were similar between treatments. Total VFA concentration in the current study agrees with Rodriguez-Hernandez (2018) when heifers were fed 10 % solvent extracted CRM. However, VFA

concentration was greater in the study conducted by Schulmeister (2019) that evaluated beef steers fed carinata meal, cottonseed meal or DDGS as protein supplements. Similarly, the total VFA was greater in a study feeding CRM with or without wheat DDGS to beef heifers in comparison to canola meal (Guidotti, 2018). The pH measurement was similar to other studies that utilized esophageal tubing to obtain rumen samples and the greater pH (range: 6.8-7.0) could potentially be due to saliva contamination (Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). The concentration of ammonia-N in rumen fluid (17.1, and 16.4 mg/dL; SE = 0.70; for CON and CRM, respectively was similar to those reported by Rodriguez-Hernandez and Anderson (2018), and in excess of the amount needed for efficient utilization of nitrogen (5 mg/dL; Satter and Roffler, 1974). An increased concentration of ammonia-N was also exhibited when heifers were fed a diet increasing in CP (16 %), which is similar to the current study diet CP % (Gabler and Heinrichs, 2003a). When protein degradation exceeds the microbial capacity to use available N, ammonia will accumulate in the rumen (NRC, 2001). This excess ammonia-N could be absorbed through the rumen epithelium and transferred to the liver where it may be used or excreted (Gabler and Heinrichs, 2003a).

The proportion of rumen acetate was not different between treatments. This does not agree with the increase in acetate for CRM fed heifers, which was found by Rodriguez-Hernandez and Anderson (2018); however, as in the current study the ratio of A: P was similar. According to Schulmeister et al. (2019), researchers found decreased proportion of propionate and an increase in A: P for CRM fed steers. Previous research (Rodriguez-Hernandez and Anderson, 2018; Schulmeister et al., 2019) found that when

CRM was fed a tendency for a lesser concentration of butyrate compared to the control diets occurred. This response in VFA proportions was not found when heifers were limit-fed 10 % solvent extracted CRM as in the current study (Rodriguez-Hernandez, 2018). Isovalerate tended to be greater ($P = 0.07$) for the CON treatment, a wk interaction was also found for this minor VFA. Although significant the slight shift in VFA profile did not alter overall rumen total VFA concentration. In addition, results of rumen samples should be considered with caution due to the method of sampling which is not ideal to accurately evaluate rumen fermentation characteristics. Since this trial was the first to evaluate 10 % CRM in a TMR fed ad libitum to dairy heifers, we believe the rumen sampling was necessary and warranted, to obtain preliminary data concerning rumen fermentation.

Metabolites and Metabolic Hormones

The plasma metabolite and metabolic hormone concentrations are presented in Table 4.7; results presented were similar to values reported for heifers limit-fed 10 % CRM (Rodriguez-Hernandez, 2018; Rodriguez-Hernandez and Anderson, 2018). Concentrations of most plasma metabolites and metabolic hormones were similar ($P > 0.05$) between treatments. Treatment by wk interactions were not found for any of the metabolites or metabolic hormones measured. There was a wk effect for all blood metabolite concentrations and concentration of T3, this is due to the normal growth of heifers over the course of the study. For heifers fed CRM concentration of plasma triglycerides tended to be greater ($P = 0.09$) than CON. This finding was unexpected as both treatment diets contained similar EE content (2.3, and 2.2 %; SE = 0.10; for CON and CRM, respectively). As heifers in the CRM treatment consumed less DMI,

potentially more production and storage of triglycerides was occurring. The same response in triglycerides concentration was not found by Rodriguez-Hernandez (2018), heifers had similar concentrations even though the actual intake of fatty acids was increased by 8.6% for the CRM treatment.

The PUN concentration (20.3, and 19.7 mg/dL; SE = 0.52; for CON and CRM, respectively) in the current study is very similar to values reported by Rodriguez-Hernandez (2018). This is somewhat expected due to CP % being similar among the feeding trials, with the exception of Rodriguez-Hernandez and Anderson (2018) in which diet CP was only 15.3-15.5 %. Although Rodriguez-Hernandez and Anderson (2018) fed less CP, the response in ammonia-N and PUN was similar to the current study. In comparison to the 10 % solvent extracted CRM limit-fed study, most plasma metabolites and metabolic hormones were similar with the exception of cholesterol which was less in the current study than previous research (Rodriguez-Hernandez, 2018). In contrast, the PUN values reported are much greater than those identified by Schulmeister et al. (2019). The ammonia-N concentration in combination with the PUN are found to be highly correlated and indicative of the energy to protein ratio in healthy cattle and as the ammonia-N concentration was increased in both treatments the increased PUN was expected (Hammond, 1996).

Previous research has found alternations in the thyroid hormone (T3 and T4) concentration when heifers or cows were fed cold pressed CRM (Rodriguez-Hernandez, 2018; Rosenthal, 2018). In this study, plasma concentrations of T3 and T4 were similar ($P > 0.05$) between treatments. Values reported in the current research were similar to those reported by Rodriguez-Hernandez (2018) in studies limit-feeding 10 % solvent

extracted CRM with grass hay to dairy heifers. As mentioned previously there was a wk effect on concentration of T3, but the response was not found for T4 indicating there were no metabolic challenges for heifers in the current study which agrees with previous research (Rodriguez-Hernandez, 2018). The glucosinolates in CRM and other Brassica species could potentially alter thyroid function and thus affect the concentration of thyroid hormones (Tripathi and Mishra, 2007; Waraich et al., 2013). The T3 and T4 concentration in the current study were well above the concentrations reported for hypothyroid beef heifers, indicating that thyroid function was not affected (Thrift et al., 1999). Feed analysis shows that CRM contains mostly sinigrin; however, the specific effects of the glucosinolate on thyroid function are not known, due to the variable results presented in previous studies (Berhow et al., 2013; Rodriguez-Hernandez, 2018; Rosenthal, 2018). Overall, heifers fed 10 % solvent extracted CRM in an ad-libitum TMR with corn silage did not experience negative effects on T3 and T4 concentrations.

Apparent Total Tract Digestion of Nutrients

The total tract digestibilities of nutrients are presented in Table 4.8. The digestibilities of DM and OM were similar between treatments. The CRM fed heifers had greater ($P < 0.05$) NDF and ADF digestion. The increased fiber digestion could be attributed to the decreased DMI of the CRM heifers, that could have potentially increased retention time in the rumen and improved fiber utilization (Loerch, 1990; Greter et al., 2008). Potentially the slight increase in NDF and ADF content (Table 4.3) of the CRM diet may also have contributed to the increased nutrient digestibility. This disagrees with Rodriguez-Hernandez and Anderson (2018), that research found heifers fed CRM had slightly lesser digestion of DM, OM, NDF, and ADF compared to the DDGS treatment.

Whereas, in studies feeding solvent extracted CRM no differences were found in nutrient digestion (Guidotti, 2018; Rodriguez-Hernandez, 2018). The greater digestion values found in previous studies with heifers of the same age is likely due to the limit-feeding strategy that was implemented (Lawrence et al., 2016; Manthey et al., 2016; Rodriguez-Hernandez, 2018). The variation in forage quality and the inclusion of corn silage in the current study compared to past research could also have played a role in nutrient digestibility values. Overall, the differences in total tract digestibility did not affect the growth performance or gain: feed of the heifers.

Conclusion

Replacing soybean meal with CRM at 10 % of the diet and feeding as a TMR for ad libitum consumption maintained heifer body frame growth while decreasing DMI. In partial agreement with our hypothesis, feeding CRM at 10 % of the diet DM in a TMR with corn silage did not affect heifer performance. Fiber digestion was improved by feeding CRM. Although there was a tendency for increased plasma triglycerides concentration for the CRM treatment, this did not affect overall BW gain or BCS, thus, heifers were not experiencing over conditioning. Results indicate that CRM is a viable option as an alternative protein source in dairy heifer diets and shows potential as a feedstuff to be included in TMR fed to dairy heifers. Proposed future research should include determining the effects of CRM in greater inclusions in heifer diets to find the optimal inclusion in dairy heifer feeding programs and evaluating CRM effects on lactating cow performance.

Table 4.1. Ingredient composition and predicted[1] nutrient composition of the control (CON) and 10% carinata meal (CRM) TMR for 12 weeks

	Diet	
Ingredients, % of DM	CON	CRM
Grass Hay	55.0	55.0
Corn Silage	15.5	15.5
Soybean Meal	13.5	4.5
DDGS	13.5	12.5
Carinata Meal	-	10.0
Mineral and Vitamin premix[2]	1.5	1.5
Salt	0.5	0.5
Limestone	0.5	0.5
Nutrients, % of DM[3]		
DM, % of diet	66.1	43.7
CP	16.0	16.0
RDP	9.3	9.5
RUP	6.7	6.4
NDF	45.5	46.2
ADF	29.6	29.9
EE	3.3	3.2
NFC	29.9	29.9
ME, Mcal/kg DM	2.4	2.4
NEg, Mcal/kg DM	0.9	0.9

[1] Based on formulation predictions of NRC (2001) when initial analyses values for samples were entered into the program.
[2] Contained: 3.2 g/kg of lasolocid sodium, 18.9% Ca, 24.3% NaCl, 1.6% Mg, 0.5% K, 3,880 mg/kg Zn, 880 mg/kg Cu, 50 mg/kg I, 25 mg/kg Se, 550,000 IU/kg Vitamin A, 110,000 IU/kg Vitamin D3, and 4180 IU/Kg Vitamin E (HeiferSmart No Phos B2909 Medicated, Purina Animal Nutrition, LLC., Shoreview, MN).
[3] % of DM, unless otherwise indicated.

Table 4.2 Nutrient composition of forages and concentrate components used in the control (CON) and 10% carinata meal (CRM) experimental diets for 12 weeks

Item[1]	Ingredients			
	Grass Hay	Corn Silage	CON Grain Mix	CRM Grain Mix
DM[2], %	86.5	36.0	90.5	91.3
CP[2]	9.1	7.2	37.2	36.0
NDF[2]	66.5	37.9	18.4	23.2
ADF[2]	36.8	20.5	7.0	8.8
EE[2]	1.3	2.7	4.0	3.8
Ash[2]	8.0	4.4	13.4	14.1
OM[2]	91.9	95.5	86.6	86.0
NFC[2,3]	15.0	47.7	27.0	23.0

[1]% of DM unless otherwise indicated.

[2] Results from analysis of monthly composites.

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

Table 4.3. Nutrient composition based on laboratory analysis for the control (CON) and carinata meal (CRM) diets fed to growing dairy heifers for 12 weeks

Item[1]	Treatment			
	CON		CRM	
	Mean	SE	Mean	SE
DM[2], %	71.7	2.60	71.9	2.60
CP[2]	17.1	1.13	16.7	1.21
NDF[2]	47.9	0.23	49.3	0.14
ADF[2]	25.5	0.31	26.0	0.30
EE[2,3] (Petroleum)	2.3	0.10	2.2	0.10
RDP[4]	11.8	-	10.3	-
RUP[4]	5.7	-	7.0	-
Forage NDF[4]	43.3	-	42.4	-
Nonforage NDF[4]	4.6	-	7.0	-
NFC[5]	23.6	1.34	22.4	4.44
Ca[6]	0.70	-	0.80	-
P[6]	0.31	-	0.40	-
Mg[6]	0.21	-	0.24	-
K[6]	1.60	-	1.50	-
S[6]	0.22	-	0.32	-
Glucosinolates[6,7], mg/g	-	-	0.40	-
ME[4], Mcal/kg DM	2.43	-	2.40	-
NE_g[4], Mcal/kg DM	0.94	-	0.91	-

[1] % DM, unless otherwise indicated.
[2] Results from analysis of monthly composites.
[3] Ether extract, analyzed with petroleum ether.
[4] Based on formulation predictions of NRC (2001) when analyses values for samples were entered into the program.
[5] % NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[6] Results from analysis of TMR study composites.
[7] Value of carinata meal from glucosinolate analysis; treatment values were calculated from glucosinolate analysis and inclusion rate of 10 % (DM basis) of the test feed in the diet (DDGS and SBM used in CON do not contain glucosinolates).

Table 4.4. Dry matter intakes, BW, and gain: feed ratios for dairy heifers fed the control (CON) and 10% carinata meal (CRM) TMR for 12 weeks

	Treatment			*P values*		
Item	CON	CRM	SEM	Treatment	Week	Treatment ×Week
Age, initial, d	242.0 ± 31.0	239.4 ± 37.3				
BW, kg						
Mean	327.1	327.0	2.53	0.95	<0.01	0.28
Initial	272.1	274.8	4.13	0.90		
Final	365.4	362.1	2.90	0.12		
ADG[1], kg/d	1.1	1.0	0.05	0.48	0.04	0.34
DMI, kg	9.2	8.4	0.25	<0.01	<0.01	<0.01
Gain: Feed	0.12	0.13	0.01	0.15	<0.01	0.20

[1] Calculated based on BW change per 2-wk intervals.

Table 4.5. Frame size measurements dairy heifers fed the control (CON) and 10% carinata meal (CRM) TMR for 12 weeks

Item	Treatment			*P values*		
	CON	CRM	SEM	Treatment	Week	Treatment ×Week
Withers Height, cm						
Mean	127.9	128.1	0.28	0.30	<0.01	0.93
Initial	122.6	122.1	1.31	0.77		
Final	131.4	132.0	0.40	0.19		
Change[1], cm/d	0.11	0.11	0.01	0.72	0.35	0.83
Hip Height, cm						
Mean	131.1	131.1	0.31	0.90	<0.01	0.99
Initial	125.9	125.8	1.38	0.97		
Final	134.5	134.5	0.50	0.90		
Change[1], cm/d	0.10	0.10	0.01	0.97	<0.01	0.94
Body Length, cm						
Mean	119.5	120.0	0.14	0.47	<0.01	0.48
Initial	112.4	111.8	1.64	0.78		
Final	126.6	125.9	0.73	0.38		
Change[1], cm/d	0.17	0.16	0.02	0.79	<0.01	0.21
Heart Girth, cm						
Mean	153.3	151.9	0.40	<0.01	<0.01	0.67
Initial	142.3	143.0	2.59	0.86		
Final	161.5	159.2	0.73	0.01		
Change[1], cm/d	0.22	0.20	0.02	0.21	0.07	0.12
Paunch Girth, cm						
Mean	186.6	187.8	1.90	0.18	<0.01	0.97
Initial	175.1	172.9	3.35	0.64		
Final	192.3	193.1	2.33	0.70		
Change[1], cm/d	0.20	0.24	0.05	0.60	0.17	0.45
Hip Width, cm						
Mean	40.0	40.4	0.24	<0.01	<0.01	0.88
Initial	36.4	36.0	0.97	0.79		
Final	42.1	42.5	0.30	0.24		
Change[1], cm/d	0.07	0.07	0.007	0.70	0.01	0.43
BCS						
Mean	2.99	2.97	0.022	0.59	<0.01	0.44
Initial	2.95	2.95	0.016	0.84		
Final	2.95	2.97	0.041	0.71		
Change[1], cm/d	-0.0003	0.0001	0.00138	0.82	0.03	0.34

[1] Calculated based on change per 2-wk intervals.

Table 4.6. Rumen fermentation characteristics for dairy heifers fed the control diet (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P values*		
Item	CON	CRM	SEM	Treatment	Week	Treatment ×Week
pH	7.0	7.0	0.04	0.86	0.65	0.70
Ammonia-N, mg/dL	17.1	16.4	0.70	0.46	0.01	0.80
Total VFA, mM	94.0	95.0	3.10	0.83	0.11	0.30
VFA, mM/100mM						
Acetate	60.7	61.2	0.43	0.40	0.15	0.58
Propionate	24.4	24.4	0.51	0.99	0.98	0.58
Butyrate	11.2	10.8	0.23	0.19	0.07	0.10
Isovalerate	2.0	1.7	0.08	0.07	<0.01	0.46
Valerate	1.7	1.7	0.07	0.70	0.11	0.32
Acetate: Propionate	2.5	2.5	0.06	0.88	0.98	0.81

Table 4.7. Plasma metabolite and metabolic hormone concentrations for dairy heifers the control diet (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P values*		
Item	CON	CRM	SEM	Treatment	Week	Treatment ×Week
Glucose, mg/dL	81.8	83.3	0.78	0.18	<0.01	0.41
PUN[1], mg/dL	20.3	19.7	0.62	0.52	<0.01	0.62
Cholesterol, mg/dL	69.5	70.6	2.18	0.73	<0.01	0.64
Triglycerides, mg/dL	20.5	22.2	0.72	0.09	<0.01	0.68
Triiodothyronine, ng/dL	128.4	130.0	4.70	0.83	<0.01	0.99
Thyroxin, µg/dL	3.0	3.2	0.23	0.65	0.22	0.15

[1]Plasma Urea Nitrogen

Table 4.8. Total tract digestion of nutrients for dairy heifers the control diet (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P value*
Item, % digested	CON	CRM	SEM	Treatment
DM	66.2	67.3	0.78	0.17
OM	69.0	70.0	0.76	0.28
NDF	57.0	60.0	1.01	0.01
ADF	54.0	56.4	1.04	0.04

Figure 4.1. Dry matter intake (DMI) of dairy heifers fed a control diet (CON) or a diet containing 10% (DM basis) carinata meal (CRM). Error bars represent SEM = 0.39. * Indicates values differ by $P < 0.05$ with Tukey's test.

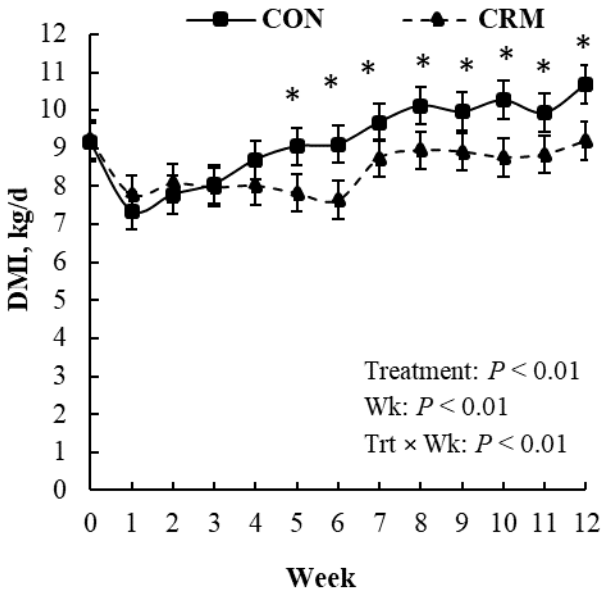
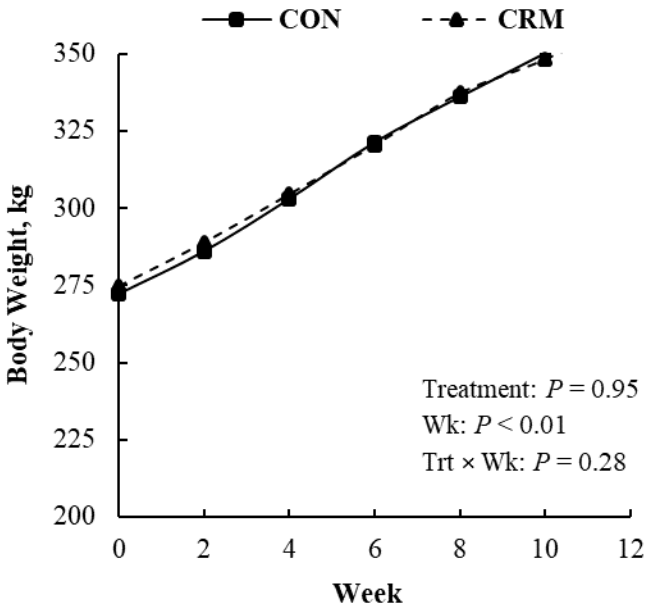


Figure 4.2. Body weight (kg) of dairy heifers fed a control diet (CON) or a diet containing 10% (DM basis) carinata meal (CRM). Error bars represent SEM = 2.53.



CHAPTER 5:

EVALUATION OF THE EFFECTS OF FEEDING CARINATA MEAL TO DAIRY COWS ON LACTATION PERFORMANCE, NUTRIENT UTILIZATION, AND METABOLIC PROFILE

Abstract

The objective of this study was to determine the effects of feeding solvent-extracted carinata meal, as a new potential protein source, to lactating cows on milk production, milk composition, rumen fermentation, metabolic profile and total tract digestibility of nutrients. Twenty Holstein cows (12 primiparous and 8 multiparous) at 83.3 ± 0.05 DIM were used in a 12-wk randomized complete block design study. Treatments included: 1) control diet with 10 % (DM basis) canola meal (**CON**) and 2) 10 % carinata meal (**CRM**). Both diets were fed as TMR using Calan gates. Cows were milked 2×/d. Body condition scores (BCS) and body weights (BW) were measured on 2 d during wk 0 and every 2 wk at approximately 4 hours post feeding, blood sample collection occurred at the same time from the coccygeal vein for analysis of metabolites related to energy partitioning and protein utilization. Milk samples were taken at each milking on the same days, in wk 12 extra milk samples were collected for milk fatty acid analysis. Immediately following blood sampling at the beginning of the trial and every 4 wk rumen fluid was collected via esophageal tube. Fecal grab samples were collected in wk 12 for total tract digestion of nutrients. In addition, blood was collected from the caudal superficial epigastric vein in wk 12, at the same time as coccygeal vein sampling to determine arteriovenous difference in amino acid composition. Dry matter intakes, BW and BCS were similar between treatments. Milk production, milk protein, milk fat yield,

and lactose yield were similar between treatments but had treatment by wk interactions. The similar milk production and DMI led to no difference in feed efficiency between treatments. Rumen fermentation characteristics were similar between treatments, with the exception of valerate concentration which tended to be greater for CRM. All blood metabolite and thyroid hormone concentrations were not different. The arterial plasma concentrations of amino acids also did not differ. Tryptophan arteriovenous difference tended to be greater for the CON treatment. Apparent total tract digestion of DM, organic matter, neutral detergent fiber, and acid detergent fiber were greater for the CRM cows. Overall results indicate that 10 % CRM can be fed to lactating dairy cows without affecting milk production and composition. Rumen fermentation and metabolic profile was not affected when CRM is fed. Based on this study, carinata meal is a high quality protein source for dairy cows and may be used in replacement of canola meal to maintain lactation performance.

Key words: carinata meal, dairy cow, milk production

Introduction

The *Brassica* family of cruciferous plants contains common food crops such as cauliflower, cabbage, kale, mustards, radish, turnips, brussel sprouts, rapeseed and canola (Moser, 2010; Waraich et al., 2013). As well as more underutilized crops such as *B. carinata* which is not grown for human consumption (Cardone et al., 2003). The common names for *B. carinata* include carinata and Ethiopian mustard. These nonfood oilseeds represent a very small percentage of the Brassica species grown worldwide, the major oilseed is *B. napus* or rapeseed and its cultivars such as canola meal (Velasco and

Fernandez-Martinez, 2009; Milazzo et al., 2013). Solvent extraction using hexane is very efficient, and the resulting meal contains less oil than extraction using a press (Goss, 1947).

A potentially negative aspect of Brassica species is that they contain anti-nutritional factors such as glucosinolates, erucic acid, phytic acid, and tannins (Fales et al., 1987; Putnam et al., 1993; Colombini et al., 2014). Carinata commonly contains only one type of glucosinolate, in the form of sinigrin (Marillia et al., 2014). The effects of these anti-nutritional factors include impaired thyroid function and possible damage to the liver and kidneys (Brown, 2015; Tripathi and Mishra, 2007). However, if the oilseed meals are utilized correctly in livestock rations, glucosinolates do not necessary decrease the value of these oilseeds as sources for biofuels. Carinata meal (CRM) that is solvent-extracted may contain lower glucosinolate content and minimal amounts of erucic acid (Brake, 2017; Rodriguez, 2018; Tripathi and Mishra, 2007), and high content of rumen degradable protein (Lawrence and Anderson, 2018). Improved CP and OM digestibility has also been found for CRM that is solvent extracted (Sackey et al., 2015).

Conventional ways to decrease concentration of glucosinolates that can be implemented by producers is the practice of ensiling *brassica* oilseeds/foliage alone or with other forages (Fales et al., 1987; Rodriguez-Hernandez, 2018). Rodriguez-Hernandez (2018) found that glucosinolates were reduced and fermentation characteristics were not affected when solvent extracted CRM (48.3 mg/g sinigrin) was ensiled with alfalfa haylage or corn silage.

Previous research in dairy cattle fed CRM includes three research trials (Rodriguez et al., 2018; Rodriguez-Hernandez, 2018; Chapter 4) using peripubertal dairy heifers and results demonstrated that growth performance and thyroid hormone concentrations are maintained when feeding CRM compared to other common protein sources, despite the glucosinolate content. Rumen fermentation, metabolic profile, and total tract digestion of nutrients were also not affected when dairy heifers, beef steers or beef heifers were fed CRM (Guidotti, 2018; Rodriguez-Hernandez, 2018; Schulmeister et al., 2019; Chapter 4). It was hypothesized that CRM would be a high quality feedstuff for lactating cows due to the increased rumen degradable protein and similar total digestible protein to soybean meal. Lactating cows are the largest sector of the dairy feeding industry and represent a lucrative potential market for CRM. Increased use of byproduct feeds for milk production will decrease pressure on arable land and improve food security within the industry (Gill et al., 2010; Wilkinson, 2011). Thus, to follow the foundational work established with heifers, the next step was to evaluate CRM versus canola meal in a feeding study with lactating cows. One aspect of CRM studies that has not been researched is plasma AA concentration and arteriovenous difference of AA. As it is a novel feedstuff the AA analysis is beneficial to determine how CRM differs from canola meal, a more common protein source in lactating diets. The objectives of this study were to determine the effects of feeding 10 % (DM basis) solvent-extracted carinata meal to lactating cows on milk production, milk composition, milk fatty acid profile, rumen fermentation, metabolic profile and total tract digestibility of nutrients.

Materials and Methods

Experimental Design

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 18-036E. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Twenty early-lactation Holsteins (DIM 83 ± 1.4) were used in a 12-wk randomized complete block design study. Cows were blocked in pairs based on parity, DIM, and milk production and randomly assigned to treatment. The feeding trial was conducted from August 2018 to December 2018 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). An adaptation period for 14 d was used for cows to adjust to the Calan gate feeding system (American Calan, Inc., Northwood, NH), followed by an experimental feeding period of 12 wk. Treatments included: 1) control diet with 10 % (DM basis) canola meal (**CON**) and 2) 10 % carinata meal (**CRM**). Canola meal was used in the control diet for comparison because it is also a Brassica oilseed and a common source of protein for lactation diets. Forage inclusion of the diet was similar (18 % alfalfa hay, 33 % corn silage), and most ingredients in the grain mix were similar, but the soybean meal and soyhulls varied slightly between treatments to make diets similar in energy content and CP (Table 5.1 and 5.2). Diets were formulated to contain 17.7 and 17.6 % CP, for CON and CRM, respectively, and to meet the requirements for a mature, lactating Holstein cow, at 680 kg body weight (BW), 95 DIM, and 43.1 kg/d of milk production, according to the 2001 Dairy NRC.

Animal Care and Feeding

Animals were observed daily for any injury or disease problems and treated according to normal farm management practices. Over the course of the study cows were housed in a free-stall barn and stalls were bedded once daily at 1700 h with chopped straw. Treatment diets were fed as a total mixed ration (TMR) using the Calan Data Ranger (American Calan, Inc., Northwood, NH) so that individual intakes could be measured. Feeding occurred once daily at approximately 0700 h, orts were weighed and recorded each morning prior to feeding, to determine individual cow daily intakes. Forages were premixed in a large TMR mixer wagon, then concentrate mix and test feeds were added into the Calan Data Ranger in the specific treatment diet, mixed, and individual ration weights were recorded for each cow. Ration mixes were adjusted weekly based on DM analysis of feed ingredients. Feed was offered for ad libitum consumption (10% refusal). Cows were allowed access to feed and fresh water at all times, except during milking. Milking occurred 2 times per day at 0600 and 1800 h in a double 8 parallel parlor and milk production was recorded at each milking and averaged by day. The CRM cows were sorted and milked last at each shift (0600 and 1800 h) and prior to applying the milking units the pipe leading into the bulk talk was disconnected and milk was discarded.

Animal Measurements and Sampling

At the start of the study and every 2 wk on 2 consecutive days throughout the experiment cows were weighed and body condition scored by 3 independent observers based on a quarter point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). On 2 consecutive days at approximately 4 h post feeding, during week 0 and every

2 wk of the feeding period, blood samples from the coccygeal vein were collected while cattle were restrained in a cattle chute, for analysis of metabolites related to energy partitioning and protein utilization. Blood samples were collected into 7 mL vacutainer tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) for glucose analysis (Cat. #: 367729) or 10 mL tubes containing potassium ethylene diamine tetra-acetic acid (K2EDTA) for all other analyses (Cat. #:366643). During wk 12 blood was also collected from the caudal superficial epigastric vein (venous sample) into 10-mL vacutainer tubes containing K2EDTA for AA analysis. Immediately after blood collection, samples were placed in ice and then brought in to the laboratory within 3 h for processing and storage. Blood collection tubes were centrifuged at 1000 × g for 20 minutes at 4°C (CR412 centrifuge, Jouan, Inc., Winchester, VA). Plasma or serum was then transferred and frozen at -20°C until metabolite analysis.

Rumen fluid was collected just after blood sample collection while cattle were still restrained via esophageal tubing for analysis of volatile fatty acids and ammonia-N. The beginning stream of rumen fluid was discarded (100 mL) in order to minimize saliva contamination. Approximately 50 mL of rumen fluid was collected into a stainless steel cup. The pH of the sample was recorded immediately (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL). A 10 mL aliquot was collected and mixed with 2 mL of 25% (w/v) meta-phosphoric acid for later determination of VFA concentrations, and a 10 mL aliquot was collected and mixed with 200 µL of 50% (v/v) sulfuric acid for later analysis of rumen ammonia nitrogen (NH3-N). The two samples from both sampling days were stored at -20° C until analysis.

On 2 consecutive days every 2 wk of the study, milk samples were taken at each milking for compositional analysis (fat, protein, lactose, milk urea nitrogen, total solids, and somatic cell counts) and sent to the Heart of America DHIA Laboratory (Manhattan, KS). During wk 12 extra milk samples were collected for fatty acid analysis.

Each week samples of the forages, grain mixes and test feeds were taken and stored at -20°C until processing and analysis could be completed as described under laboratory analysis. During wk 12 of the feeding period samples for analysis of total tract digestibility of nutrients were collected. Orts and fecal grab samples were collected over 3 d. Fecal grab sample collections were scheduled so that samples would ultimately represent every 3 h over the 24 h period relative to time of feeding. Orts and fecal samples were stored at -20°C until processing and analysis could be completed. Total tract digestibility of nutrients was then calculated using the internal marker acid detergent insoluble ash (ADIA) according to equation provided by Merchen, 1988. Nutrients in fecal samples were analyzed using similar procedures as used for analysis of feed samples.

Laboratory analysis

To determine DM content, feed samples were dried for 24 h at 105°C every 2 wk, to check ingredient inclusion rates in the ration and determine DMI. For processing, feeds were thawed and samples from four consecutive weeks were composited on an as-fed basis by volume. Composite samples were dried in duplicate for 48 h at 55°C in a Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN). Composites of the forage were ground to a 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA). Ground forages and the concentrates were reground to a 1

mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To correct analysis to 100% DM, 1 g aliquot of sample was dried for 4 h in a 105°C oven (AOAC, 1998; method 935.29). Ash content was analyzed by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (AOAC, 1998; method 942.05). Organic matter (OM) was then calculated as OM = (100 - % Ash). The TMR composites and feed ingredient composites were sent to a commercial lab for proximate analysis and mineral analysis (Dairyland Laboratories Inc., Arcadia, WI). All samples were analyzed for nitrogen (N) content via combustion analysis (AOAC, 2002; method 990.03). The resulting nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (AOAC 2002.04) and ADF (AOAC 973.18) were analyzed for all samples. Ether extracts (EE) were analyzed using diethyl ether (AOAC, 2002; method 920.39) in an Foss Soxtec 2047 (Foss Analytics, Eden Prairie, MN). Non-fibrous carbohydrates were calculated as % NFC= 100 - (% Ash + % CP + %NDF + % EE) as described by the NRC (2001). The TMR composites were analyzed for starch using a modified method of glucose analysis completed on an YSI 2700 Select Biochemistry Analyzer (YSI Biochemistry Analyzer, YSI Inc., Yellow Spring, OH). Mineral analyses included Ca, P, Mg, K, Na (method 985.01), S (method 923.01), and Cl (method 915.01) (AOAC, 1998).

Overall study composites of the diets fed were formed into 2 subsamples for AA and glucosinolate analysis. Carinata meal and canola meal were also composited into an overall study composite and analyzed for AA analysis, and glucosinolate quantification since the study only required 1 batch of oilseed meals the composition of the meal did not differ over the course of the trial. A subsample was sent to the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for complete AA

profile analysis (method 982.30; AOAC International, 2006) and a second subsample sent to the USDA Agricultural Research Service (ARS; Peoria, IL) for glucosinolate analysis.

Glucosinolate analysis and quantitation was conducted by Dr. Mark Berhow of the USDA ARS. Analysis methods performed on the carinata meal and freeze dried milk samples were similar to those described by Berhow et al. (2013). Quantitation was completed using a modified method for HPLC developed by Betz and Fox (1994). The preparation of sinigrin standards (Sigma-Aldrich Co., St. Louis, MO) was done on a molar concentration basis to determine standard curve and lower detection limits. Dried ground samples were extracted with methanol and analyzed using liquid chromatic mass spectrometry to evaluate glucosinolate composition and reversed-phase HPLC at 237 nm was used to determine concentrations of individual glucosinolates.

Milk samples collected at both milking times on 2 d every 2 wk were sent to Heart of America DHIA Laboratory (Manhattan, KS) for component analysis. Milk composition analysis was conducted according to AOAC (1995). Milk true protein, fat, and lactose were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments). Energy-corrected milk was determined using the equation: [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] (Orth, 1992). Also, composites from 2 d of wk 12 milk samples were prepared for analysis of milk fatty acid composition. Fatty acid profiles were analyzed via direct

butylation method as originally described by Sukhija and Palmquist (1988) with adaptations described by (Abdelqader et al., 2009). Prepared fatty acid samples were analyzed via gas chromatography (Hewlett Packard 6890, Palo Alto, CA) as also described by Abdelqader et al. (2009).

For analysis of rumen fluid, it was first thawed and vortexed to completely mix contents before pipetting 2 ml into a microcentrifuge tube to be centrifuged at 10,000 × g for 20 min in a micro centrifuge (Model A-14, Jouan Inc., Vinchester, VA). Samples acidified with sulfuric acid were used analyzed for Ammonia-N concentration using the assay described by Chaney and Marbach (1962). Volatile fatty acid concentrations were measured in samples acidified with meta-phosphoric acid using 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) with 2-ethylbutyrate used as an internal standard. The flow rate was 1.3 ml/min of helium and the column and detector temperature were maintained at 140°C and 250°C, respectively.

Blood metabolites (glucose, cholesterol, triglyceride and PUN) were analyzed with commercially available enzymatic or colorimetric assay kits on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA.). Serum glucose was analyzed using glucose oxidase reagent as described by Trinder (1969) (Cat. #: G7521; Pointe Scientific, Inc., Canton, MI). Total cholesterol was analyzed using cholesterol esterase and oxidase (Cat. #: C7510; Pointe Scientific, Inc., Canton, MI) as described by Allain et al. (1974). Plasma urea nitrogen (PUN) was analyzed using diacteylymonoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Triglycerides concentrations were

determined using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) and Trinder (1969).

Thyroid hormone concentrations, total T3 and total T4 were analyzed in duplicate according to the manufacturer's protocol using solid phase RIA and Coat-A-Count kits (MP Biomedicals, Orangeburg, NY). The sensitivity, intra- and interassay coefficients of variation were respectively, 2.95 ng/dL, 8.7% and 4.1% for T3, and 1.74 μg/dL, 14.8 and 5.9% for T4.

Plasma samples from 1 day in wk 12 from the coccygeal and caudal superficial epigastric vein were shipped on dry ice for analysis. Plasma concentrations of free AA were analyzed by the University of Missouri Agriculture Experiment Station Chemical Laboratories (Colombia, MO) according to AOAC Official method 982.30 E (1,b; AOAC International, 2006). Arteriovenous difference was calculated for each AA as arterial plasma concentration minus venous plasma concentration (Cant et al., 1993). Extraction efficiency was calculated as extraction efficiency (%) = arteriovenous difference/arterial concentration × 100. Amino acids were classified into EAA and NEAA based on their importance for milk protein synthesis (Clark et al., 1978). The EAA were Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; NEAA were Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr; and branched-chain AA (BCAA) were Ile, Leu, and Val. The total AA content was calculated as the sum of EAA and NEAA.

For digestibility analysis, fecal and orts samples were composited on an as-is basis by volume for each cow. Samples were processed (dried and ground) as described for the feed composites. Fecal and orts samples were also analyzed for DM, Ash, CP, NDF and ADF as previously described for feeds. Acid detergent insoluble ash (ADIA)

analyses was conducted on all feed composites, fecal samples, and orts. The method for ADIA analysis consists of analyzing the sample for ADF digestion (Robertson and Van Soest, 1981) and then determining the ash percentage using a modified procedure of the AOAC (1998) method 935.29. Digestibility calculations were performed according to Merchen (1988).

Statistical Analysis

Feed nutrient means and standard errors were calculated using the MEANS procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The total dietary nutrient values were calculated based on analysis of concentrate mixes and hay for each treatment.

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Lactation performance data were analyzed as a randomized complete block design with week as the repeated measure and cow (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, parity and the interactions of all terms. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

The MIXED procedures of SAS were also used for analysis of the data for milk fatty acid profile, total-tract digestibility of nutrients, arterial AA concentration and arteriovenous difference of AA. As all parameters were a single time point, the models

included only treatment with cow (block) as a random variable. Least square means are reported for each treatment in the tables and means were compared using Tukey's test.

Results and Discussion

Feed Composition

The nutrient compositions of concentrate mixes, forages (alfalfa hay and corn silage) and test feeds are presented in Table 5.3. Canola meal was found to have a similar nutrient composition when compared to the literature and CRM was found to be similar to results of previous feeding trials (Brito and Broderick, 2007; Broderick et al., 2015; Jayasinghe, 2014; NRC, 2001; Piepenbrink and Schingoethe, 1998; Rodriguez-Hernandez, 2018; Schulmeister et al., 2019). In contrast, the canola meal in the current study was found to have less CP and EE than values reported by Mulrooney et al. (2009).

The glucosinolate composition of test feeds varied greatly between the content of specific glucosinolates (Table 5.4). Comparable to previous research, CRM contained primarly sinigrin and a very small proportion of sinablin (4-Hydroxybenzyl) (Ban et al., 2017; Rodriguez-Hernandez, 2018; Rosenthal, 2018). Canola meal contained a small amount of sinigrin and a more variable glucosinolate profile including: gluconapin, glucobrassicin, glucobrassicanapin, and gluconasturtiin. In contrast to previous research, the phenylethyl glucosinolate (gluconasturtiin) was not found in CRM (Ban et al., 2017). The total amount of glucosinolates in the current study are less compared to previous research evaluating CRM and canola meal. Mailer et al. (2008) found a greater concentration of total glucosinolates for canola meal at 9-169 μm/g, canola meal used in treatment diets of this research contained only 3.13 μm/g. In addition, Mailer et al. (2008)

and Ban et al. (2017) both found greater total glucosinolates for CRM at 64-167 μm/g and 115.2 μm/g. Treatment diet concentrations were only 0.11 μm/g for CRM and 0.03 μm/g for CON, because test feeds were only included at 10 % (DM basis) in the diet.

The nutrient composition of the diets fed based on laboratory analysis is presented in Table 5.5. The CON treatment actual nutrient composition was closer to the formulated diets, compared to CRM. The CRM diet (16.4 % CP) had less CP than originally formulated for (17.6 % CP), this did not affect overall milk production as explained by similar yield of milk between treatments. The optimum dietary concentration of CP has been found to be 17 %, the ideal CP % for milk and protein yield has been found to be between 16.7 and 17.1 % CP (Olmos Colmenero and Broderick, 2006). The NDF and ADF of the treatment diets matched closely with what was formulated. Actual nutrient composition was slightly greater for ADF; however the NFC was still similar to diet formulations. The energy content of the treatment diets was slightly elevated compared to diet formulations but did not differ between treatments.

The test feeds were analyzed for total AA composition and results are presented in Table 5.6. Previous studies (Jayasinghe, 2014; Piepenbrink and Schingoethe, 1998) have reported AA content of canola meal, however, since CRM is so new and underdeveloped the AA profile is to the author's knowledge currently unpublished. In comparison, AA were mostly similar between test feeds, CRM did have a greater amount of total AA. Individual AA that were less in CRM compared to canola was limited to Lys. The Leu concentration of test feeds was similar and all other AA were greater in CRM. The total AA as a % of CP was found to be 38.0 versus 43.9 %, for CON and CRM, respectively. Canola meal AA values were similar to published research

(Jayasinghe, 2014; Piepenbrink and Schingoethe, 1998). The minimal differences in test feed AA composition did not alter overall treatment diet AA content (Table 5.7). Total AA were very similar between treatments (14.84 versus 14.54 % of DM) and differed due to minor differences in EAA and NEAA between diets.

Animal Performance

Dry matter intake (25.0, and 24.3 kg/d, for CON and CRM, respectively; SEM = 0.72) and BW (675.0, and 670.0 kg; SEM = 5.54) are presented in Table 5.8 and were similar ($P > 0.05$) between treatments. Body condition scores were also similar, and indicative (BCS = 3) of cows maintaining condition over the course of the study. As this study was the first to evaluate CRM versus canola meal in a lactating diet, it is difficult to compare performance parameters with literature. The DMI intake of treatment diets is similar to the values reported in studies feeding canola meal (Brito and Broderick, 2007; Broderick et al., 2015; Mulrooney et al., 2009). The similar intake response is a very positive finding because it has been found that feeding CRM requires an adaptation period when the diet is limit fed to dairy heifers, and this response was not apparent in the current study (Rodriguez-Hernandez, 2018). The decreased palatability of oilseed meals containing glucosinolates is due to bitterness and may reduce intake which could result in decreased performance (Putnam et al., 1993; Tripathi and Mishra, 2007). Sinigrin and progroitrin, specifically their degradation products are what causes the bitterness and mustard taste of brassica oilseed meals (Fenwick et al., 1982). The progoitrin found in meals is a non-bitter compound, however when broken down during processing (heating, crushing) or ingestion by myrosinase it is converted to goitrin a very bitter substance (van Doorn et al., 1998). As BW were similar between treatments and cows were not

mobilizing fat reserves due to similar BCS, the reported averages for BW disagree with Mulrooney et al. (2009) when researchers fed a similar TMR with less canola meal (6.6 versus 10.0 % DM), and found a greater BW (708 kg versus 675 kg). This could be attributed to cows in the previous study feeding canola being later in the lactation cycle and past peak lactation (100 DIM). The average BW is in agreement with Jayasinghe, (2014) in comparison to the treatment diet fed consisting of 100:0 (Corn: Barley) and supplemented with 12.2 % (DM basis) canola meal.

Milk production (Table 5.8) was also similar between treatments, however; there was a significant treatment × week interaction ($P = 0.01$) for milk production (Figure 5.1). Milk yield was not affected by feeding CRM and the average yield is comparable to studies feeding canola meal as an alternative protein source (Brito and Broderick, 2007; Mulrooney et al., 2009). Feeding *Camelina sativa*, an oilseed in the same Brassica family as carinata, to lactating cows also maintained milk production when fed as the seed, meal or oil (Halmemies-Beauchet-Filleau et al., 2011; Hurtaud and Peyraud, 2007). As milk yield and dry matter intakes (Figure 5.2) were similar between treatments, feed efficiency (1.55 and 1.46; SEM = 0.08) was also similar and treatment × wk interactions were not found. Milk component percentages and yields were similar between treatments, which in addition to milk yield disagrees with published research. Milk protein yield was found to have a treatment × week interaction ($P = 0.01$). In comparison, studies feeding camelina meal or seed (brassica oilseed) resulted in milk protein decreasing slightly while milk fat and yield decreased in greater proportions (Hurtaud and Peyraud (2007). Two meta-analyses based on results of published peer-reviewed journals reported an increase of yields of milk and milk components, and a reduction in milk urea N (MUN) when canola

meal was supplemented in replacement of soybean meal (Martineau et al., 2013, 2014). As MUN has been found to be a good indicator for protein metabolism, even though treatments did not decrease overall MUN they were still similar to literature and slightly below the normal range of 10 to 16 mg/dL (Roseler et al., 1993; Jonker et al., 1998). A lower MUN value could be attributed to a protein deficiency in the diet, as witnessed by Mulrooney et al. (2009), however, MUN reported herein are comparable to diets containing similar levels of CP as reported by Broderick et al. (2015).

Milk fatty acid profile presented in Table 5.9 was similar ($P > 0.05$) between treatments, however specific fatty acids were numerically different between treatments. The major fatty acids in CRM according to Rodriguez-Hernandez (2018) include: C18:2 cis-9, cis-12 (25.87%), C16:0 (12.59%) and C16:1 (12.59%). Canola meal major fatty acids are C18:1 cis-11 (39.09%) and C18:2 cis-9, cis-12 (27.05%; Rodriguez-Hernandez, 2018). The increased SCFA could potentially be attributed to increased de-novo synthesis by the mammary gland (Akers, 2002). The long chain fatty acids (LCFA) were not different and as these are preformed fatty acids derived from feed we can determine that fatty acid profile did not differ drastically between treatments. A greater concentration of MUFA and conjugated linoleic fatty acids was identified in the milk for cows fed camelina meal (Hurtaud and Peyraud, 2007). The major fatty acid of concern when feeding CRM is C22:1, results demonstrate CRM fed cows produced a very low amount of C22:1 at 0.058 mg/100mg FA compared to 0 mg/100mg for the CON treatment. Even though toxicological studies are not available, epidemiologic studies have indicated that erucic acid may accumulate in human myocardium in specific areas where vegetable oils containing erucic acid are consumed (FSANZ, 2003). Thus, it was imperative we

evaluated erucic acid in the milk to determine levels in cows fed CRM, in rats the toxicological dose is 1500 mg/kg of BW/d and 900 mg/kg of BW/d for piglets (FSANZ, 2003).

Glucosinolate quantification of milk samples is difficult to undertake and there are many confounding factors. Immediately as the meal is incorporated into the TMR enzyme reactions begin, dependent upon myrosinase, epithiospecifer protein and thio forming protein (Berhow, personal communication). Once ingested the degradation products are subject to changes in pH and microflora in mouth and GIT. As degradation products may be modified by the rumen and change structure from isothiocyanates, nitriles, and thiocyanates, because it is not known if they are absorbed intact or how long degradation products remain intact (Berhow, personal communication). The isothiocyanates could potentially react with proteins lysine and cysteine to form thiocarbamates and thio ureas (Walker and Gray, 1970). Currently there is not a proven method to analyze the metabolized form of degradation products (Berhow, personal communication). Detectable concentrations of isothiocyanates or nitriles were not found in freeze dried eggs or milk (Kakani et al., 2012; McGuire, unpublished). An AAFO/FDA petition for feeding carinata meal to dairy cattle in Canada presented information based on mustard allergen testing in milk and results were negative, in addition isothiocyanates and nitriles were not detected in CRM fed cows (Lortie, personal communication). It is very likely that due to dilution of CRM in the TMR, followed by dilution in the rumen and animal, there is a extremely small proportion able to enter the milk, which would volatilize during handling for analysis (Berhow, personal communication).

Rumen Fermentation Characteristics

Rumen fermentation characteristics are presented in Table 5.10. Rumen pH (6.7, and 6.6; SEM = 0.07) was found to be similar between treatments. The pH values are similar to those reported by other studies that used cannulated cows to sample rumen contents (Brito and Broderick, 2007; Broderick et al., 2015); and implemented the same sampling technique (Jayasinghe, 2014). The greater pH found in this study may be attributed to sampling method, esophageal tubing to obtain rumen samples has the likelihood of saliva contamination. Use of rumen-fistulated cows was beyond the scope of this initial study, thus rumen fluid samples were only collected every 4 wk.

The ammonia-N concentration was similar between treatments. For both treatments, concentrations of ammonia–N are greater than those reported by Mulrooney et al. (2009) and Broderick et al. (2015). The increased ammonia–N concentration may have been affected by sampling method, approximately 4 h post feeding is when samples were collected via esophageal tube, which could also potentially be when concentrations are at their peak (Owens and Zinn, 1988). The greater concentrations of ruminal ammonia-N can also be attributed to greater RDP supplied from the test feeds (Lawrence and Anderson, 2018). Ruminal ammonia is highly correlated with MUN and MUN is correlated with PUN (Schwab and Broderick, 2017), however, increased ammonia-N did not elicit a drastic increase in either parameter measured, as explained by a relatively normal MUN and PUN concentration (Mjoun et al., 2010).

The total concentration of VFA (Table 5.10) did not differ (106.5, and 101.2 mM; SEM = 5.50) between treatments and was greater than the concentrations reported by in previous studies feeding canola meal (Brito and Broderick, 2007; Broderick et al., 2015;

Jayasinghe, 2014; Mulrooney et al., 2009). Acetate: propionate ratio (A:P) was similar between treatments and was found to have a wk effect ($P = 0.01$). The proportions of all other VFAs are similar between treatments, with the exception of valerate. Valerate concentration (1.41, and 1.51 mM) tended to be greater ($P = 0.06$) for the CRM fed cows. In contrast, the concentration of isovalerate tended to be less for the CRM fed heifers in Chapter 4. However, these are considered minor VFA and these differences do not convey changes of biological significance. Rumen fermentation characteristics were not affected when cows were fed CRM compared to CON.

Metabolites, Metabolic Hormones and Plasma Amino Acids

Blood metabolite concentrations are presented in Table 5.11. Treatment by week interactions for any of the blood metabolite concentrations measured were not found. All blood metabolites were similar between treatments. Most metabolite values are similar to published literature (Mjoun et al., 2010), it is difficult to gain an understanding of overall metabolic profile as most studies do not measure all metabolites as Mjoun et al. (2010). Cholesterol concentrations were less in the current study compared to Mjoun et al. (2010), this is due to the lower fat content fed in this trial at 4.8 % and 4.9 % EE, for CON and CRM, respectively. Blood cholesterol levels are subject to increase when high fat diets are fed, specifically linoleic acid, which is a precursor for arachidonic acid and cholesterol (Palmquist, 1994), as reported by Mjoun et al. (2010) in the trial focused on feeding diets with DDGS. Due to similar EE of treatment diets a response was not found in plasma concentration of cholesterol. Compared to other studies feeding dairy heifers canola meal and CRM (Rodriguez-Hernandez, 2018), values for PUN are reduced, this is to be expected as a mature dairy cow is more efficient at N utilization (Lobley, 1992).

Thyroid hormone concentration of triiodothyronine (T3) and thyroxin (T4) are not commonly measured for adult dairy cattle. Plasma concentrations of T3 and T4 were similar ($P > 0.05$) between treatments. Thyroid hormone concentration was analyzed in the current study due to previous research feeding CRM to dairy heifers or beef cows reporting alternations in the thyroid hormone (T3 and T4) concentrations (Rodriguez-Hernandez, 2018; Rosenthal, 2018). Values reported in the current research were similar to those reported by Rodriguez-Hernandez (2018) in studies limit-feeding 10 % cold press or solvent extracted CRM with grass hay to dairy heifers. The glucosinolates in CRM and other Brassica species could potentially alter thyroid function and thus affect the concentration of thyroid hormones (Tripathi and Mishra, 2007; Waraich et al., 2013). As thyroid hormones are important for many metabolic processes it was vital we determined the effect of CRM on lactating cow thyroid hormones. Primiparous cows could potentially be impacted more severely due to growth demands while undergoing the first two lactation cycles. The T3 and T4 concentration in the current study was above the concentrations reported for hypothyroid beef heifers, and we can determine that thyroid function was not affected (Thrift et al., 1999).

The concentrations of free AA in arterial plasma are presented in Table 5.12. Amino acids were classified into EAA and NEAA based on their importance for milk protein synthesis (Clark et al., 1978). The EAA included Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; NEAA was composed of Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr; and branched-chain AA (BCAA) were calculated as the sum of Ile, Leu, and Val. Total AA was calculated as the sum of EAA and NEAA. As this is the first study to evaluate AA composition in plasma of in lactating dairy cows when fed CRM it

was difficult to find literature for comparisons. The arterial plasma concentrations reported are similar to those of published research (Mjoun et al., 2010; Mulrooney et al., 2009). As the AA composition of the treatment diets was similar it was expected to find similar free AA in arterial plasma for cows fed CON versus CRM. The profile of plasma AA has been used as an indicator of metabolizable AA used for milk protein synthesis, only essential AA composition is presented (Doepel et al., 2004).

For arteriovenous (A/V) differences in all EAA and NEAA (Table 5.12) concentrations were similar between treatments. indicating that AA removal by the mammary gland was similar for both treatments as supported by similar milk protein % and yield. A trend (P = 0.08) was identified for Trp, where CON had a greater A/V difference compared to CRM. This was also apparent in the extraction efficiency (%) of Trp which was 13.1 % versus 10.3 %, for CON and CRM, respectively. Calculating extraction efficiency may be the ideal method to evaluate AA requirements, it accounts for the entire EAA needs of the mammary gland (Derrig et al., 1974). It includes AA extracted for all needs such as protein synthesis and catabolism (Schingoethe, 1996; Nichols et al., 1998; Kleinschmit et al., 2007), whereas transfer efficiency takes into consideration only AA relative to secretion as milk protein (Kleinschmit et al., 2007). The popularity of this method stems from the reduction of error associated with measuring/calculating mammary blood flow. The amino acid with the greatest percentage extracted by the mammary gland is considered the first limiting AA for milk protein synthesis (Derrig et al., 1974). In the current study the first limiting AA was determined to be Met for the CON treatment and Lys for CRM. The results were expected based on previous research on canola meal (Piepenbrink et al., 1998; Piepenbrink and Schingoethe,

1998), because the CON treatment was greater in Lys, indicating that Met should be the first limiting. The inverse was true for second limiting AA, the third limiting AA was Phe for CON and Arg for CRM, the results agree with those of Mulrooney et al. (2009) for diets fed with canola meal and DDGS.

Apparent Total Tract Digestion of Nutrients

Apparent total tract nutrient digestibility is presented in Table 5.14. Crude protein digestibility was the only nutrient not affected by treatment. All other nutrients (DM, OM, NDF, and ADF) were found to have greater ($P < 0.05$) digestion in CRM fed cows. The reported values are greater in percent digested than those published by Brito and Broderick (2007) for the canola meal fed cows. In comparison, dairy heifers fed CRM in Chapter 4 had increased fiber digestibility compared to the CON treatment without CRM supplementation, although heifer and lactating cow rations are vastly different in concentrates and digestible forages the increased digestibility was attributed to decreased passage rate and greater ruminal fermentation of forages. In situ and in vitro studies on CRM have demonstrated the meal is a good source of degradable protein in the rumen and has a total protein digestibility comparable to soybean meal (Lawrence and Anderson, 2018). Total digestibility of protein is greater for CRM than canola meal and distillers dried grains with solubles (Lawrence and Anderson, 2018; Ban et al., 2017; Xin and Yu, 2014). Therefore, we attribute the increased nutrient digestion to CRM supplying a more digestible source of RDP that allowed microbial populations in the rumen to increase ruminal fermentation of forages and improve overall DM and OM digestibility. Carinata meal has less RUP compared to canola meal, thus, the intestinal supply of AA of CON was able to overcome limitations in RDP and perform similarly, as proven by

similar CP digestion and similar milk yield. If CRM was used in combination with a feed greater in RUP such as DDGS, the increased nutrient availability might increase milk production or milk protein, as the diet will be satisfying limiting AA more successfully as witnessed by Mulrooney et al. (2009) when canola meal was fed with DDGS. The overall differences in nutrient digestibility were not great enough to impact cow performance.

Conclusion

Feeding 10% CRM maintained lactation performance, milk composition, blood metabolic profile, metabolic hormones, or composition of plasma AA. The hypothesis was partially proven, CRM maintained cow performance and improved total tract digestion of nutrients. Rumen fermentation was not different and total tract digestion of nutrients increased, except CP, when CRM was fed to early lactation cows. The increased digestibility was due to the greater content of RDP in CRM, and the response was not carried through to increasing milk production. Successfully feeding 10% CRM to lactating dairy cows and maintaining performance provides foundational research proving CRM is viable as an alternative feedstuff in the dairy industry. Further research is warranted to determine the optimal inclusion of CRM in lactating diets and more studies are necessary for future approval of CRM as an AAFCO accepted feed ingredient. Based on this study, carinata meal is a high quality protein source for dairy cows and may be used in replacement of canola meal to maintain lactation performance.

Table 5.1. Formulation of the control (CON) and carinata meal (CRM) TMR fed during the lactation study[1]

	Diet	
Item	CON	CRM
Ingredients, % of DM		
Alfalfa Hay	18.00	18.00
Corn Silage	33.00	33.00
Ground Corn	25.00	25.00
Soybean Meal	3.60	1.60
Soy Hulls	2.00	4.00
Soybest	5.00	5.00
Canola Meal	10.00	-
Carinata Meal	-	10.00
Salt	0.50	0.50
Calcium Carbonate	0.50	0.50
JPW Vitamin Premix[2]	0.10	0.10
JPW TM Premix[3]	0.10	0.10
Magnesium Oxide	0.15	0.15
Vitamin E	0.05	0.05
Energy Booster (Rumen inert fat)	2.00	2.00

[1] Based on formulation predictions of NRC (2001) when initial analyses values for samples were entered into the program.
[2] Contained: 25.8 % Ca (DM basis) 3,405 IU/lb Vitamin A, 852 IU/lb Vitamin D, and 10,640 IU/lb Vitamin E (JPW Vitamin Premix, JPW Nutrition).
[3] Contained 11.7 % Ca (DM basis), 1.96 % S, 10,527 ppm Fe, 63,158 ppm Zn, 12,632 ppm Cu, 63,158 ppm Mn, 325 ppm Se, 632 ppm Co, and 1,053 ppm I (JPW Vitamin Trace Mineral Mix, JPW Nutrition).

Table 5.2. Formulated nutrient compositions[1] for the control (CON) and carinata meal (CRM) treatments fed during the lactation study

	Diet	
Item[2]	CON	CRM
DM, %	57.7	57.9
CP	17.7	17.6
Fat[3]	4.9	4.7
RDP	10.9	10.8
RUP	6.8	6.8
ADF	15.8	15.5
NDF	25.6	26.1
Forage NDF	17.9	17.9
NFC[4]	47.8	47.9
Ca	0.67	0.65
P	0.44	0.42
Mg	0.36	0.36
Cl	0.45	0.44
K	1.29	1.31
Na	0.23	0.23
S	0.22	0.31
Glucosinolates[5], mg/g	0.40	0.01
ME, Mcal/Kg DM	2.54	2.55
$NE_{L,}$ Mcal/Kg DM	1.62	1.63

[1] Based on Dairy NRC (2001) when initial analyses values or program values for feeds were entered into the program.
[2] % of DM, unless otherwise indicated.
[3] Ether extract.
[4] NFC (non-fibrous carbohydrate) = 100-(NDF + CP + EE + Ash) (NRC, 2001).
[5] Value of test feeds from glucosinolate analysis; values for the CRM and CON treatments were calculated from glucosinolate analysis and inclusion rate of 10 % (DM basis) of the test feed in the diet.

Table 5.3. Nutrient composition of forages and concentrate components used in the experimental diets for 12 weeks during the lactation study

Item[1]	Ingredients						
	Alfalfa Hay	Corn Silage	Base Grain Mix	Soybean Meal	Soyhulls	Canola Meal	Carinata Meal
DM[2], %	85.0	34.2	87.6	87.7	88.0	89.0	90.7
CP[2]	21.5	7.6	13.9	53.6	12.0	41.5	50.0
NDF[2]	38.0	36.9	11.6	8.6	68.0	25.6	19.1
ADF[2]	31.5	23.4	5.6	6.2	49.4	18.6	10.7
EE[2]	2.1	3.7	7.7	1.7	1.6	3.3	1.2
Ash[2]	10.6	4.2	5.9	6.6	4.8	7.6	7.6
OM[2]	89.4	95.8	94.1	93.4	95.2	92.4	92.4
NFC[2,3]	28.0	48.0	60.9	29.5	13.7	22.0	22.0

[1]% of DM unless otherwise indicated.

[2] Results from analysis of monthly composites.

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

Table 5.4. Glucosinolate composition[1] based on laboratory analysis for the canola meal and carinata meal fed during the lactation study

Item[1]	Oilseed Meal			
	Canola Meal		Carinata Meal	
	Mean	SD	Mean	SD
µM/g				
Sinigrin	1.0	0.20	10.5	0.95
Sinablin	-	-	0.6	0.06
Progoitrin	0.2	0.01	-	-
Gluconapin	0.6	0.03	-	-
Glucobrassicin	0.4	0.06	-	-
Glucobrassicanapin	0.8	0.14	-	-
Gluconasturtiin	0.2	0.001	-	-
Total, µM/g	3.13	-	11.13	-
mg/g				
Sinigrin	0.3	0.07	3.8	0.34
Sinablin	-	-	0.3	0.03
Progoitrin	0.1	0.003	-	-
Gluconapin	0.2	0.01	-	-
Glucobrassicin	0.2	0.03	-	-
Glucobrassicanapin	0.3	0.05	-	-
Gluconasturtiin	0.1	0.0004	-	-
Total, mg/g	1.22	-	4.03	-

[1] Based on the oilseed meal study composites.

Table 5.5. Nutrient composition based on laboratory analysis for the control (CON) and carinata meal (CRM) treatments fed during the lactation study

Item[1]	Treatment			
	CON		CRM	
	Mean	SE	Mean	SE
DM[2], %	56.7	0.15	57.8	0.24
OM[2]	93.7	0.14	93.8	0.10
Ash[2]	6.3	0.14	6.1	0.10
CP[2]	17.4	0.30	16.4	0.17
ADF[2]	17.8	0.40	18.1	0.41
NDF[2]	22.8	3.24	26.5	0.64
EE[2,3]	4.8	0.32	4.9	0.87
NFC[2,4]	48.6	2.94	46.1	0.90
RDP[5]	10.5	-	10.9	-
RUP[5]	5.7	-	6.0	-
Forage NDF[5]	19.0	-	19.0	-
Nonforage NDF[5]	3.8	-	7.5	-
Starch[2]	28.1	0.60	31.0	0.70
Ca[2]	0.82	0.032	0.73	0.065
P[2]	0.40	0.018	0.40	0.012
Mg[2]	0.40	0.014	0.39	0.001
K[2]	1.34	0.044	1.34	0.062
S[2]	0.23	0.001	0.30	0.009
Na[2]	0.25	0.009	0.28	0.018
Cl[2]	0.40	0.006	0.43	0.030
Glucosinolates[5], mg/g	0.40	-	0.01	-
ME[6], Mcal/kg DM	2.60	-	2.60	-
NE_L[6], Mcal/kg DM	1.65	-	1.65	-

[1] % DM, unless otherwise indicated.
[2] Results from analysis of monthly TMR composites.
[3] Ether extract, analyzed with diethyl ether.
[4] % NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[5] Value of test feeds from glucosinolate analysis (Table 5.4); values for the CRM and CON treatments were calculated from glucosinolate analysis and inclusion rate of 10 % (DM basis) of the test feed in the diet.
[6] Based on formulation predictions of NRC (2001) when analyses values for samples were entered into the program.

Table 5.6. Analyzed amino acid composition of the canola meal and carinata meal used in treatment diets at 10% (DM basis) during the lactation study[1]

	Oilseed Meal	
Item	Canola Meal	Carinata Meal
Amino Acid, % of DM		
Arg	2.46	3.45
His	1.12	1.28
Ile	1.73	2.01
Leu	1.32	1.32
Lys	2.40	1.83
Met	0.82	0.92
Phe	1.70	2.00
Thr	1.74	1.90
Trp	0.48	0.63
Val	2.20	2.43
Total EAA[2]	18.00	20.20
Total NEAA[3]	20.00	23.73
TAA[4]	38.00	43.93

[1] Based on the oilseed meal study composites.
[2] Total essential amino acids.
[3] Total nonessential amino acids.
[4] Total amino acids calculated as the sum of EAA and NEAA.

Table 5.7. Analyzed amino acid composition of the control (CON) and carinata meal (CRM) TMR fed during the lactation study[1]

	Diet	
Item	CON	CRM
Amino Acid, % of DM		
Arg	0.81	0.84
His	0.37	0.37
Ile	0.71	0.68
Leu	1.32	1.32
Ly	0.80	0.71
Met	0.26	0.25
Phe	0.75	0.74
Thr	0.65	0.62
Trp	0.18	0.16
Val	0.85	0.84
Total EAA	6.84	6.70
Total NEAA	8.00	7.84
TAA	14.84	14.54

[1] Based on AA profile analysis of TMR study composites.
[2] Total essential amino acids.
[3] Total nonessential amino acids.
[4] Total amino acids calculated as the sum of EAA and NEAA.

Table 5.8. Dry matter intake, milk yield and composition, efficiency calculations, and body characteristics for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

Item	Treatment			*P values*		
	CON	CRM	SEM	Treatment	Week	Treatment ×Week
DMI, kg/d	25.0	25.3	0.72	0.74	<0.001	0.50
Milk kg/d	37.0	36.0	1.63	0.65	<0.001	0.01
Fat, %	2.84	3.05	0.35	0.74	0.20	0.24
Fat, kg/d	1.05	1.10	0.10	0.39	0.29	0.38
Protein, %	3.30	3.38	0.04	0.50	0.46	0.40
Protein, kg/d	1.25	1.23	0.06	0.70	<0.01	0.01
Lactose, %	4.96	4.94	0.03	0.99	0.40	0.41
Lactose, kg/d	1.25	1.23	0.06	0.66	<0.001	0.01
MUN, mg/dL	9.10	8.70	0.30	0.31	<0.001	0.16
SCC, 10^5/mL	56.50	81.97	27.70	0.59	0.69	0.60
ECM[1], kg/d	35.00	34.60	2.03	0.37	0.12	0.45
Feed Efficiency[2]	1.55	1.46	0.08	0.72	0.06	0.39
Body Weight, kg	675.0	670.0	5.54	0.46	0.52	0.49
Body Condition Score[3]	3.00	3.04	0.04	0.08	0.13	0.17

[1] Energy corrected milk (ECM) = [(0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein)] (Orth, 1992).

[2] Feed efficiency= ECM/ DMI.

[3] Body condition score is on a scale of 1 to 5 with 1 being emaciated and 5 being obese (Wildman et al, 1982).

Table 5.9. Milk fatty acid composition for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P-value*
Item[1], mg/100 mg FA	CON	CRM	SEM	Treatment
C4:0	1.986	2.171	0.381	0.73
C6:0	2.036	1.823	0.160	0.35
C8:0	1.127	1.049	0.103	0.59
C10:0	3.065	2.985	0.263	0.82
C12:0	3.692	3.658	0.269	0.91
C14:0	12.07	12.22	0.311	0.72
C14:1	1.327	1.328	0.127	0.99
C16:0	32.91	32.03	1.141	0.58
C16:1 *cis*	1.720	1.174	0.297	0.20
C18:0	8.636	8.996	0.409	0.46
C18:1 *trans* 11	0.970	1.000	0.090	0.82
C18:1 *cis* 9	19.29	20.57	0.983	0.36
C18:1 *cis* 11	2.142	2.033	0.132	0.55
C18:2 *cis* 9 *cis* 12	2.544	2.545	0.178	0.99
C18:2 *cis* 9 *trans* 11 (CLA)	0.082	0.085	0.012	0.87
C18:2 *trans* 10 *cis* 12 (CLA)	0.060	0.099	0.018	0.13
C18:3 *alpha*	0.336	0.348	0.047	0.80
C20:0	0.275	0.277	0.045	0.98
C22:1	0.000	0.058	0.039	0.31
C22:2	0.014	0.011	0.008	0.76
SCFA[2]	24.43	27.27	1.116	0.91
LCFA[3]	72.62	72.80	1.103	0.88
Saturated FA[4]	68.86	68.28	1.513	0.78
MUFA[5]	26.23	27.27	1.347	0.58
PUFA[6]	4.972	4.988	0.836	0.68
Others[7]	4.195	4.482	0.296	0.49

[1] Number of carbons: number of double bonds.
[2] Short Chain Fatty Acids, <C16:0.
[3] Long Chain Fatty Acids, ≥ C16:0.
[4] Saturated FA= saturated fatty acids.
[5] Monounsaturated fatty acids.
[6] Polyunsaturated fatty acids.
[7] Others: sum of C7:0, C9:0, C11:0, C11:1, C12:1, C13:0, C15:0, C15:1, C16:1trans, C17:0, C17:1, C19:0, C18:2 trans, C18:3ɣ, C20:2, C20:3, C20:4, C22:3, C22:4.

Table 5.10. Rumen fermentation characteristics for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

Item	Treatment			*P values*		
	CON	CRM	SEM	Treatment	Week	Treatment ×Week
pH	6.7	6.6	0.07	0.87	<0.01	0.27
Ammonia-N, mg/dL	8.5	7.0	0.84	0.15	0.77	0.80
Total VFA, mM	106.5	101.2	5.50	0.65	0.35	0.55
VFA, mM/100mM						
Acetate	54.7	54.3	1.26	0.40	0.04	0.73
Propionate	33.5	34.1	1.60	0.54	0.01	0.60
Butyrate	9.1	8.7	0.52	0.98	0.04	0.24
Isovalerate	1.2	1.3	0.09	0.20	0.05	0.79
Valerate	1.41	1.51	0.13	0.06	0.71	0.47
Acetate: Propionate	1.67	1.67	0.15	0.77	0.01	0.62

Table 5.11. Plasma metabolites for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

Item	Treatment			*P value*		
	CON	CRM	SEM	Treatment	Week	Treatment ×Week
Glucose, mg/dL	63.0	62.5	1.20	0.60	0.05	0.44
PUN[1], mg/dL	13.7	13.9	0.59	0.86	0.29	0.70
Cholesterol, mg/dL	130.6	137.6	5.20	0.65	0.92	0.20
Triglycerides, mg/dL	13.8	14.2	0.51	0.34	0.86	0.34
Triiodothyronine, ng/dL	137.7	141.3	4.72	0.30	0.35	0.40
Thyroxin, µg/dL	1.8	1.9	0.05	0.30	0.24	0.17

[1]Plasma Urea Nitrogen

Table 5.12. Arterial plasma amino acid and arteriovenous difference[1] for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P value*
Item	CON	CRM	SEM	Treatment
Arterial plasma, µg/mL				
EAA				
Arg	13.0	14.7	1.20	1.42
His	8.9	9.8	0.55	1.40
Ile	16.7	18.4	0.93	0.19
Leu	23.2	25.0	1.40	0.35
Ly	14.2	14.2	1.40	1.00
Met	3.3	3.6	0.22	0.22
Phe	7.7	8.3	0.43	0.15
Thr	12.7	13.0	0.68	0.71
Trp	8.2	8.7	0.32	0.26
Val	35.1	38.0	1.75	0.19
EAA	115.0	123.0	5.74	0.33
NEAA[2]	135.5	141.3	6.10	0.50
BCAA[3]	75.0	81.4	3.93	0.22
TAA[4]	250.0	264.5	10.33	0.33
Arteriovenous difference[5], µg/mL				
EAA				
Arg	6.0	6.8	0.41	0.18
His	1.9	2.0	0.14	0.80
Ile	6.0	6.0	0.43	0.97
Leu	9.0	9.2	0.62	0.85
Ly	7.7	7.9	0.48	0.80
Met	2.0	2.1	0.14	0.50
Phe	3.7	3.7	0.21	0.90
Thr	3.7	3.9	0.24	0.63
Trp	1.07	0.90	0.074	0.08
Val	6.8	6.8	0.44	0.96
EAA	60.7	60.4	3.55	0.96
NEAA	30.8	32.1	2.20	0.67
BCAA	21.7	22.0	1.46	0.91
TAA	91.5	92.5	5.35	0.90

[1] Week 12 Plasma collected from the coccygeal artery (arterial) and caudal superficial epigastric vein (venous) to calculate arteriovenous difference.
[2] NEAA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr.
[3] BCAA = Branched-chain AA (Val, Ile, and Leu).
[4] TAA = EAA + NEAA
[5] Arteriovenous = arterial plasma concentration – venous plasma concentration (Cant et al., 1993).

Table 5.13. Essential AA extraction efficiency[1] (%) of treatment diets

	Treatment			*P value*
Item	CON	CRM	SEM	Treatment
Extraction Efficiency, %				
Arg	47.0 (4) [2]	47.6 (3)	4.06	0.90
His	23.0 (8)	21.0 (8)	1.93	0.40
Ile	35.4 (6)	32.7 (6)	2.60	0.46
Leu	39.5 (5)	37.3 (5)	2.74	0.53
Lys	57.3 (2)	58.3 (1)	4.75	0.90
Met	62.4 (1)	58.2 (2)	4.54	0.46
Phe	49.1 (3)	44.8 (4)	3.77	0.38
Thr	30.0 (7)	30.4 (7)	2.44	0.85
Trp	13.1 (10)	10.3 (10)	0.95	0.04
Val	19.5 (9)	18.1 (9)	1.40	0.44
Tyr	41.0 [5] [3]	41.2 [5]	3.90	0.96

[1] Extraction efficiency = arteriovenous difference of AA × 100/concentration of AA in plasma of coccygeal artery.

[2] Numbers in parentheses indicate the apparent order of limiting AA.

[3] Numbers in brackets are the ranking of Tyr if it were considered an essential AA.

Table 5.14. Total tract digestion of nutrients for cows fed the control (CON) and 10% carinata meal (CRM) diets for 12 weeks

	Treatment			*P value*
Item, % digested	CON	CRM	SEM	Treatment
DM	74.7	78.0	1.05	0.04
OM	76.0	80.0	1.11	0.01
CP	76.0	76.5	0.87	0.65
NDF	48.1	56.6	2.23	0.01
ADF	48.1	57.5	2.03	<0.01

Figure 5.1. Milk yield (kg) for Holstein cows fed the control diet (CON) and 10 % carinata meal (CRM) diets for 12 weeks. Error bars represent SEM = 0.65.

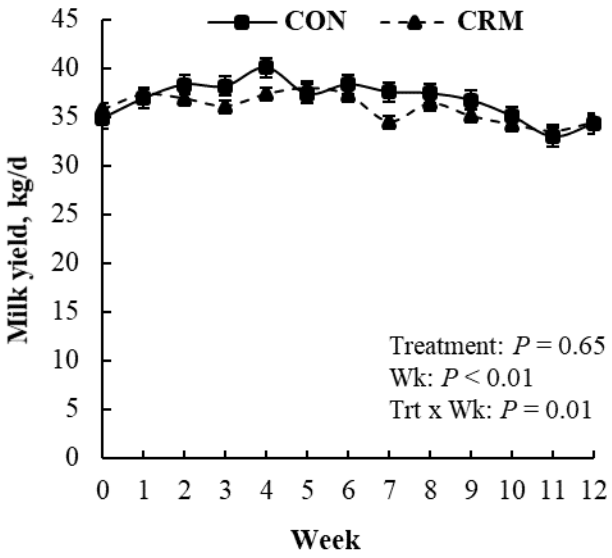
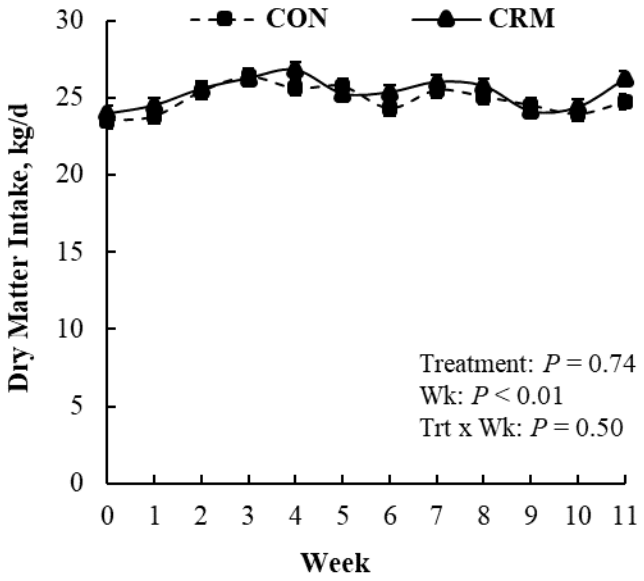


Figure 5.2. Dry matter intake (kg/d) for Holstein cows fed the control diet (CON) and 10% carinata meal (CRM) diets for twelve weeks. Error bars represent SEM = 0.72.



OVERALL SUMMARY AND CONCLUSION

The research presented accomplished our overall objective, which was to determine the effects of feeding alternative feedstuffs: hydroponic barley sprouts and CRM on dairy cattle performance. The alternative feeds were evaluated in growing dairy heifer diets and lactating cow diets. As both stages of life are important to the success of a dairy operation, feeds were evaluated during the growth phase of life and the lactating phase, both of which are vital areas to examine when considering new feedstuffs. In Chapter 2 and 4, we determined how heifer growth, metabolic profile, rumen fermentation and total tract digestion of nutrients were affected by feeding the test feed in comparison to control diets not supplemented. Once test feeds were determined to have not negatively affected heifer performance, the feeds were used in lactating cow diets (Ch. 3 and 5). In Chapter 3 and 5, we evaluated how alternative feeds affected milk production, composition, milk fatty acid profile, rumen fermentation, and blood metabolic profile. In addition, for CRM fed cows (Ch. 5) we also evaluated AA composition of diets and plasma samples. All studies implemented were conducted as a randomized complete block design to better evaluate the effect of time feeding treatment diets on animal performance.

Heifers were fed hydroponically grown barley sprouts at 14 % (DM basis) in a TMR fed for ad libitum intakes in Ch. 2. Results indicate that replacing corn and some soybean meal with hydroponic barley sprouts maintained heifer frame growth without affecting blood metabolic profile or rumen fermentation. Decreased gain: feed was found for heifers fed HYD, attributed to the high moisture content in the HYD TMR. Apparent total tract digestion of nutrients was not affected by feeding hydroponic barley. The

proper incorporation of barley sprouts has yet to be identified, cutting the sprouts by hand is not feasible for dairy producers and a method for chopping needs to be investigated.

In agreement with Ch. 2, feeding hydroponic barley sprouts (8 % DM basis) to lactating cows indicated that hydroponically grown barley sprouts can replace a portion of the corn and some soybean meal in diets of mid-lactation cows and maintain production performance. Fatty acid profile was similar between treatments. Rumen fermentation characteristics and blood metabolic profile were not affected by feeding hydroponic barley sprouts. Total tract digestibility of DM and OM was increased. Diets were conservative on inclusion amount of the hydroponic feeds, and a lesser inclusion rate was chosen to avoid effects caused from increased moisture of the TMR. It is suggested that more research is needed on the optimum inclusion rates of the test feed.

In Chapter 4, it was determined that CRM could be fed as a protein supplement in an ad libitum TMR with corn silage to growing dairy heifers and increase fiber utilization, acetate production, and concentration of plasma triglycerides. Although DMI was decreased, results have shown that CRM when fed at 10 % of the diet maintained growth performance, metabolic profile, and digestibilities of nutrients compared to more common protein sources (soybean meal). It was hypothesized that growth performance would be enhanced in heifers fed CRM because of the increased RDP and total digestibility, however growth was only maintained, not disproving the hypothesis entirely. In this study and the lactation trial, the concentration of glucosinolates in the CRM treatment were determined to be very low 4.03 mg/g and this did not affect growth, when fed at 10% (DM basis) it only contributed 0.40 mg/g of glucosinolates (sinigrin and sinablin). Carinata meal shows potential as a feedstuff to be included in TMR fed to dairy

heifers. Proposed future research should include determining the effects of CRM in greater inclusions in heifer diets to find the optimal inclusion of this alternative feedstuff in dairy heifer feeding programs and evaluating CRM effects on lactating cow performance.

Feeding 10% CRM in lactation diets in replacement of canola meal maintained lactation performance, milk composition, milk fatty acid profile, blood metabolic profile, thyroid hormones, or composition of plasma AA in Chapter 5. Cow performance and improved total tract digestion of nutrients, partially proves our hypothesis in that cows maintained production performance. Rumen fermentation was not different and total tract digestion of nutrients (DM, OM, NDF, and ADF) increased. Lactating cows were able to successfully adapt to 10 % CRM in the diet, this was demonstrated by similar limiting AA compared to the CON diet and similar yields of milk components. According to results of this study, CRM could be utilized in the dairy industry as a high quality protein source for dairy cows and it can replace canola meal to maintain lactation performance.

In conclusion, hydroponic barley sprouts and carinata meal can be fed to growing heifers and lactating cows without affecting performance. The water soluble carbohydrates and readily fermentable carbohydrates in barley sprouts make it an ideal supplement to reduce concentrate use. Barley sprouts are high in moisture and can decrease the DM of TMR, which can affect feed intake and performance. Reducing cost of production in the dairy industry is becoming more viable with use of alternative feedstuffs. Carinata meal is a low cost, high quality protein source for the dairy sector. The aforementioned carinata meal studies will serve as the foundation for carinata meal utilization in the dairy industry.

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