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Evaluation of Carinata Meal in Dairy Heifer Feeding Programs

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EVALUATION OF CARINATA MEAL IN DAIRY HEIFER FEEDING PROGRAMS

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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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This Dissertation is dedicated to God.

To my Mom, and in memory of my Father.

To all the heifers that were part of my experiments.

Happiness is not a goal, it is a by-product - Eleanor Roosevelt.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADIA	Acid detergent insoluble ash
ADG	Average daily gain
AI	Artificial insemination
AFC	Age at first calving
BCS	Body condition score
BW	Body weights
CAN	Canola meal treatment diet
CRM	Carinata meal treatment diet
CON	Control treatment diet
Ca	Calcium
Cl	Chloride
cm	centimeters
dL	deciliter
DDGS	Distillers dried grains with solubles

DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
IGF-1	Insulin-like growth factor -1
K	potassium
K_2EDTA	potassium ethylene diamine tetra-acetic acid
Mcal	Megacalories
ME	Metabolizable energy
Mg	Magnesium
mg	milligrams
µg	micrograms
µM	micromoles
µU	microunits
N	Nitrogen
Na	Sodium
NaFl	Sodium fluoride

NDF	Neutral detergent fiber
NFC	Non-fibrous carbohydrate
ng	nanograms
NEg	Net energy of gain
OM	Organic matter
P	Phosphorus
pg	Picograms
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RIA	Radioimmunoassay
RUP	Rumen undegradable protein
S	Sulfur
SEM	Standard error of the mean
wk	week

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ABSTRACT

EVALUATION OF CARINATA MEAL IN DAIRY HEIFER FEEDING PROGRAMS

KARLA RODRIGUEZ-HERNANDEZ

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The main purpose of this body of research was to evaluate carinata meal as a feedstuff for dairy heifers and lay the foundations for its future evaluation in lactating dairy cow diets. This research focused on evaluating the taste preference of carinata meal compared with other oilseeds meals and distillers dried grains with solubles (DDGS). Despite containing glucosinolates, carinata meal was preferred similarly to canola meal, had greater preference compared to camelina meal, and less preference compared to the other meals. Next, we used a limit-feeding strategy in two different feeding studies, to determine the effect of feeding carinata meal cold-pressed and solvent-extracted on DMI, nutrient digestibility, growth rate, metabolic profile and the onset of puberty of growing Holstein heifers compared to other protein sources such as DDGS, canola meal, and soybean products. Feeding cold-pressed or solvent extracted carinata meal at 10% of the diet (dry matter basis) maintained growth performance of dairy heifers without negatively affecting the metabolic profile, thyroid function nor the onset of puberty and was comparable to DDGS, canola meal, and soybean products. However, a period of adaptation to cold-pressed carinata meal should be allowed for the heifers to adapt. The final study demonstrated the viability of ensiling as an on-farm option to decrease the

glucosinolate content of carinata meals either cold-pressed or solvent extracted with increased protein content and quality of corn silage and alfalfa silage without affecting the fermentation characteristics of the silage.

INTRODUCTION

Feeding co-products from the biofuel industry such as canola meal and distillers dried grains with solubles (**DDGS**), which are less expensive than soybean meal, is a frequently used strategy to reduce costs of raising heifers while supplying good quality crude protein to maintain growth performance.

Environmental contamination and rising oil prices are the driving forces for exploring new feedstocks to produce renewable fuels. Government agencies and commercial airlines have committed to the reduction of the use of petroleum-derived jet fuel and are joining with universities, research centers, and crop developers to promote most of the research on this subject. High oil content in oilseeds with very-long chain fatty acids (**VLCFA**) provides a unique platform to produce jet biofuel that can replace non-sustainable petroleum as fuel source (Cahoon et al., 2007). After oil extraction, the remaining seed materials may be burned to generate energy or used as feedstuffs, which is a desirable option since it increases the marketing value of the co-products.

One oilseed of new interest is carinata (*Brassica carinata*). Carinata is an oilseed well adapted to dry climates and can grow in regions inadequate for other crops (Marillia et al., 2014). Carinata oilseeds have been selected for higher concentrations of VLCFA, which can be used to produce biofuels and bio-oils (Enjalbert et al., 2013; Cardone et al., 2003). Its co-product known as “carinata meal” has better nutritive characteristics than canola meal, according to in situ and in vitro experimental results (Marillia et al., 2014; Yu et al., 2014; Lawrence and Anderson, 2018).

As with most co-products of the biofuel industry, the extraction process increases the content of nutrients, as well as anti-nutritive substances on the meal, which in excess may negatively affect animal performance. Examples are sulfur, mycotoxins and fat content in DDGS, trypsin and lectin in soybean meal, and glucosinolates and erucic acid in canola meal. Carinata, similar to other Brassicas oilseeds (canola, rapeseed, camelina), contains erucic acid and glucosinolates. Research with laboratory animals suggests that erucic acid can cause abnormal fat accumulation on the heart (FSANZ, 2003). Plant glucosinolates are self-defense substances of brassicas which are innocuous by themselves. However, when the plant is damaged or the glucosinolates are exposed to heat, water or changes on pH, they are hydrolyzed, and their endproducts can cause a bitter taste, affect thyroid gland function, and therefore growth and reproduction. Concentration, glucosinolates type, and the effects of its hydrolysis endproducts differ among species and, in some cases, anticarcinogenic effects have been reported (Tripathi and Mishra, 2007; Clark, 2010; Marillia et al., 2014). As an option to reduce the content of glucosinolates, Fales et al. (1987) ensiled fresh or wilted rape forage and observed a glucosinolate reduction (0.3% to 0.03%) with only the fresh ensiled material. The reason of this effect could be attributed to the production of lactic acid during fermentation which decreased the pH and promoted glucosinolate hydrolysis (Bones and Rossiter, 2006).

In vivo evaluation of the nutritive value of carinata meal is needed; however, the young calf's digestive system is not completely developed (Baldwin et al., 2004; Anderson et al., 1987) and their tolerance to anti-nutritive substances may be reduced. During pregnancy and lactation dairy cows' hormonal and metabolic status may complicate the

evaluation and objective measurement of negative effects of carinata meal, if any. Heifers also present hormonal and metabolic changes related to growth and puberty, but the effects of milk production and fetal growth are not present, so they are the most viable option for the introduction of carinata meal to the dairy industry.

In the last decades, extensive research has been conducted on the nutritional management and feeding programs for growing dairy heifers with the objective of increasing growth performance and reduce rearing costs. Limit-feeding programs have been shown an excellent tool to test new feedstuffs for growing heifers (Lascano et al., 2012; Anderson et al., 2015a; Lawrence et al., 2006; Manthey et al., 2017). Restricting dry matter intake (DMI) allows to separate the effects of gut fill, rate of passage, forage to concentrate and protein to energy ratios, and growth rate. Therefore, evaluating carinata on growing dairy heifers using a limit-feeding program may be the best option.

The main purpose of this body of research was to evaluate carinata meal as a feedstuff for dairy heifers and lay the foundations for its future evaluation in lactating dairy cows. This research focused on evaluating the taste preference of carinata meal compared with other oilseeds meals and DDGS. A limit-feeding strategy was used to determine the effects of feeding cold-press and solvent-extracted carinata meal on DMI, nutrient digestibility, growth rate, metabolic profile and onset of puberty of growing Holstein heifers, when compared to other protein sources such as DDGS, canola meal, and soybean products. Ensiling was also evaluated as an on-farm option to decrease the glucosinolates content of carinata meal. It was hypothesized that as CRM has high crude

protein content and quality, its inclusion in the diet will maintain or enhance the growth performance of dairy heifers and age at puberty without negatively affecting health and thyroid hormone concentrations. Secondly, since the content and types of glucosinolate vary depending on the oilseed meal, the taste preference could be different, affecting the intake of dairy heifers. And third, it was hypothesized that the fermentation process during ensiling of carinata meal with forages would decrease the glucosinolates content without affecting the fermentation characteristics of the silage.

CHAPTER 1. LITERATURE REVIEW

Growth performance of dairy heifers

Dairy farms have two highly important animal groups, the dairy cows and the replacement heifers. Both groups depend on each other, the management of the cow herd determines the number of available replacements and the number of replacements that will stay on the farm (Tozer and Heinrichs, 2001). However, as dairymen do not see the financial return of the replacement herd until first calving and the start of lactation, one of their priorities to decrease the cost of raising heifers (Gabler et al., 2000). Therefore, research needs to address how to create strategies that optimize the heifer growth and minimize costs without sacrificing future productivity (Hoffman and Funk, 1992; Mourits et al., 1997).

Cost of raising dairy heifers is the second or third largest expense depending on the farm (Tozer and Heinrichs, 2001; Heinrichs et al., 2013). Feeding is the largest expense representing approximately 73% of total rearing costs (Heinrichs et al., 2013). Costs have increased over time and in 2018 feed prices are twice as much as in 2000 (Gould, 2018) with overall costs of raising heifers having increased almost 10% (Gabler et al., 2000).

The first strategy to reduce cost and increase economic returns to the farm is to shorten the non-productive period through decreasing the age at first calving (**AFC**) which depends mostly of the growth rate of the heifers (Mourits et al., 1997; Heinrichs, 1993; Gabler et al., 2000; NRC, 2001). Additionally, growth rate of replacement heifers

impacts feed costs and influences future milk production (Heinrichs and Tozer, 2001). According to USDA-NAHMS (2014), the average AFC in U. S. dairy farms is 25 mo with a range of 23.4 to 26.4 mo depending on the system and herd size. A study by Gill and Allaire (1976) showed that total performance decreased as AFC increased beyond 22.5 to 23.5 mo and although milk/day-life increased when AFC was 25 mo, the profit/day of life declined. Heinrichs et al. (2013) confirmed these previous results as they observed that efficient dairy farms had AFC of 23.7 mo when feed costs and efficient labor use were optimized.

Growth rate and puberty

Puberty in heifers can be defined as the physiological state where first ovulation is triggered. This happens when the hypothalamic-pituitary axis loses its sensitivity to the negative feedback effect of estradiol and results in a surge of luteinizing hormone (**LH**) and formation of a corpus luteum (**CL**; Moran et al., 1989). Before the onset of puberty, hypothalamic gonadotropin releasing hormone (**GnRH**), pituitary follicle stimulant hormone (**FSH**), and LH are produced and released, promoting follicular growth and with it estradiol synthesis. During prepuberty low-frequency LH pulses occur (1-4/24 h) and the frequency of these pulses start to increase during the 50 days before the onset of puberty. The few days before puberty frequencies of 24 pulses/24 h can be observed (Kinder et al., 1995). The progression of events that leads to puberty is controlled largely by genetic and environmental factors, among which nutrition has a major influence. Nutritional signals of sufficiency are perceived by a variety of neurons in the

hypothalamus which interact with estradiol-receptive neurons and GnRH-ergic neurons (which become less sensitive to estradiol negative feedback) increasing high-frequency GnRH release and with it a surge in LH and ovulation. (Amstalden et al., 2014).

Therefore, to reach the benchmark of AFC between 23 and 24 mo with a heifer weighing 82% of her mature weight, heifers should be pregnant between 14 and 15 mo of age (NRC, 2001). Growth rate directly impacts onset of puberty; in 1960 Menge et al. found that heifer weight at 6 mo was negatively correlated with age at puberty and that attainment of puberty at a later age negatively affects the first 90 days of milk production, and the average butterfat percentage of the first lactation.

Very poor growth rates can delay the onset of puberty after 15 mo of age, as was observed in beef heifers that grew at a rate of 0.15 kg/d from 6 to 14-15 mo of age and did not attain puberty but after 50 to 64 days after their feed regimen was increased (alfalfa hay ad libitum and 2-3 kg of shelled corn/head/d) (Gonzalez-Padilla et al., 1975). Additionally, low growth rates not only negatively affect the age at puberty but the percentage of heifers that can reach it at a certain age. Lammers et al. (1999) observed a higher percentage (85 vs. 67%) of pubertal heifers by 12 mo of age when fed diets for accelerate growth (1.0 kg/d of ADG) compared with heifers fed for a standard growth regimen (0.7 kg/d of ADG). Similarly, only 60% heifers with ADG 0.57 kg/d between 6 to 14 mo of age reached puberty before 14 mo. Conversely, 100% of heifers fed to grow 1.0 kg/d reached puberty before 13 mo of age (Cardoso et al., 2014).

Although heifers can reach puberty before 12 mo of age, it does not mean heifers should be bred at that age. In beef heifers, conception rates were improved as the number of estrous cycles increased after onset of puberty (pubertal estrus = 57% vs. third estrus = 78%) (Byerley et al., 1987).

Growth rate and future milk yield

Growth rate between 6 and 12 mo of age affects the onset of puberty, age at breeding, and mammary gland growth. During this period the mammary gland growth is allometric, which means it grows at a higher rate than other body tissues. Therefore, below or above optimal can affect future milk production (Sinha and Tucker, 1969; Sejrsen et al., 1982; Zanton and Heinrichs, 2005). It is widely accepted that a growth rate of 0.8 kg/d (range 0.7 – 0.9 kg/day) of average daily gain (**ADG**) for heifers during the peripubertal period is optimal because it allows heifers to be bred between 55-60% of their mature body weight and calve near to 24 mo of age without negatively affecting milk production and by decreasing the rearing costs (Gardner et al., 1988; Sejrsen and Purup, 1997; NRC, 2001; Zanton and Heinrichs, 2005). Growth rates in prepubertal heifers above optimal, resultant from high energy intake, can negatively affect future milk production because negative effects on mammary gland parenchymal growth (epithelial secretory tissue of the gland) with increased amount of adipose tissue and decreased amount of parenchyma (Sejrsen et al., 1982; Sejrsen, 1994; Sejrsen and Purup, 1997; Radcliff et al., 2000).

The negative effect of rapid weight gains on future milk production does depend on the interaction of protein and energy in the diet. When heifers were fed corn silage-based diets at accelerated growth rates (1.0 kg/d; ME 20 Mcal/d; 16 % CP) it increased the mammary gland weight, and the percentage of adipose tissue, and it decreased the percentage of epithelial cells observed. However, heifers fed alfalfa silage-based diets at high growth rates (1.0 kg/d; ME 20 Mcal/d; 22% CP) resulted in lighter mammary gland and no increase of percentage of adipose tissue. Additionally, the amount of parenchyma or milk production during first lactation did not differ with either treatment (low and high growth rate, corn or alfalfa silage; Capuco et al., 1995; Waldo et al., 1997; Waldo et al., 1998). In another experiment, diets with high energy-high RUP were fed to produce ADG of 1.2 kg/d, and no differences were observed in parenchymal content of the mammary gland. There was, however, more adipose tissue compared with the heifers fed low energy-low RUP diet (Radcliff et al., 1997). Van Amburgh et al. (1998a, 1998b) demonstrated that in prepubertal heifers with ADG of 0.94 kg/d (ME 14.4 Mcal/d; 15.5% CP) first lactation milk yield was not compromised; however, heifers in that treatment calved lighter and earlier which reduced their FCM by 5% and 305-d milk yield compared with heifers with ADG of 0.68 kg/d (ME 9.6 Mcal/d; 15.4% CP). Therefore, the negative effects on milk yield could be more related to weight and age at calving. Fisher et al. (1983) observed that calving weight explained the variation in milk yield during the first 240 days of lactation, and age at calving was related to total milk yield and not as much to weight. Later, Keown and Everett (1986) observed that heifers that

calved with body weight ≤ 408 kg produced 806 kg less milk compared with heifers that calved with BW between 544 and 567 kg.

Silva et al. (2002) analyzed the data from two studies to identify factors, within a dietary treatment group, that would account for variation in first lactation milk production or amount of mammary gland development. With high energy diets, and even when evaluated independently of the dietary treatment, heifers that grew faster did not have impaired mammary development. Furthermore, increased body fatness was a better predictor of impaired mammary development than growth rate. Similarly, Anderson et al. (2015a, 2015c) did not observe negative effects on milk fat, and energy-corrected milk yield on prepubertal heifers limit-fed at 2.45% of the body weight with ADG of approximately 0.96 kg/d and similar body condition score (**BCS**).

Heifer feeding programs

Our knowledge about how to efficiently use and combine nutrients to produce optimal growth rates is increasing, resulting in different feeding programs that can be used depending on the resources available to producers.

Conventional program

Dairy heifers are usually fed ad libitum diets in general as total mixed rations (**TMR**) with predominantly forages and a small amount of concentrate. This high forage may be a handicap for the growth efficiency of heifers depending on the quality of the fiber and the energy to protein ratio (Moody et al., 2007; Heinrichs, 2017). Heifers fed

poor quality grass hay in stacks and restricted concentrate intake had lower ADG (0.64 kg/d) compared with those fed free choice good quality chopped alfalfa hay (ADG 0.76 kg/d) (Clark et al., 1984). Heifers on ad libitum high forage diets (72-76%) that included corn crop residue silage (ammoniated or with brassica forage), alfalfa haylage, cracked corn with or without urea as N source had ADG of 0.65 kg/day (Lopez-Guisa et al., 1991). However, when conventional diets (92-95% of DM offered as forage) consisted of good quality forages (corn silage, legume silage, small grain silage and sorghum silage) and were balanced for energy and protein according to the NRC (2001) growth rate near to optimal (0.85 kg/d) was observed with AFC of 24 mo and milk 305 d 8,700 kg (Bjelland et al., 2011).

Stair-step program

Stair-step programs are based on the compensatory growth response, combining both restriction and subsequent over allowance of energy that induces enhanced growth rate (Park et al., 1987; Park et al., 1998; Heinrichs et al., 2017). During compensatory growth, greater body weight (**BW**) gains are observed compared with heifers in a control group. This increase in BW is the result of increased appetite and feed intake, but as basal metabolic rate was depressed during the restricted-fed phase, the energy required for maintenance is less, and the spared energy is directed to tissue growth, improving the overall efficiency of growth (Park et al., 1998; Hornick et al., 2000; Park, 2005).

Each step in the program includes one restriction phase followed by one phase of over allowance of energy (Park, 2005). During the restriction phase the diet usually is

formulated for an allowance of ADG of near to 0.9 kg/day according to the NRC for all nutrients except protein (12-14 % CP) and energy which is restricted between 15 to 30%; additionally, the amount of DM offered was 30% below the control group. During the over allowance phase, the diet was fed ad libitum and formulated to offer 30 to 40% more energy than the restricted diet and 16-18% of crude protein. These changes are made mainly by changing the forage to concentrate ratios and including high-fat oilseeds such as sunflower seeds. The stair-step program can have 1 to 3 steps and the duration of each restriction phase vary from 3 to 5 mo, each over allowance phase lasting between 2 to 3 mo (Park et al., 1985; Park et al., 1987; Park et al., 1989; Peri et al., 1993; Choi et al., 1997; Ford and Park, 2001) which coincides with allometric growth phases (pre-puberty, puberty or late gestation; Tucker, 1987).

In general, dairy heifers fed under stair-step programs had ADG between 0.9-2.1 kg/d during the over allowance phases, but during the restriction phase, the ADG varies widely between 0.25 to 0.77 kg/d. Overall, stair-step fed heifers compared with control group animals eat less, have growth rates similar or slightly over controls, and its reproduction performance and AFC are similar. However, the development of the mammary gland of stair-step fed heifers is better; the tissue content of ADN, RNA, and protein higher, and the lipid is lower compared with the control heifers. Additionally, heifers produce 6 to 10% more milk during the first lactation (Park et al., 1985; Park et al., 1987; Park et al., 1989; Choi et al., 1997; Park, 2005) and this difference can last to the 2nd lactation (Ford and Park, 2001). The biggest issue with this feeding strategy is

management logistics as some farms have complications dividing heifers in feeding groups and keeping track of the different diets.

Bulk forage feeding program

Bulk forage feeding programs consist on feeding ad libitum diets with forages low in energy and with high NDF content that will physically limit the dry matter intake through gut fill (Greter et al., 2008; Coblentz et al., 2012). Research with this program has been conducted with pregnant heifers (Coblentz et al., 2012; Coblentz et al., 2015; Su et al., 2017; Coblentz et al., 2018) and prepubertal heifers (Greter et al., 2008). In general, ad libitum TMR diets based on corn silage, alfalfa haylage and a high NDF forage (eastern gamagrass haylage, wheat straw, rye straw, corn fodder or alfalfa stemlage) are fed on different pen stocking rates (100, 120, 125 or 130%). With this feeding system, dietary NDF content usually is over the 45% recommended in the NRC (2001).

Overall, DMI and energy intake are reduced, but ADG maintained between 0.79 to 1.04 kg/d. However, heifers sort for long particles and NDF (thus fibrous material). Also sorting may be more severe when straw is fed compared with eastern gamagrass silage or corn fodder (Greter et al., 2008; Coblentz et al., 2012; Coblentz et al., 2015; Su et al., 2017; Coblentz et al., 2018).

Limit-feeding program

Limit-feeding programs were originally developed for the beef industry. High-energy diets are fed in restricted amounts of DM increasing the digestibility of nutrients, decreasing manure output and reducing costs (Loerch, 1990). Lammers et al. (1999a, 1999b) applied the concept of limit-feeding to test the effect of accelerated growth on puberty of dairy heifers and future milk yield performance. A nutrient-dense diet formulated to allow 1.0 kg/d of ADG was fed ad libitum (accelerated group) or the same diet was limit-fed at 2.5% of BW to allow a standard ADG of 0.7 kg/d (restricted group). Although the heifers on the restricted ADG group attained puberty later than the ones on the accelerated group, no differences on AFC were observed and a 7% higher milk yield was observed on the standard growth rate heifers.

Limit-feeding programs have been shown as an excellent tool to study the digestive physiology of growing heifers as allowed for the separation of the effects of amount of DMI and to control the forage to concentrate and CP to ME ratios. Lammers and Heinrichs (2000) used a limit-feeding strategy to avoid the confounding effects of DMI and test the effects of different CP to ME ratios on growth performance. Using 4-mo old heifers, researchers limit-feeding at 2.45% of BW and evaluated three CP to ME ratios (46:1, 54:1, and 61:1 g of CP/Mcal of ME). The heifers on the high ratio of CP to ME had better feed efficiency, frame growth, and slower rate of BCS gain than the heifers with low and medium ratios. To evaluate the effects CP to ME ratio and degradability of the CP, Gabler and Heinrichs (2003a, 2003b, and 2003c) conducted a

series of studies with heifers between 125 and 234 kg of BW using limit-feeding at 2.0% of BW. Researchers observed that the CP to ME ratio of 63:1 g CP/Mcal of ME with more availability of soluble CP, increased feed efficiency without affecting N digestibility or excretion, synthesis of microbial protein, and DM digestibility. Additionally, Anderson et al. (2015a) limit-fed heifers of 4 mo of age at 2.45% of BW to evaluate diets of 64.7% forage and 33.3% concentrate with a corn-soybean, low- or high-fat distillers dried grains with solubles (**DDGS**). The diets had a CP to ME ratios of 64:1, 63:1 and 67:1 g CP/Mcal of ME, for corn-soybean, low- and high-fat DDGS, respectively. And observed feed efficiencies (0.151 kg gain/kg DM) and ADG (0.96 kg/d) were similar to the ones observed by Lammers and Heinrichs (2000). Moreover, Manthey et al. (2016) demonstrated that the increase of the inclusion rate of DDGS (30, 40, and 50% of the diet DM) in prepubertal limit-fed heifers had a linear positive effect on feed efficiency without differences in BCS or frame growth among treatments. Additionally, for all the groups a precocious onset to puberty (238 d of age and 254 kg of BW) was observed (Manthey et al., 2017) without negatively affecting AFC (23.3 mo of age), weight at calving (623.5 kg BW) or milk production (28.5 kg/d) during the first three months of lactation (Manthey and Anderson, 2017).

Hoffman et al. (2007) limit-fed gravid dairy heifers at 10 and 20% the DM offered compared to a control diet fed ad libitum; although the diets could be considered high in forage, the forage to concentrate ratio was changed (94:6, 80:20, and 63:37) augmenting the nutrient density as the restriction and the concentrate rate increased. However, the

ADG was not different, which caused a tendency for feed efficiency to be higher for the restricted-fed groups, and no differences on milk yield from 0 to 150 DIM were observed.

Overall, limit-feeding or target-feeding programs for dairy heifers allow higher feed efficiency, maintain growth rates near to the optimal (0.8 kg/d) both with high forage (80:20) or low forage diets (60:40), without negative effects on age at puberty, AFC, body weight at calving, and milk production when diets are offered to prepubertal or pregnant heifers (Hoffman et al., 2007; Zanton and Heinrichs, 2007; Lascano et al., 2009; Kase et al., 2010; Zanton and Heinrichs, 2010; Anderson et al., 2015b; Anderson et al., 2015b; Manthey et al., 2017; Manthey and Anderson, 2017). Diets formulated to target specific ADG with 0.8 kg/d are usually the most common. The amount of DM is limited by percentage from the actual DMI of a reference group, usually between -20 to -10% (Hoffman et al., 2007; Kruse et al., 2010); by limiting the amount of ME per kg of metabolic weight ($BW^{0.75}$) usually to 0.22 Mcal. This is also referred as precision-feeding (Lascano et al., 2009; Lascano and Heinrichs, 2009; Lascano et al., 2012a; Lascano et al., 2009b; Lascano et al., 2011) or by limiting the DM offered at a specific percentage of BW which can be as low as 1.5% (Zanton and Heinrichs, 2008) as high as 2.65% (Lawrence et al., 2016; Manthey and Anderson, 2018).

One of the concerns with this system are some behavioral changes since the heifers spent less time eating and more time standing and vocalizing; however, time laying was not different between groups (Hoffman et al., 2007; Kitts et al., 2011). Provision of straw has been used as an option to increase the time eating; however, feed

efficiency is decreased (Kitts et al., 2011) and the number of displacements at the feed bunk increased 2- fold to 6-fold depending on how the straw was offered (mixed vs. as choice; Kitts et al., 2011; Greter et al., 2011). Other research demonstrated limit-fed heifers at 2.05% BW are more motivated to eat than heifers fed ad libitum (Greter et al., 2015). Another option to decrease the number of displacement and increase the time eating was proposed by Greter et al. (2013a) by increasing feed delivery times/d. In their experiment delivery time (2×) decreased the number of displacements by half. However, it also decreased eating time and feed efficiency. Modifying the bunk space did not have any effects on number of displacements or eating time (Greter at al., 2011; Greter et al., 2013a). One of the common characteristics of these studies is that limit-fed diets were restricted between 1.8 to 2.0% of BW.

Moreover, the use of limit-fed diets allows for less inclusion of feedstuffs high in NDF (corn stover, wheat straw, ensiled wet distillers grain with solubles and soybean hulls, sorghum silage) without sorting such as an issue as with bulk feeding (Lascano et al., 2011, Ding et al., 2015; Lascano et al., 2016; Anderson et al., 2009; Pino et al., 2017; Pino and Heinrichs, 2017). Limit-feeding at 2.45% of BW concentrate grain mix or DDGS alone and fed grass hay ad libitum to prepubertal heifers (220 d old) allowed ADG of 0.98 kg/d without fattening (BCS of 3.1), feed efficiencies of 0.16, and DM digestibilities between 57 to 60 % (Manthey and Anderson, 2018).

Alternative feedstuffs for dairy heifers

The use of by-products as feedstuffs for heifers is a good option to reduce feed cost and promote heifer growth (Clark et al., 1984). Using limit-fed programs allows researchers to evaluate these alternative feeds safely since these new feedstuffs can have high amounts of NDF, fat, or antinutritive components. In ad libitum diets these compounds could negatively affect heifer performance, impede their nutritive evaluation, and probably hide their potential and future marketability. One of the best examples are DDGS which used to have high fat content. In one experiment heifers were limit-fed at 2.45% of BW with low- or high-fat DDGS replacing part of ground corn or soybean meal of the control diet. Although ADG was 0.94 kg/d during the peripubertal phase of growth, they had similar growth performance, digestibility of nutrients, maintained metabolic status, without negative effects on onset to puberty or milk yield pubertal (Anderson et al., 2015a, Anderson et al., 2015b, Anderson et al., 2015b).

Lawrence et al. (2016) evaluated the use of camelina meal a co-product of the oil extraction from *Camelina sativa* oilseed, which has a high content of glucosinolates an antinutritive compound which can cause bitter taste and may have negative effects on growth performance of animals. Heifers were limit-fed at 2.65% of BW with a diet containing camelina meal (at 10% of the DM). Growth performance, metabolic profile, and digestibility of nutrients were comparable with heifers fed linseed meal or DDGS.

Brassica carinata

Brassica carinata (from the Latin “carina”- keel of a ship, keeled, for the form of the valves of the fruit), usually known as carinata, is an oilseed crop that is part of the mustard family (Brassicaceae or Cruciferae) which includes over 3700 species, grouped in over 338 genres (Seegeler, 1983; Al-Shehbaz et al., 2006; Oguntoyinbo et al., 2016). Other common names are Ethiopian mustard, Abyssinian mustard or African Kale. The Brassicaceae family includes ornamental and crop species such as vegetables, including food and non-edible oilseeds like rapeseed and camelina, forage and condiments (Al-Shehbaz et al., 2006). Eurasia and Middle-East are the presumptive points of origin of the Brassica species, which may be related to their good adaptation to semi-arid regions of the World (Barthet, 2008; Marillia et al., 2014).

Carinata crop has a variety of agronomic characteristics that make it thrive in environmental conditions where other crops cannot succeed. This crop can grow in marginal or fallow lands because it has a short growing season, off-season growing, has cold weather tolerance, low input requirements, and is a good rotational crop for small grain crops such as wheat. It is also resistant to aphids, flea beetles and blackleg disease (Drenth et al., 2014; Marillia et al., 2014; Zhao et al.,2016; Basili and Rossi, 2018). It has moderate salinity resistance and has been shown to efficiently uptake heavy metals in soils irrigated with sewage water which makes it a friendly-environment option for phytoextraction of soil contaminants (Seegeler, 1983; Quartacci et al., 2007; Fiorentino et al., 2014). Compared with other oilseeds from the same family, carinata has greater grain

yields than canola (*Brassica napus*) under low rainfall conditions and high air temperatures (Cardone et al., 2003; Xin and Yu, 2013). Originally grown in the Ethiopian highlands and North of Kenya, *Brassica carinata* can grow in the Mediterranean and semi-arid climates such as Italy, Spain, Canada and India (Seegeler, 1983; Johnson et al., 2013).

The average oil content of carinata seeds is between 38-44% (Ban et al., 2018; Del Gatto et al., 2015; Atabani et al., 2013); however, environment plays a big role in determining its oil content which can be as low as 12% in extreme drought conditions or as high as 52% in humid temperate conditions (Alberti, 2017). Approximately, 48% of the carinata oil is composed of erucic acid, followed by oleic (20%), linoleic (11%), and palmitic acids (8%) (Zhao et al., 2016); however, its fatty acid composition can be modified through conventional, non-transgenic breeding approaches allowing the development of germplasm with a wide range of oilseed types such as high oleic acid (> 80%), low linolenic acid (< 2%), and high erucic acid content (> 55%) (Nabloussi et al., 2008). This makes carinata an advantageous feedstock for production of non-petroleum jet-fuel, biofuel, bio-oil, and other high-value added components and chemicals (Marillia et al., 2014; Enjalbert et al., 2013; Cardone et al., 2003; Jadhav et al., 2005). Additionally, carinata oil can be used to produce on-farm fuel as a triglyceride blend (the straight vegetable oil is mixed with another less viscous fuel as E10), and used as a substitute for petroleum diesel (Drenth et al., 2015). Furthermore, carinata is a potential crop for biodiesel and biochemicals production because of its low production cost, ability

to grow in semi-arid climate fallow lands without competing with food crops, and its non-edible oil (Marillia et al., 2014; Zhu et al., 2016).

Therefore, carinata crop has several advantages over conventional oilseed crops used as feedstocks for biofuels, which has been increasing the interest on carinata oil as a biofuel feedstock in the United States (Great Plains and Pacific Northwest), Canada, and Italy (Cardone et al., 2003; Marillia et al., 2014; Drenth et al., 2015; Zhu et al., 2016). To date, field tests of *Brassica carinata* have been successful across South Dakota, Minnesota, North Dakota, Montana, Mississippi and Florida (Gesch et al., 2015; Zhao et al., 2016; Alberti, 2017).

The use of brassicas oilseeds as feedstock spurred research interest in the last few years because of the cost of petroleum-derived jet fuel. In 2016, U. S. commercial airlines used approximately 18 billion gallons of jet fuel (EIA, 2017). Additionally, the relationship between jet fuel prices with petroleum prices and the dependence of commercial airlines has increased the interest in the development of bio-jet fuel alternatives (Biello, 2008). The commercial aviation industry has set a goal of carbon-neutral growth by 2020 and a reduction of 50% of petroleum-derived jet fuel use for 2050 compared with the 2005 use (Gersch et al., 2015; Chu et al., 2017).

In 2009, the U. S. Navy started flight test with biojet fuel using blends of camelina oil (*Camelina sativa*) another member of the Brassica family (Biello, 2009b). In the same year, Japan Airlines flew a Boeing 747 using a blend of camelina and other vegetable oils for 90 minutes, and the Dutch airline KLM tested in a one-hour flight a

blend with 50% camelina oil in one of its four engines (Biello, 2009c). However, since 2012, the U.S. military has shown interest in the carinata oilseed as feedstock and begun flight trials with 100% carinata based jet fuel, where without modification of the engine, a 50% reduction in aerosol emissions compared with conventional jet fuel was observed (NRC Canada, 2012; Marillia et al., 2014). Furthermore, in 2018, the Australian airline Qantas used biojet fuel from carinata oil in a 15-hour intercontinental flight from Los Angeles, US to Melbourne, Australia (Qantas, 2018).

However, the economic success of a new industrial oil crop not only depends on the oil but requires finding a use and a market for the residue once the oil is extracted (Matthäus and Angelini, 2005; Van Dyne and Raymer, 1992). The co-product of carinata oilseed resulting from oil extraction is known as carinata cake or meal which can be used for power stations by fast pyrolysis (Sonnek, 2015; Fiorentino et al., 2014) or as a protein meal that could be used as feedstuff for livestock (Marillia et al., 2014). However, marketing the meal as a source of protein for animals feed makes more economic sense (Matthäus and Angelini, 2005).

Carinata meal

Carinata meal is the co-product of the oil extraction from carinata seeds. This meal is attractive as feedstuff for livestock because it is a good source of protein (48%), rich in sulfur-containing amino acids, and with less fiber content compared with canola meal (co-product of another brassica) (Marillia et al., 2014; Yu et al., 2014). However, similar to other protein meals derived from crops part of the family of Brassicas (canola,

camelina, rapeseed, mustard), it has anti-nutritional substances as erucic acid (depending of the crop variety) and glucosinolates (Marillia et al., 2014; Nabloussi et al., 2008) which restrict the inclusion rate of mustard meals used in non-ruminant animal diets to 5-10% (Brown, 2015; EFSA, 2008) and to 10 % of the diet for cattle and sheep (AFFCO, 2014).

The in situ and in vitro nutritional evaluations of carinata meal as a feedstuff for dairy cattle demonstrate it is a good source of degradable protein in the rumen and has a total protein digestibility comparable to soybean and linseed meals (Lawrence and Anderson, 2018). It has a better total digestibility of protein than canola meal and distillers dried grains with solubles (Lawrence and Anderson, 2018; Ban et al., 2017; Xin and Yu, 2014). Additionally, evaluation of carinata meal pelleted with peas, showed total protein digestibility being superior to canola meal pellets (Guevara-Oquendo et al., 2018). However, to date, all studies that evaluated the nutritional quality of carinata meal for dairy cattle have been based on small scale in situ and in vitro measurements lacking an in vivo evaluation component. Ruminants can generally tolerate mustard meal dietary inclusions of up to 10% (*Brassica juncea*, *B. nigra*, or *B. alba* or *Sinapis alba*; AAFCO, 2014; Durge et al., 2014). Previous research with Holstein dairy heifers fed camelina meal that has a similar nutrient profile, but has different glucosinolates compared to carinata meal, showed no differences in growth performance compared with heifers fed DDGS or linseed meal (Lawrence et al., 2016).

Anti-nutritional substances of carinata oilseeds and meal

Content and type of anti-nutritional substances on Brassicaceae plants can be affected by several factors such as plant species, cultivar, agronomic settings, and environmental conditions (Fahey et al., 2001; Björkmann et al., 2011). Lower content of glucosinolates have been observed in brassicas plants grown during autumn and winter, under sulfur and nitrogen deficiencies, and when grown in extreme temperatures or with deficient irrigation rather than the optimal (Björkmann et al., 2011).

Fatty acid profile and glucosinolates content of carinata seeds can be modified by conventional plant breeding; therefore, varieties with low- or high-content of erucic acid, glucosinolates or both can be produced (Barro et al., 2002; Nabloussi et al., 2008). Processing during oil extraction may affect the amount of erucic acid and glucosinolates on the meal as the amount of residual oil varies, and heat, water, and solvents degrade or bind glucosinolates to different extents (Bones and Rossiter, 2006; Clark, 2010). Oil extraction can be performed by mechanical and mechanical-chemical processes. Mechanical oil extraction process is known as cold-pressing which can be divided into two steps: 1) preparation, which depending on the oilseed can include cleaning, dehulling, crushing, rolling, or flaking; 2) oil extraction, where the seeds are squeezed through a screw press. Pressure forces out oil and the rest of the seed (meal) exits the press through a nozzle. The exit nozzle's diameter and the rotational speed of the screw can vary. Oil recovery percentages are between 60-80%. Therefore residual oil on the meal can be greater than 5%. Although cold-press oil extraction does not require external

heat, because internal friction heat is generated but is not allowed to reach up to 120˚C (Sackey, 2015). Cold-pressing can be performed in small-scale biodiesel facilities or at farm level (Hristov et al., 2011; Drenth et al., 2015). Mechanical-chemical processing is known as solvent-extraction and can be divided in three steps: 1) mechanical oil extraction, cold-pressing of the seeds; water addition, dry heating at approximately 90˚C, and depending on the plant, flaking before the cold-press process is performed; 2) the solvent oil extraction which consists in percolating the extruded seeds in a solvent solution (usually hexane) where the solvent binds with the residual oil; and 3) in the solvent recovery step, a desolventizer-toaster evaporates the residual solvent on the meal, after the meal is dry cooled. Time, temperature, and use of dry heat or steam may vary depending on the plant. Oil recovery percentages are between 95.0 to 99.5% (Newkirk et al., 1997; Newkirk and Classen, 2002; Xin et al., 2014; Sackey, 2015).

Erucic acid

To our knowledge, there is no research reporting the content of erucic acid in carinata meal. Erucic acid effects have been studied in laboratory animals and results suggest deleterious effects such as potential myocardial lesions that reduce the contractility of myocardium and abnormal fat accumulation (Björkman et al., 2011; FSANZ, 2003). The doses of erucic acid associated with myocardial lipidosis are 1,500 mg/kg BW/d in rats and 900 mg/kg BW/d in nursing pigs (FSANZ, 2003). In dairy cattle fed rapeseed meal high in erucic acid (42% of total lipids) at an inclusion rate of 12% of the diet dry matter decreased feed intake and a reduction in milk production was observed

(Hristov et al., 2011). There are no toxicologic studies in humans, but epidemiologic studies indicate that erucic acid may occur in human myocardium in geographic areas where vegetable oils containing erucic acid are consumed; however, evidence of an association between erucic acid intake and myocardial lessons was not observed (FSANZ, 2003).

Glucosinolates

Glucosinolates are not harmful by themselves and are important plant defense metabolites. Cellular breakdown because of mechanical damage, infection or insect attack, expose glucosinolates to hydrolysis by degradative enzymes known as myrosinases or thioglucosidases. The hydrolysis products (isothiocyanates, thiocyanates, nitriles, and epithioalkanes) are the ones that may cause some issues (Duncan and Milne, 1993; Bones and Rossiter, 2006; EFSA, 2008; Björkman et al., 2011). The glucosinolates amount, their profile, and their hydrolysis products (**Table 1.1**) vary between Brassica species and cultivars (Fahey et al., 2001; Zukalová and Vašák, 2006; Björkmann et al., 2011; Berhow et al., 2013).

Carinata meal as other co-products derived from the Brassicaceae family (camelina, rapeseed, canola) has high concentrations of sinigrin, which when hydrolyzed forms allyl isothiocyanate, allyl cyanide and allyl thiocyanate, substances that may cause bitter taste potentially decreasing oilseed meals palatability and health problems which limit their use as feedstuffs (Marillia et al., 2014; Tripathi and Mishra, 2007; Tsao et al., 2000).

Table 1.1. Some of the glucosinolates (**GSL**) found in Brassica species (Adapted from Zukalová and Zukalová and Vašák, 2006; Vaughn and Berhow, 2005; Clarke, 2010; Berhow et al., 2013)

Systematic name	Common name	Hydrolysis products
Group I		
Aliphatic		
2-propenyl (allyl)-GLS	Sinigrin	Isothiocyanates
3-butenyl-GLS	Gluconapin	
4-pentenyl-GLS	Glucobrassicanapin	
Group II		
Hydroxy-aliphatic		
2-hydroxy-3-butenyl-GLS	Progoitrin	Oxazolidine-2-thiones
2-hydroxy-4-pentenyl-GLS	Napoleiferin	
Group III		
Cyclic		
4-hydroxybenzyl-GLS	Sinalbin	
Heterocyclic (indolyl)		
3-indolylmethyl-GLS	Glucobrassicin	
1-methoxy-3-indolylmethyl-GLS	Neoglucobrassicin	
Group IV		
Sulfur chains		
9-(sulfinyl)-nonyl-GLS	GS9 or glucoarabin	3-butenyl isothiocyanate
10-(methylsulfinyl)-decyl-GLS	GS10 or glucocamelinin	
11-(methylsulfinyl)-undecyl-GLS	GS11	

Health problems observed after the ingestion of mustard (*Brassica* spp.) seeds and glucosinolates by cattle and the subsequent release of mustard oils are lesions in the gastrointestinal tract including profuse edema of the forestomachs and abomasum, mucosal necrosis, and hemorrhage of the cecum and colon (Majak, 2001). Specifically, thiocyanates resulting from the breakdown of glucosinolates, are goitrogenic agents that cause hyperplasia and hypertrophy of the thyroid gland. Thiocyanates inhibit uptake of inorganic iodide by the thyroid gland, apparently in a competitive way since the inhibition can be reversed with iodide supplements (Brown, 2015; Tripathi and Misra,

2007; Majak, 2001) and consequently cause hypothyroidism. Although, induced hypothyroidism on prepubertal Brahman heifers increased 1.7-fold ADG and almost 1 unit of BCS compared with control heifers (Thrift et al., 1999). Hypothyroidism could negatively affect follicular steroidogenesis as observations in vitro demonstrated that triiodothyronine in the presence of follicle stimulating hormone and insulin increases the synthesis of androstenedione in theca cells (Spicer, 2001).

Isothiocyanates irritate mucous membranes (EFSA, 2010), and nitriles or cyanides cause growth depression, and lesions in the liver and kidney (Brown, 2015; Tripathi and Misra, 2007). However, these isothiocyanates also occur naturally in food such as horseradish and mustard and are readily cleared from rat and mouse tissues so that within 24 hours after administration only less than 5% of the total dose was retained in tissues. The clearance is even faster in humans (2 h; EFSA, 2010). There are some clinical reports of photosensitivity in cattle caused from brassicas, generally turnip (*Brassica rapa*) or kale (*Brassica oleracea*) used as a fodder in New Zealand, specifically nitrile product from glucosinolate hydrolysis, where the skin hardened, cracked and sloughed, occasionally presenting jaundice and subcutaneous edema of the lower limbs, and liver damage after 3 or 4 days of having access to the brassica forage (Collett and Matthews, 2014).

Rationale and significance

As the research and use of carinata oilseeds as a biofuel source increases, and the crop does not compete with others such as corn and soybeans, availability of carinata

meal as a source of crude protein for the dairy industry will increase in the Great Plains and South Eastern areas of the US. Additionally, if the carinata crop is produced locally, it may become a good competitor of imported canola meal. Therefore, evaluation of the use of carinata meal for growing heifers and its effects on taste preferences, intake, nutrient digestibility, and growth performance and their possible impacts on thyroid and metabolic hormones, and onset of puberty will also impact future research and evaluation of the meal on lactating cows and other options for use of this co-product. Additionally, testing the use of ensiling to reduce the glucosinolate content will offer an option to increase the quality of silages and provide greater latitude to include more of the meal in dairy heifers and lactating cow diets.

CHAPTER 2. SHORT-TERM TASTE PREFERENCE OF CARINATA MEAL COMPARED WITH OTHER OILSEED MEALS AND DISTILLERS DRIED GRAINS WITH SOLUBLES

ABSTRACT

Our objective was to determine if the type and content of glucosinolates in carinata meal affected dairy heifer short-term preference and intake compared to canola meal, camelina meal, linseed meal and distillers dried grains with solubles. Six Holstein heifers (7.2 ± 0.3 mo old; 234.7 ± 15.7 kg of body weight [**BW**]) were used in a sequential elimination taste preference study to compare five different grain mixes containing each 27.4 % dry matter (**DM**) basis of cold-pressed carinata meal (**CRM**), cold-pressed camelina meal (**CAM**), solvent-extracted canola meal (**CAN**), solvent-extracted linseed meal (**LIN**), and distillers dried grains with solubles (**DDGS**). Heifers were kept in individual pens (3.7 × 4.5 m) with a row of 7 feed containers. Grain mixes were offered for 30 min in the morning and evening. Intake of each grain mix and feeding behavior were registered at each feeding time. At each feeding time, the positions of grain mixes were randomized, and the two end containers were left empty to nullify the effects of placement. Grass hay was fed at 1.6% of BW throughout the day in a separate tub. To determine preference ranking, during phase 1, all 5 grain mixes were offered for 5 d, and the most preferred by each heifer was removed at the end of the phase. In the subsequent phases, days and number of grain mixes were reduced sequentially, until only 2 grain mixes were offered during 2 d. Preference ranking by

heifer was then based on intake amounts. Kendall's coefficient of concordance (***W***) was calculated to evaluate the agreement of preference among heifers. Type, total content, and profile of glucosinolates was different on the Brassica oilseed-derived meals. Total DM intakes (**DMI**) were 3.90 ± 1.74, 5.91 ± 1.39, 6.60 ± 1.47, and 6.49 ± 1.16 kg/d for phases 1, 2, 3, and 4, respectively. During phase 1, when all grain mixes were offered, grain mix DMI/d were 1.58 ± 0.57, 0.20 ± 0.43, 0.16 ± 0.17, 0.14 ± 0.57, and 0.07 ± 0.13 kg/d for DDGS, LIN, CRM, CAN, and CAM, respectively. Heifers preferred DDGS first, LIN second, CRM and CAN were tied for third, and CAM was fourth with $W = 0.64$ and $P = 0.009$ indicating agreement in preference rankings among heifers. Despite greater glucosinolate content, CRM was comparable in taste preference to CAN, had greater preference compared to CAM, and less preference compared to DDGS or LIN.

Keywords: dairy heifer, taste preference, carinata meal, glucosinolates, Brassica

Introduction

Carinata oilseed crop (*Brassica carinata*) is being developed as a new feedstock for biofuel production. After extraction of the oil, the co-product meal is of interest to be used as a livestock feed. It is a good source of rumen degradable protein, with a total tract digestibility of the protein similar to soybean and linseed meals (Lawrence and Anderson, 2018) and it has a better total digestibility of protein than canola meal and distillers dried grains with solubles (Lawrence and Anderson, 2018; Ban et al., 2017; Xin and Yu, 2014). Carinata meal, as well as other protein meals derived from Brassica species such as canola, camelina, and rapeseed, has glucosinolates (Marillia et al., 2014; Lawrence et al.,

2016). Glucosinolates by themselves are non-harmful but are precursors of secondary metabolites which may have anti-nutritional properties which vary depending on the chemical structure of the original glucosinolates (Fahey et al., 2001; Majak, 2001; Bones and Rossiter, 2006; Singh et al., 2007). To date, more than 120 different glucosinolates have been described but 8 are the most common in Brassicas (Fahey et al., 2001; Zukalová and Vašák, 2006; Clarke, 2010). Presence of glucosinolates in oilseed meals are associated with bitter taste which may affect the palatability of the meal (Tripathi and Mishra, 2007), and potentially decrease the intake or require the animal be given an adjustment period (Chapter 3). Beef cows fed canola meal or carinata meal obtained by two different oil extraction processes (cold-pressing or solvent-extraction) as supplement in amounts to supply the RDP requirement of the cows during a 56-d trial, consumed 14% less of the cold-pressed carinata meal, than the cows supplemented with canola meal or solvent-extracted carinata meal (Rosenthal et al., 2017). In contrast, no intake differences of cold-pressed camelina meal, linseed meal or DDGS were observed on limit-fed dairy heifers with diets that included 10% of the diet dry matter (DM) during a 12 wk trial (Lawrence et al., 2016). Long-term intake differences of oilseed meals derived from Brassicas have been attributed to the glucosinolates content and the bitter taste caused after their hydrolysis (Tripathi and Mishra, 2007), but attributing long-term intake differences to taste preferences may not be accurate when the animal does not have feed choices (Marten, 1978; Nombekela and Murphy, 1995).

To our knowledge, there is no information about short-term or initial taste preference of carinata meal compared with different oilseed meals for dairy heifers. This

would be very valuable to alert dairy producers of possible initial intake issues when these feedstuffs are used in rations. Therefore, our objective was to identify the short-term or initial taste preferences of cold-pressed carinata meal compared with different oilseed meals and DDGS using a sequential elimination taste preference study. This experimental design has been used by several researchers to evaluate cow and calf taste preferences (Nombekela et al., 1994; Erickson et al., 2012; Chapman et al., 2016). It was hypothesized that as the content and type of glucosinolates vary depending on the oilseed meal, the taste preference would also be different, affecting the dry matter intake (DMI) of dairy heifers.

Materials and Methods

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 15-060A. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01. Heifers were observed daily for any injury or disease problems and treated according to normal farm management practices at the Dairy Research and Training Facility (Brookings, SD).

Experimental Design

To meet our objective a 14-d sequential elimination taste preference study was conducted using 6 Holstein heifers [7.2 ± 0.3 mo of age and 235±16 kg body weight (BW)]. Test feeds were offered in isonitrogenous and isoenergetic grain mixes to avoid

the effect of preference over nutritive characteristics of the test feeds (Miller-Cushon et al., 2014). Five different grain mixes containing 27.4% (DM basis) of carinata meal (CRM), camelina meal (CAM), canola meal (CAN), linseed meal (LIN), or distillers dried grains with solubles (DDGS) were tested. To avoid feed familiarity effects on initial diet selection (Miller-Cushon et al., 2011), instead of soybean meal which is a common ingredient of starter and grower pellets, linseed meal was chosen as control oilseed meal as it contains no glucosinolates, whereas DDGS was chosen as a non-oilseed control. The inclusion amount of test feeds in grain mixes targeted 10% (DM basis) of the test feed in the total ration when forage was also included (**Table 2.1**). Diets were formulated using the dairy NRC software (2001), and the remainder of the grain mixes were comprised of ground corn, soybean meal, and mineral mix, inclusion rate varying slightly to make isonitrogenous and isoenergetic grain mixes and avoid the effect of preference over nutritive characteristics of the test feeds (Miller-Cushon et al., 2014). Based upon previous research (Lawrence et al., 2016) grass hay was offered at 1.6% of BW and half of total grass hay was fed in the morning and the other half was fed in the evening and left in the pen in a separate tub to be consumed throughout the day. Orts of hay were weighed and recorded in the morning before feeding. Each heifer was housed individually (3.7 × 4.5 m pens) with a row of 7 feed containers for the grain mixes. Five containers (27.5 x 27.5 x 26.5 cm) each with one of the grain mixes were positioned randomly at each feeding in the 5 middle spots of the manger and two empty containers were included on each end to nullify border and position effects. At each feeding, grain mixes were weighed individually for each heifer. Grain mixes were offered ad libitum

during 30-minutes in the morning and evening at approximately 0800 h and 1800 h. After each of the 30-minute periods, the feeders were collected, and orts weighed and recorded. Water was available ad libitum. Each pen was roofed and bedded with straw as a manure pack.

Heifers were adapted to research pens and feeders for 2-d, followed by an experimental period of 14-d. During phase 1, all 5 diet grain mixes were offered from d 1-5. After the fifth day of data collection, the treatment with the overall greatest consumption (first place preference) was removed and replaced by an empty container. Phase 2 was comprised of another 4 days of data collection, the treatment with the overall greatest consumption (second place preference) was removed and replaced by an empty container. Phase 3 was another 3 days of collection, the treatment with the overall greatest consumption (third place preference) was removed and replaced by an empty container. Phase 4 was the last 2 d of the experiment with only the remaining 2 least liked treatments fed to determine the 4th and 5th preferences. Preference ranking for each heifer was based on intake amounts. Rankings were determined by giving 1 to the grain mix the heifer preferred the most (consumed the most DMI during the first 5-d phase when all the treatments were given) up to 5 for the grain mix the heifer preferred the least. Rankings were averaged by the number of heifers used, to determine overall rankings.

Animal Measurements and Sampling

Three samples of each grain mix and hay were collected during the study and stored at -20°C until processing and analysis could be completed as described under laboratory analysis. At the beginning and the middle of the study, 500 g samples of hay and individual concentrate mix ingredients (corn, soybean meal, CRM, CAN, CAM, LIN, and DDGS) were collected and dried by duplicate for 24 h at 105°C for DM analysis to adjust dietary ingredient inclusion amounts of grain mix and determine dry matter intakes (DMI).

At the beginning and end of the study, body growth measurements including BW, withers height, hip height, hip width, heart girth, paunch girth, and body length were recorded to characterize the heifers. The measurement for body length was taken from the top point of the withers to the end of the ischium. Body condition scores were recorded by 4 independent observers based on a quarter-point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). Body weight was measured during 2 consecutive days at the beginning of the study to determine the amount of hay to offer to heifers.

Laboratory Analysis

Total dietary nutrient concentrations were calculated based on analyses of grass hay and grain mix for each treatment. Hay and grain mix samples were thawed and composited on as-fed basis by weight and sent to a commercial laboratory for nutrient analysis (Dairyland Laboratories Inc., Arcadia, WI). Samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC International, 2002, method 990.03).

Nitrogen content was then multiplied by 6.25 to calculate crude protein (CP). Acid detergent fiber (ADF) (AOAC International, 2002, method 973.18). For neutral detergent fiber (NDF), heat-stable α-amylase and sodium sulfite were used (AOAC International, 2002, method 2002.04 2005). Lignin (AOAC International, 1996, method 973.18). Petroleum ether was used to determine ether extract (EE; AOAC International, 2002, method 920.39) in a Foss Soxtec 2047 fat analysis system (FOSS, Hilleroed, DK). Nonfibrous carbohydrates were calculated as % NFC = 100 – (% ash + % CP + % NDF + % EE) according to the NRC (2001). Organic matter was calculated as OM = (100 - % ash). Minerals (Ca, Cl, Mg, P, K, Na, and S) were analyzed and dietary cation-anion difference (DCAD) calculated. Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC International, 2002). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY).

Glucosinolate analysis and quantitation were performed by another laboratory under the supervision of Mark Berhow (USDA, Agricultural Research Service). Analysis methods performed on the original test feeds (CRM and DDGS) 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 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 chromatography mass spectrometry to find

glucosinolate composition, and reversed-phase HPLC at 237 nm was used to determine concentrations of individual glucosinolates.

Statistical Analysis

Kendall's coefficient of concordance (*W*) was calculated to evaluate the agreement of preference among heifers (Nombekela et al., 1994; Erickson et al., 2012; Chapman et al., 2016).

Results and discussion

Inclusion amounts of ground corn and soybean meal were slightly different to balance the diet to be isonitrogenous and isoenergetic (**Tables 2.1** and **2.2**). Nutrient composition of all grain mixes (**Table 2.3**) was comparable with the formulated for CP, ME, and NFC for each grain mix (coefficient of variation [CV] < 5%). Contents of ADF and NDF in grain mixes offered varied slightly (CV between 5 to 9%) but differences were small compared with the formulated except for CAN grain mix which were greater than in the formulated grain mix. The nutrient that varied the most was EE (CV between 8 to 15%) being greater for CRM, CAM, and DDGS grain mixes and less for the LIN and CAN mixes. We attribute the variation to be from differences in the fat content of the meals and the slight differences in the inclusion rates of corn and soybean meal among grain mixes.

Content of glucosinolates (μM/g and mg/g) of the test feeds is presented in **Table 2.4**. The greatest content of glucosinolates was observed in CRM, followed by CAM, and

CAN; LIN and DDGS do not contain glucosinolates. Carinata and canola meal shared the type of glucosinolates (sinigrin, progoitrin, and gluconapin), but had a different profile. Sinigrin was the predominant glucosinolate in CRM (83%) followed by progoitrin (10%) and gluconapin (7%). The distribution of glucosinolates was more uniform for CAN (43%, 31%, and 27%, sinigrin, progoitrin, and gluconapin, respectively). Camelina meal had a unique composition of glucosinolates, the greatest proportion was glucocamelin (60%) with similar proportions of glucoarabin (19%) and camelinin (15%). Total content of glucosinolates for CRM and CAN were below the values reported by Mailer et al. (2008), which tested glucosinolates content on laboratory solvent-extracted meals from different brassicas oilseeds from Australia and other parts of the World (B. carinata [64 – 167 μM/g] and B. napus [9 – 169 μM/g]). They also differed from the ones reported by Ban et al. (2017) for cold-pressed carinata meal (168.5 μm/g), solvent-extracted carinata meal (115.2 μm/g) and canola meal (3.4 μm/g). Lawrence and Anderson (2018) reported profiles and total content of glucosinolates for canola meal (2.7 mg/g), camelina meal (12.4 mg/g) and carinata meal (48.6 mg/g). The three meals in the present experiment had values below those observed by the previous authors; however, the profile of glucosinolates for CAN and CAM were consistent between studies, with the exception of sinigrin found as the only glucosinolate in carinata meal. In general, glucosinolates profile and total content for CAM are consistent with the ones observed in seeds of different genotypes (18.7 – 36.2 μM/g; Schuster and Friedt, 1998; 15.2 – 24.6 μM/g) and meal (12.45 mg/g; Lawrence et al., 2016).

Overall, total dry matter intake increased throughout the experiment (**Figure 2.1**) indicating that the removal of the most preferred feed did not adversely affect total DMI. Total DMI by heifer during phase 1 (5-d) of the experiment is in **Table 2.5**. The grain mix with DDGS had top preference suggested by the amount consumed by all heifers; it was therefore ranked in 1st place. A similar table was prepared for each phase until preference rankings for the five grain mixes where completed by each heifer (**Table 2.6)**. Heifers preferred DDGS first, LIN second, CRM and CAN were tied for third, and CAM was fourth with $W = 0.64$ and $P = 0.009$, indicating agreement in preference rankings among heifers.

In **Table 2.7**, the average DMI for individual heifers by each phase is shown; the average DMI of four of the heifers increased in phases 1 to 3. During phase 3, DMI of two heifers decreased, ranking LIN 2nd. Therefore their choices were the three brassica oilseed meals. For the last phase, all heifers had CAM grain mix and the three heifers that ranked LIN 3rd decreased DMI, of these heifers two ranked CAM and one ranked CRM as 5th.

Nombekela et al. (1994) observed that cows preferred bitter after sweet flavor, and that sour and salty flavors were less preferred. Glucosinolates are reported to produce a bitter taste when they are hydrolyzed (Duncan and Milne, 1993; Majak, 2001). Glucosinolate content in foods is associated with bitter flavor perception in humans, principally from sinigrin (D'Antuono et al., 2009), which is consistent with our results. The similar preference for CRM and CAN may be due to both having the same

glucosinolates, but in different proportions, with sinigrin predominating in both. Although in a long-term experiment, CAM did not affect negatively DMI (Lawrence et al., 2016), it appears that given a choice heifers would prefer feeds other than CAM.

Conclusions

Results of the literature review for this experiment showed it to be the first study on short-term preference of Holstein heifers fed glucosinolates-containing oilseeds meals. Content of glucosinolates was greatest in CRM, although this meal was comparable in preference with CAN, which had the least content of glucosinolates, and is already commonly used as a feedstuff for dairy cattle. It appears the glucosinolates profile is the main factor affecting preference, at least in the short-term. Findings of this study are important because dairy producers need to be aware that taste preference may cause heifers to need an adjustment period to different oilseed meals or may consume them better if they are mixed with other, more palatable feeds.

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Table 2.1. Ingredient and nutrient composition of the formulated diets with forage included to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)[1]

	Diet[1]				
Item, % DM	CRM	CAN	CAM	LIN	DDGS
Grass hay	63.39	63.39	63.55	63.39	63.49
Carinata meal	10.00	-	-	-	-
Canola meal	-	10.00	-	-	-
Camelina meal	-	-	10.00	-	-
Linseed meal	-	-	-	10.00	-
DDGS	-	-	-	-	10.00
Ground corn	14.31	15.48	15.32	14.52	12.89
Soybean meal	10.65	9.52	9.52	10.48	12.01
Vitamin and mineral pre-mix[2]	0.65	0.65	0.65	0.65	0.65
Calcium carbonate	0.48	0.48	0.48	0.48	0.48
Salt	0.48	0.48	0.48	0.48	0.48
Nutrient					
CP	16.0	16.1	16.0	16.0	16.0
ADF	30.4	31.3	31.2	31.4	30.9
NDF	47.5	48.3	49.1	48.3	48.7
EE	4.3	2.4	3.0	2.5	3.0
NFC	29.0	30.3	29.2	30.6	29.7
ME, Mcal/kg DM	2.43	2.40	2.37	2.35	2.37
NEg, Mcal/kg DM	0.92	0.86	0.88	0.87	0.88
Glucosinolates, mg/g	2.23	0.085	1.18	-	-

[1]Formulated (NRC, 2001).

[2]Contained: Ca 18.9%, NaCl 24.5%, Mg 1.6%, K 0.5%, Cu 880 mg/kg , I 50 mg/kg, Se 25 mg/kg, Zn 3,880 mg/kg, vitamin A 551,146 UI/kg, vitamin D_3 110,229 UI/kg, and vitamin E 4,189 UI/kg (HeiferSmart No Phos, Purina Animal Nutrition LLC, Shoreview, MN).

Table 2.2. Nutrient composition of the formulated grain mixes offered to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)

	Grain mix[1]				
Nutrient, % DM	CRM	CAN	CAM	LIN	DDGS
CP	29.1	29.5	29.3	29.1	29.1
ADF	5.9	8.4	7.7	8.3	6.8
NDF	10.2	12.7	14.1	12.2	13.1
EE[2]	7.9	2.8	4.5	3.1	4.4
NFC[3]	46.4	48.4	45.8	49.5	47.3
ME[4], Mcal/kg DM	3.32	3.02	3.12	3.06	3.12

[1]Formulated (NRC, 2001).
[2]EE = Ether extract.
[3]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).
[7]Values calculated based on glucosinolate analysis (Table 5) and inclusion rate (10%) of CRM.
[4]ME = Metabolizable energy; values calculated based on inputting sample nutrient analysis into ration formulations in the Dairy NRC (2001).

Table 2.3. Nutrient composition of grass hay and the grain mixes offered to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)

	Grain mix					
Nutrient, % DM	CRM	CAN	CAM	LIN	DDGS	Hay
DM, %	88.17	87.31	87.32	86.26	91.82	88.68
Ash	8.10	8.44	7.65	8.81	7.96	10.31
OM	91.90	91.56	92.35	91.19	92.04	89.69
CP	28.71	28.29	28.28	27.22	28.23	6.39
ADF	5.28	9.54	7.51	7.35	6.28	41.87
NDF	8.58	13.11	11.67	11.25	12.20	62.12
Lignin	0.19	2.86	1.25	1.62	0.80	4.26
EE	8.08	3.17	5.54	3.20	5.20	2.50
NFC	46.55	48.21	46.87	49.73	46.71	21.98
Ca	1.22	1.46	1.21	1.50	1.08	0.33
P	0.57	0.51	0.56	0.52	0.53	0.13
Mg	0.26	0.30	0.28	0.32	0.27	0.18
K	1.25	1.12	1.16	1.14	1.27	1.64
S	0.48	0.32	0.38	0.24	0.32	0.14
Na	0.66	0.68	0.59	0.67	0.80	0.02
Cl	1.10	1.12	1.10	1.08	1.24	0.45
DCAD[6], mEq/100 g	-0.67	6.30	0.23	12.94	11.99	21.50
TDN[1]	88.01	76.11	82.02	78.07	82.18	56.28
ME[2], Mcal/kg DM	3.45	2.94	3.20	3.03	3.21	2.10
Glucosinolates, g/kg	6.12	0.23	3.24	-	-	-

[1]Total digestible nutrients (calculated from ingredients).
[2]Estimated: ME = 1.01x (TDN*0.04409)-0.45, Eq. 2-2 from NRC (2001).

Table 2.4. Content and profile of glucosinolates in test feeds carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)

	CRM[1]		CAN[2]		CAM[1]		LIN[3]		DDGS[3]	
Glucosinolate	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total, μM/g	61.64	1.475	2.28	0.023	22.74	2.264	0.00	-	0.00	-
Progoitrin, μM/g	6.05	0.036	0.69	0.014	-	-	0.00	-	0.00	-
Sinigrin, μM/g	51.23	2.975	0.97	0.054	-	-	0.00	-	0.00	-
Gluconapin, μM/g	4.36	0.042	0.61	0.031	-	-	0.00	-	0.00	-
Glucoarabin, μM/g	-	-	-	-	4.42	0.416	0.00	-	0.00	-
Glucocamelin, μM/g	-	-	-	-	14.92	1.558	0.00	-	0.00	-
GS11, μM/g	-	-	-	-	3.39	0.290	0.00	-	0.00	-
Total, mg/g	22.32	0.528	0.85	0.008	11.84	1.179	0.00	-	0.00	-
Progoitrin, mg/g	2.35	0.014	0.27	0.005	-	-	0.00	-	0.00	-
Sinigrin, mg/g	18.34	1.065	0.35	0.019	-	-	0.00	-	0.00	-
Gluconapin, mg/g	1.63	0.016	0.23	0.012	-	-	0.00	-	0.00	-
Glucoarabin, mg/g	-	-	-	-	2.24	0.211	0.00	-	0.00	-
Glucocamelin, mg/g	-	-	-	-	7.78	0.813	0.00	-	0.00	-
GS11, mg/g	-	-	-	-	1.82	0.160	0.00	-	0.00	-

[1]Cold pressed meal: content of glucosinolates in a solvent-extracted meal could be different.
[2]Solvent-extracted meal.
[3]Feeds that naturally do not contain glucosinolates but were tested.

Table 2.5. Total dry matter intake (DMI) by heifer and average DMI for grass hay, and each grain mix offered to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS) during phase 1 (5-d) of the experiment

	Grain mix					Hay
Heifer	CRM	CAN	CAM	LIN	DDGS	
1	0.97	0.32	0.97	1.04	4.93	21.13
2	0.73	3.21	0.24	0.24	9.14	16.79
3	1.12	0.24	0.00	0.00	8.89	19.60
4	1.54	0.16	0.40	0.16	10.19	12.45
5	0.32	0.24	0.40	4.40	8.09	14.58
6	0.08	0.16	0.08	0.24	6.15	15.10
Total DMI[1] 5-d, kg	4.86	4.33	2.09	6.08	47.38	99.6
Mean DMI 5-d, kg	0.81	0.72	0.35	1.01	7.90	16.61
Mean DMI, kg/d	0.16	0.14	0.07	0.08	1.58	3.32

[1]Total DMI by heifer of each grain mix during phase 1 (5-d) of the experiment was calculated to determine which mix was the most preferred by intake amounts and therefore ranked 1st; a similar table was prepared for each phase (not showed) and ranks where assigned until having the overall preference ranking of the five grain mixes by each heifer

Table 2.6. Overall rankings[1] of treatments for taste preference[2] of test feeds carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)

Heifer	DDGS	LIN	CRM	CAN	CAM
1	1	3	2	5	4
2	1	2	5	3	4
3	1	3	5	2	4
4	1	3	2	4	5
5	1	2	5	3	4
6	1	3	2	4	5
Average	1.0	2.7	3.5	3.5	4.3

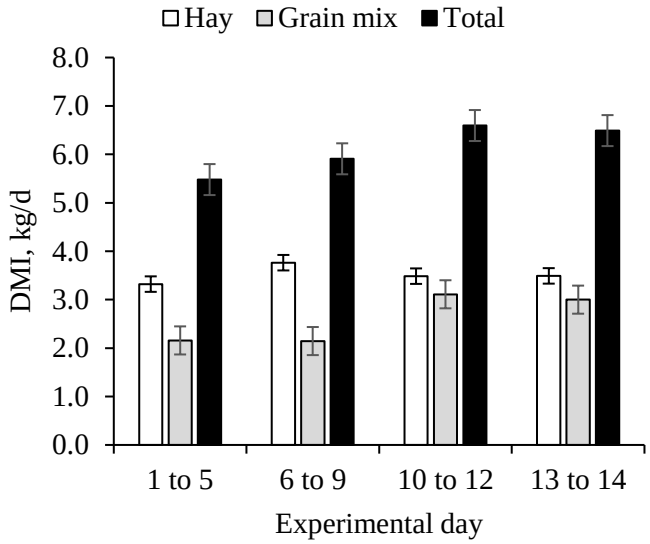
[1]Preference ranking for each heifer based on intake amounts. Rankings were determined by assigning 1 to the grain mix the heifer preferred the most (consumed the most DMI during the first 5-d phase when all the treatments were given) and up to 5 for the grain mix the heifer preferred the least.

[2]Rank of treatment diets is given with 1 = most preferred and 5= least preferred.

Table 2.7. Average dry matter intake (DMI, kg/d) by heifer for each phase of the experiment to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS)

	Experiment	Heifer					
Phase	days	1	2	3	4	5	6
1	1 to 5	5.87	6.07	5.99	4.98	5.61	4.36
2	6 to 9	5.28	7.59	6.33	4.60	6.92	4.72
3	10 to 12	4.55	7.55	7.54	7.26	5.30	7.37
4	13 to 14	6.77	7.74	5.18	6.32	5.39	7.54
Mean		5.62	7.24	6.26	5.79	5.80	6.00

Figure 2.1. Total dry matter intake (DMI) of grass hay, grain mixes offered to test taste preference of carinata meal (CRM), canola meal (CAN), camelina meal (CAM), linseed meal (LIN), and distillers dried grains with solubles (DDGS) for phases 1, 2, 3, and 4.



CHAPTER 3. EVALUATION OF CARINATA MEAL AS A FEEDSTUFF FOR GROWING DAIRY HEIFERS: EFFECTS ON GROWTH PERFORMANCE, RUMEN FERMENTATION, AND TOTAL TRACT DIGESTIBILITY OF NUTRIENTS

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ABSTRACT

Our objective was to determine the effects of feeding carinata meal (**CRM**) compared with distillers dried grains with solubles (**DDGS**) on growth performance, rumen fermentation, and nutrient utilization in peripubertal dairy heifers. A 16-week randomized block design experiment with 24 Holstein heifers [6.6 ± 0.7 mo and 218 ± 27 kg of body weight (**BW**)] was conducted. Treatments diets were: 1) 10% cold-pressed CRM, and 2) 10% DDGS on a dry matter (**DM**) basis. The remainder of the diets consisted of grass hay, ground corn, soybean meal and mineral mix. Diets were formulated to be isonitrogenous and isocaloric. Heifers were individually fed using a Calan gate feeding system, and the rations were limit-fed at 2.65% of BW on DM basis to target a 0.8 kg/d average daily gain. Heifers were weighed every 2 wk and the ration amount offered was adjusted accordingly. Frame sizes, BW, and body condition scores were measured 2 d every 2 wk throughout the study. During week 12 and 16, rumen fluid

samples were collected via esophageal tubing for pH, ammonia N, and volatile fatty acid analyses. In week 16, fecal grab samples were collected for apparent total tract digestibility estimation. Heifer DM intake, BW, average daily gain, and gain:feed were similar between treatments. There were no differences between treatments in frame measurements or body condition scores. Rumen pH tended to be greater in CRM compared to DDGS. Rumen ammonia N, and total volatile fatty acid concentration were not different between treatments. Apparent total tract digestibility of DM, neutral detergent and acid detergent fiber was decreased in CRM compared with DDGS. A tendency was detected for reduced organic matter digestibility in CRM. There was no difference between treatments for crude protein total tract digestibility. However, these differences in total tract nutrient digestibility were not large enough to influence growth performance. Overall, results demonstrated that growing heifers can be limit-fed diets with 10% CRM and maintain growth performance compared to a control diet containing 10% DDGS.

Keywords: dairy heifer, carinata meal, growth performance

Introduction

One of the priorities for dairy producers is to decrease the cost of raising heifers through strategies that optimize the growth of the heifers and minimize cost without sacrificing productivity (Gabler et al., 2000; Tozer and Heinrichs, 2001). One option to decrease costs is to use less-expensive co-products from the growing renewable biofuels industry. Co-products such as distillers dried grains or canola meal are 67.8% and 23.6%, respectively, less expensive than soybean meal (Gould, 2017). Additionally, government programs are focused on increasing the use of renewable fuels (U.S. Department of Energy, 2015). These circumstances are encouraging the development of new feedstocks to produce biofuels, and hence the possibility of new feedstuffs that could be used to feed dairy replacement heifers.

One feedstock of new and increasing interest in the Great Plains is carinata (*Brassica carinata*) because of its high oil content and unique fatty acid profile that is favorable for biofuel production. It also has promising agronomic properties, such as good adaptation to dry climates and could be grown in areas where other more common crops cannot adapt (Marillia et al., 2014). Carinata oilseeds have been genetically selected to have very high concentrations of very-long-chain fatty acids, such as erucic acid (C22:1), which can be used to produce biofuels and bio-oils more efficiently compared to other oilseeds (Cardone et al., 2003; Enjalbert et al., 2013). After the oil extraction, the resulting carinata meal has a high content of rumen degradable protein, which has a total protein digestibility comparable to that of soybean and linseed meals (Lawrence and Anderson, 2015).

A disadvantage of carinata meal is that it contains glucosinolates, which are anti-nutritional compounds. Glucosinolates are present in plants of the Brassicaceae family (rapeseed, camelina, and carinata). By themselves glucosinolates are innocuous, but when the vegetative parts of the plant are damaged, they are degraded and may cause bitter taste and irritate the mucous membranes (Duncan and Milne, 1993; Majak, 2001). Therefore, potentially decreasing the palatability of these oilseed meals. Although, ruminants generally can tolerate diets of 10% rapeseed meal which also contains glucosinolates (Brown, 2015). Some glucosinolates decrease the thyroid function through interference with iodine uptake, potentially affecting the growth and animal performance (Forss and Barry, 1983; Duncan and Milne, 1992; Geertmann et al., 1994; Tsao et al., 2000; Tripathi and Mishra, 2007; Marillia et al., 2014).

Thus, the objective of this research was to conduct an initial study to determine the effects of feeding carinata meal on growth performance, rumen fermentation, and nutrient utilization of peripubertal dairy heifers compared with a control diet containing distillers dried grains with solubles. We hypothesized that, as carinata meal has high crude protein content and quality, its inclusion in the diet at 10% (DM basis) would maintain or enhance the growth performance of dairy heifers without negatively affecting rumen fermentation or nutrient digestibility compared with the control diet.

Materials and Methods

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 15-060A. The

institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Experimental Design

To meet our objectives a 16-wk randomized complete block design feeding study was conducted using 24 Holstein heifers (6.6 ± 0.7 mo of age and 218 ± 27 kg BW) with 2 treatment diets. The feeding study was conducted over 11 mo from August 2015 to June 2016 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). Heifers were blocked in groups of two based on birthdate and body weights. Heifers were randomly assigned to treatment after assignment to block. Heifers were added on the study in groups of 6 animals or 3 blocks at different times based on age and availability with a target starting age of 6.5 mo. Heifers were habituated to the research barns and feeding system for 2 wk, followed by an experimental feeding period of 16 wk.

Treatments diets were (1) cold-pressed carinata meal (**CRM**), and (2) distillers dried grains with solubles (**DDGS**) both at 10% of the diet on a dry matter basis. The DDGS was chosen as a control diet, and for comparison as it has been shown it can replace corn and soybean meal in dairy heifer diets (Anderson et al., 2015; Manthey et al., 2016). Additionally, the fat content of DDGS also allowed for a closer total fat content between diets when compared to other common protein sources. The remainder of the diets were comprised of grass hay, ground corn, soybean meal and mineral mix to meet nutrient requirements and formulated to allow for similar intakes of protein and

energy between treatments (**Table 3.1**). The dietary inclusion of 10% as CRM was used as it is the limit established by the FDA for rapeseed meals which are from a similar plant family and have similar glucosinate concentrations (Benz, 2010). The two treatment diets were both limit-fed at 2.65% of BW.

Animal Care and Feeding

Heifers were observed daily for any injury or disease problems and treated according to normal farm management practices at the Dairy Research and Training Facility. Heifers were housed in pens in groups of 6 heifers. Each pen had an inside roofed area (7 m x 4 m) and an outside soil exercise lot (7 m x 23.5 m). The inside areas of the pens were manure pack and bedded with straw once per week to discourage consumption of straw.

Heifers had ad libitum access to fresh water. Feeding occurred once daily at 0600 h using the Calan gate feeding systems (American Calan Inc., Northwood, NH). Every morning before feeding, any orts were weighed, and the individual intakes were measured. As mentioned, rations were formulated using the NRC (2001) to be limit-fed at 2.65% of BW (DM basis) to meet requirements of a heifer weighing 250 kg and to target 0.8 kg/d of average daily gain (**ADG**) as recommended by Hoffman (1997) and Zanton and Heinrichs (2005). The 250 kg of BW was a pre-estimated average BW for heifers during the study based on age and previous herd data. Rations were adjusted every 2 wk based on the BW recorded on the last 2 d of the previous 2-wk interval and DM of feeds. At each feeding, coarsely ground brome grass hay and grain mix were

individually weighed for each heifer into a large tub, hand mixed, and then placed in the Calan boxes. As rations were limit-fed, heifers consumed all of their rations between daily feedings during the majority of the experimental period, and sorting was not an issue. Each week samples of the grass hay and grain mixes were taken. Each month samples of individual concentrate mix ingredients (corn, soybean meal, CRM, and DDGS) were also taken. All feed samples were stored at -20°C until processing and analysis could be completed as described under laboratory analysis.

Animal Measurements and Sampling

Body growth measurements including BW, withers height, hip height, hip width, heart girth, paunch girth, and body length were taken on 2 consecutive days at the beginning of the study and then every 2 wk during the study at 4 h post-feeding. Body length was measured from the top point of the withers to the end of the ischium (Hoffman, 1997). Body condition scores were recorded every 2 wk by 4 independent observers based on a quarter-point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). Rumen fluid was collected via esophageal tubing during wk 12 and 16 on 2 consecutive days, at 4 h post feeding at the same time as body measurements were being taken. After discharging the first 200 mL of fluid to minimize saliva contamination, approximately 50 mL of rumen fluid was collected. The pH of the samples was immediately measured using a pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10 mL) were acidified with either 200 μL of 50% (vol/vol) sulfuric acid or 2 mL of 25% (wt/vol) meta-phosphoric acid and stored

at -20°C until later analysis of ammonia N (**NH_3-N**) and volatile fatty acid (**VFA**). During 3 consecutive days in wk 16 of the feeding period, fecal grab and ort samples were collected for analysis of total tract digestibility of nutrients using acid detergent insoluble ash (**ADIA**) as an internal marker. Fecal sampling time points were scheduled so that the samples represented every 3 h in a 24-h feeding cycle. Samples were stored at -20°C until processing and analysis.

Laboratory Analysis

Total dietary nutrient concentrations were calculated based on analyses of grass hay and grain mix for each treatment. Every 2 wk throughout the study an aliquot of feed samples was dried for 24 h at 105°C for DM analysis to adjust dietary ingredient inclusion amounts and determine dry matter intakes (**DMI**). Samples of ground corn, soybean meal, CRM, DDGS, grass hay, CRM grain mix, and DDGS grain mix were collected once weekly and frozen at -20°C until analysis. Feeds and grain mix weekly samples were thawed and samples from 4 consecutive weeks were composited on as-fed basis by weight. Composite samples were dried in duplicate for 48 h at 55°C in a Dispatch oven (Style V-23, Dispatch Oven Co., Minneapolis, MN), ground to 4-mm particle size with a Wiley Mill (model 3, Arthur H. Thomas Co., Philadelphia, PA), and further ground to 1-mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To correct nutrient analyses to 100% DM, 1-g aliquots of ground feed samples were dried for 4 h in a 105°C oven (Model 28, Precision Scientific Co., Chicago, IL). Ash content (AOAC International, 2002 method 942.05) was determined

by incinerating a 1-g sample for 8 h at 450°C in a muffle furnace (Model F1730, Thermolyne Corp., Dubuque, IA; temperature controller Model Wheelco 293, Barber-Colman Co., Rockford, IL). Organic matter was calculated as **OM** = (100 - % ash). Samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC International, 2002, method 968.06), on a rapid N Cube (Elementar Analysensysteme, GmbH, Hanau, Germany). Nitrogen content was then multiplied by 6.25 to calculate crude protein (**CP**). Neutral detergent fiber (**NDF**) (Van Soest et al., 1991) and acid detergent fiber (**ADF**) (Robertson and Van Soest, 1981; AOAC International, 2002, method 973.18) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable α-amylase and sodium sulfite were used (AOAC International, 2002, method 2002.04). Petroleum ether was used to determine ether extract (**EE**; AOAC International, 2002, method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Nonfibrous carbohydrate was calculated as % **NFC** = 100 – (% ash + % CP + % NDF + % EE) according to the NRC (2001).

Dried and ground samples of grass hay, CRM, and DDGS grain mixes were further composited into 5 and 6 mo composites and sent to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for analysis of minerals (Ca, Cl, Mg, P, K, Na, S, Fe, Mn, Mo, Cu, and Zn) and dietary cation-anion difference (**DCAD**). Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC International, 2002). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY).

Glucosinolate analysis and quantitation in the CRM were performed by a laboratory at the USDA, Agricultural Research Service (Peoria, IL). Analysis methods performed on the original test feeds (CRM and DDGS) 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 feed samples were extracted with methanol and analyzed using liquid chromatic mass spectrometry to find glucosinolate composition, and reversed-phase HPLC at 237 nm was used to determine concentrations of individual glucosinolates.

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 x *g* for 20 min at 4°C (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for NH_3-N using a colorimetric assay performed on a microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) according to Chaney and Marbach (1962). Rumen fluid samples that were preserved with 25% meta-phosphoric acid were thawed and centrifuged at 30,000 x *g* for 20 min at 4°C and analyzed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations using an automated GC (model 6890, Hewlett-Packard Co., Palo Alto, CA) using a flame ionization detector. Volatile fatty acids were separated on a capillary column (15 m x 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethyl butyrate as an internal standard. The split ratio of 100:1 in the injector port was at a

temperature of 250°C with flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140 and 250°C, respectively.

Fecal samples from all collection time points were composited for each heifer on an as-is basis by volume. Aliquots of 100 mL of fecal samples were taken from each time point and composited. Orts (if any were left) were collected three days during the fecal collection period. Orts were composited based on proportions of weight from each day for any heifers that had orts on multiple days. Fecal and orts composites were the dried and ground and were analyzed for DM, ash, CP, NDF, and ADF as previously described for feed composites. Analysis of ADIA consisted of determination ADF content (Robertson and Van Soest, 1981) and the analysis of the ash content using a modified procedure of AOAC method 935.29 (AOAC International, 2002) for all feed composites, fecal and orts samples. Apparent total tract digestibility calculations for DM, OM, CP, NDF, and ADF were determined according to Merchen (1988).

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The MEANS procedure of SAS was used to estimate the means and standard errors of the nutrients of the monthly feed composites.

To determine ADG for body weight and change per day for body frame measurements the difference was found between each data collection time point and the previous time point and then divided by the number of days in the time period [i.e. (wk 2-wk 0)/14 d]. Gain to feed ratio was calculated as the ratio of ADG of body weights to

total DMI for each heifer during each 2-week time interval between weight and frame measurements.

Heifer intakes, gain:feed, growth data, ADG, and rumen fermentation data were analyzed as a randomized complete block design with week as the repeated measure and the term heifer (block) as the subject using PROC MIXED procedures of SAS (Littell et al., 2006). Initial (week 0) body weights and frame measurements were used as covariate terms for each respective variable. The model included treatment, week, and treatment x week interactions. 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 $0.05 < P \leq 0.10$. Least squares means are reported for each treatment in the tables. The slice option was used to determine if differences between treatments were significant at individual weeks or time points of measurements.

The MIXED procedures of SAS were used for the analysis of total tract digestibility of nutrients. As total tract digestibility was analyzed only during wk 16, the model only included treatment with block included as a random variable.

Results and Discussion

Feed Analysis

Inclusion amounts of ground corn and soybean meal were slightly different to balance the diets to be isonitrogenous and isoenergetic (**Table 3.1**) because of the variation in nutrient composition among the two test feeds (**Table 3.2**). The nutrient composition of the grain mixes and grass hay (**Table 3.2**) was consistent during the study. One exception was a slight variation on DM of grass hay over the course of the study was due to changes in season and humidity; however, as the dietary inclusion amount of grass hay was similar between diets these DM changes did not affect our interpretation of treatment effects. The CRM grain mix EE was greater than the DDGS grain mix because of the EE content of the carinata meal which was cold pressed. However, the amount of EE of the grain mix did not markedly increase the fat content of the CRM diet (**Table 3.3**) compared to the original formulation of the CRM diet. Generally, diets were consistent with the formulated diets; however, CP of both diets was 1% less than formulated, this was because the CP of the hay was slightly less during the study than values used for initial diet formulations. When the nutrient composition of the ingredients based on analysis was reentered into the NRC (2001) software, the energy values of the analyzed diets were consistent with the original formulations between treatments for most nutrients.

Heifer Growth Performance

During the study, one heifer died from pneumonia, which was unrelated to treatment. Without any replacement heifer of similar size and age available, the DDGS treatment had a total of 11 heifers and the CRM treatment had 12 heifers.

Dry matter intake, BW, ADG, and gain:feed results are presented in **Table 3.4**. There were time effects for these variables as expected in growing heifers, but there were no effects of treatment. There was an interaction of treatment by week for DMI, and gain:feed ratio because during the first week of the study the DMI of the heifers on the DDGS diet was 1 kg greater than the heifers in the CRM diet (**Figure 3.1**). However, CRM intakes quickly rebounded for the rest of the study. This demonstrated that after the initial adjustment to the treatment ration, intakes were not compromised by feeding CRM and agreed with findings by Lawrence et al. (2016) who fed camelina meal to heifers. Although the CP content of the diet was less than the originally formulated diets, the targeted ADG of 0.8 kg/d was still achieved. This was because the CP of the diets still was above the ideal amount of CP to achieve maximum microbial fermentation (Tamminga, 1992).

Frame size measurements and BCS are shown in **Table 3.5**. No treatment by week interactions were found for any of the frame growth measurements. There was an increase over the time for the frame size measurements as expected in growing animals. Additionally, there were no effects of treatment in change per day for any of the growth variables measured. To the extent of our knowledge, this is the first study on the effects of feeding CRM to growing dairy heifers. There is only one study where carinata meal

pellets were fed to Angus crossbred beef heifers (Schulmeister et al., 2016) at 0.3% of the BW (as fed basis) where the researchers observed an increase of 57% in ADG compared with beef heifers fed with only grass. Although we did not observe differences between treatments in the current study, both groups of heifers achieved the target ADG of 0.8 kg/d, which is the optimal rate of gain for growing heifers (Zanton and Heinrichs, 2005). Additionally, studies with dairy heifers (Lawrence et al., 2016) and beef heifers (Grings et al., 2014) on feeding camelina meal (which has comparable nutrient composition to CRM) compared to DDGS also found no differences in growth performance. However, the ADG observed in the present study is greater than the observed in dairy heifers fed camelina meal (Lawrence et al., 2016). Overall frame growth and size were normal and comparable to other feeding studies by our research group with heifers in this age range (Anderson et al., 2015; Lawrence et al., 2016; Manthey et al., 2016).

Rumen Fermentation Characteristics

Collection of rumen samples via esophageal tubing at a single time point in a day is not an optimal or ideal method of collection. However, as this was one of the first feeding studies on feeding CRM to heifers we considered it valuable to determine at a preliminary level if rumen fermentation was affected (**Table 3.6**). There was concern that the glucosinolates and long-chain fatty acids in the CRM diet may negatively affect rumen microorganism and fermentation. Although acetate concentrations were greater in the rumen fluid samples of the heifers fed CRM and butyrate rumen fluid concentrations were greater in heifers fed DDGS, no differences were found for propionate

concentrations between treatments. Additionally, the volatile fatty acids proportions and ammonia concentrations were comparable to other studies that also collected rumen fluid via esophageal tubing by our research group with heifers of this age (Lawrence et al., 2016; Manthey et al., 2016). However, because of sampling methodology, these results should be regarded with caution and more research is warranted with cannulated heifers or cows to substantiate that feeding CRM at 10% of diet DM does not negatively alter rumen fermentation.

Apparent Total Tract Digestion of Nutrients

Apparent total tract nutrient digestibilities of nutrients are presented in **Table 3.7**. Crude protein digestibility was similar among treatments, whereas digestibility of DM, OM, NDF, and ADF was greater for the DDGS diet. Overall apparent total tract nutrient digestibility values were also comparable to other studies with this age of dairy replacement heifers (Anderson et al., 2015; Lawrence et al., 2016; Manthey et al., 2016). The relatively small differences between treatments in fiber and consequentially DM and OM digestibility could be attributed to the difference in non-forage fiber content between the two test ingredients. Another possibility, is the difference of DCAD between DDGS and CRM grain mixes. Martins et al. (2016) found a positive association between DCAD and NDF total tract digestibility which could be attributed to major activity of cellulolytic bacteria. In this study, the greater butyrate proportion in the rumen fluid of the heifers in DDGS may support this hypothesis. However, the differences in total tract digestibility were not large enough or of enough biological significance to affect the overall growth

performance or gain:feed of the heifers. We hypothesized that the crude protein total tract digestibility on the CRM diet may be greater compared to DDGS diet because of differences in the RDP content (Lawrence and Anderson, 2015), but found in the current study that CP digestibility was similar. It is speculated that the difference in RDP was compensated for by the high digestion of RUP in the intestines (Kleinschmit et al., 2007).

Conclusions

This research study is one of the first, which we are aware of, on feeding CRM to growing dairy heifers. In this study, we showed that despite containing some glucosinolates, heifers can adapt to the taste of CRM and DMI will not be affected for long. However, producers need to be aware that heifers may initially need a week or two of adaptation period to adjust to CRM flavor. Although rumen fermentation and total tract digestibility of nutrients had some minor differences compared to the DDGS diet, body frame growth and ADG were maintained at recommended rates throughout the study. In this initial study, a limit-feeding strategy was utilized to control overall intakes. More research may be warranted using other feeding strategies such as in diets fed ad libitum as TMR. Also, more research is needed to determine interactions with other types of feeds and evaluate feeding cold-pressed versus solvent-extracted carinata meal. Overall, this initial research on feeding carinata meal demonstrated that it is a viable protein and energy source for dairy heifers that can maintain growth performance when included at 10% of diet DM. Carinata meal shows potential as a by-product of the biofuels industry that can be used as a new feedstuff for growing dairy heifers.

Acknowledgments

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Table 3.1. Ingredient composition of diets with carinata meal (CRM), and distillers dried grains with solubles (DDGS) fed to growing dairy heifers[1]

	Diet[1]	
Ingredient, % DM	CRM	DDGS
Grass hay	63.53	63.53
Carinata meal	10.00	-
DDGS	-	10.00
Corn grain, ground	14.51	13.01
Soybean meal, solv. 48% CP	10.51	12.01
Vitamin and mineral pre-mix[2]	0.65	0.65
Calcium carbonate	0.40	0.40
Salt	0.40	0.40

[1]Formulated (NRC, 2001).

[2]Contained: 18.9% Ca, 24.5% NaCl, 1.6% Mg, 0.5% K, 880 mg/kg of Cu, 50 mg/kg of I, 25 mg/kg of Se, 3,880 mg/kg Zn, 551,146 UI/kg of vitamin A, 110,229 UI/kg of vitamin D_3, and 4,189 UI/kg of vitamin E (HeiferSmart No Phos, Purina Animal Nutrition LLC, Shoreview, MN).

Table 3.2. Nutrient composition of the test feeds (carinata meal and distillers dried grains with solubles) and ration components (grain mixes and forage) used to make the carinata meal (CRM), and distillers dried grains with solubles (DDGS) diets fed to growing Holstein heifers

	Test feeds				Concentrate mixes				Forage	
	Carinata meal		DDGS		CRM		DDGS		Brome grass hay	
Item[1]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]
DM[2], %	91.6	0.20	89.6	0.37	88.2	0.30	88.0	0.26	86.1	0.99
Ash[2]	7.3	0.12	5.6	0.09	8.5	0.09	8.3	0.13	8.6	0.34
OM[2]	92.7	0.25	94.4	0.09	91.5	0.09	91.7	0.13	91.4	0.34
CP[2]	38.7	0.00	30.1	0.02	28.7	0.25	28.0	0.26	7.8	0.18
ADF[2]	12.0	0.84	10.2	0.22	5.3	0.15	4.6	0.13	38.2	0.34
NDF[2]	20.0	1.96	28.8	0.35	11.3	0.44	13.9	0.27	66.2	0.58
EE[2,3]	20.1	0.32	9.0	0.17	6.9	0.12	3.8	0.11	1.5	0.08
NFC[2,4]	13.9	2.39	26.5	0.63	44.6	0.70	45.9	0.54	15.9	0.50
Ca[5]	0.54	0.005	0.08	0.000	1.1	0.15	1.2	0.10	0.37	0.000
P[5]	1.2	0.00	0.91	0.000	0.67	0.015	0.60	0.005	0.14	0.010
Mg[5]	0.48	0.000	0.38	0.005	0.27	0.000	0.26	0.000	0.17	0.020
K[5]	1.68	0.010	1.3	0.01	1.34	0.02	1.34	0.04	1.7	0.14
S[5]	1.44	0.02	0.71	0.005	0.57	0.015	0.40	0.020	0.14	0.015
Na[5]	0.01	0.000	0.29	0.000	0.73	0.020	0.77	0.040	0.02	0.000
Cl[5]	0.13	0.000	0.19	0.010	1.1	0.03	1.1	0.05	0.45	0.055
Mo[5], mg/kg	0.47	0.015	0.86	0.095	1.1	0.13	1.5	0.10	5.6	1.40
Mn[5], mg/kg	40.5	0.50	16.5	0.50	74.5	0.50	73.5	3.50	48.5	1.50
Zn[5], mg/kg	64.0	1.00	55.0	0.00	124.5	11.50	118.0	6.00	30.5	1.50
Cu[5], mg/kg	2.0	0.00	3.0	1.00	23.5	5.50	30.0	1.00	3.0	1.00
Fe[5], mg/kg	91.5	1.50	72.0	6.00	89.5	3.50	104.5	14.50	78.5	1.50
DCAD[6], mEq/100 g	-49.8	1.19	-4.8	0.47	-0.27	0.745	12.6	2.27	22.9	1.20
Glucosinolates[7], mg/g	20.6	0.81	-	-	5.6	-	-	-	-	-

[1]% of DM, unless otherwise indicated.
[2]Results from monthly composite samples.

[3]EE = Ether extract.
[4]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).
[5]Results calculated from the analysis of 5 and 6 mo composites of the ration components.
[6]DCAD = dietary cation-anion difference.
[7]Value of the test feed from glucosinolate analysis; value for the CRM grain mix was calculated from glucosinolates analysis and inclusion rate (10%) of the test feed in the diet. Distillers dried grains with solubles and brome grass hay do not contain glucosinolates.
[8]The MEANS procedure of SAS was used to estimate the means and standards errors of the nutrients of the monthly feed composites, and 5 and 6 mo composites of the ration components.

Table 3.3. Overall nutrient composition of diets containing 10% carinata meal (CRM) or 10% distillers dried grains with solubles (DDGS)

	Diet			
	CRM		DDGS	
Item[1]	Mean[8]	SE[8]	Mean[8]	SE[8]
DM[2], %	86.9	0.68	86.8	0.65
Ash[2]	8.5	0.21	8.5	0.22
OM[2]	91.5	0.21	91.5	0.22
CP[2]	15.5	0.15	15.3	0.15
ADF[2]	26.0	0.27	25.7	0.27
NDF[2]	46.0	0.35	46.9	0.38
EE[2,3]	3.5	0.05	2.4	0.06
NFC[2,4]	26.5	0.45	27.0	0.37
Forage NDF[2]	41.7	0.37	41.7	0.37
Nonforage NDF[2]	4.2	0.16	5.2	0.10
Ca[2,5]	0.65	0.055	0.69	0.035
P[2,5]	0.33	0.012	0.31	0.008
Mg[2,5]	0.21	0.013	0.20	0.013
K[2,5]	1.6	0.09	1.6	0.10
S[2,5]	0.29	0.015	0.23	0.002
Na[2,5]	0.28	0.007	0.30	0.015
Cl[2,5]	0.68	0.046	0.67	0.053
Mo[5], mg/kg	3.9	0.93	4.0	0.85
Mn[5], mg/kg	58.1	1.13	57.8	0.35
Zn[5], mg/kg	65.3	5.20	62.9	3.17
Cu[5], mg/kg	10.6	1.41	13.0	1.00
Fe[5], mg/kg	82.6	0.35	88.1	6.31
DCAD[5], mEq/100 g	14.3	0.48	19.1	1.59
Glucosinolate[6], mg/g	2.06	-	-	-
ME[7], Mcal/kg of DM	2.38	-	2.34	-
Neg[7], Mcal/kg of DM	0.87	-	0.85	-

[1]% of DM, unless otherwise indicated.
[2]Results from monthly composite samples.
[3]EE = Ether extract.
[4]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).
[5]Results calculated from the analysis of 5 and 6 mo composites of the ration components.
[6]Value was calculated based on glucosinolate analysis (Table 2) and inclusion rate (10%) of the test feed on the CRM diet.
[7]Values are calculated based on inputting sample nutrient analysis into ration formulations in the Dairy NRC computer program (2001, Washington, DC).
[8]The MEANS procedure of SAS was used to estimate the means and standards errors of the nutrients of the monthly feed composites, and 5 and 6 mo composites of the ration components.

Table 3.4. Dry matter intake (DMI), BW, ADG, and gain:feed ratios for heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

Item	Treatment		SEM	*P*-values		
	CRM	DDGS		Treatment	Week	Treatment × Week
BW, kg						
Mean	269.9	268.9	1.4	0.61	<0.01	0.99
Initial	221.0	214.8	9.5	0.89		
Final	321.5	313.8	8.33	0.74		
ADG, kg/d	0.837	0.825	0.0282	0.76	<0.01	0.97
DMI, kg/d	6.55	6.42	0.159	0.58	<0.01	<0.01
Gain:feed	0.131	0.130	0.0037	0.93	<0.01	<0.01

Table 3.5. Frame size measurements and BCS for Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Item	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Withers height, cm						
Mean	122.8	123.4	0.53	0.46	<0.01	0.22
Initial	115.7	115.6	0.85	0.57		
Final	129.0	128.3	0.94	0.60		
Change, cm/d	0.11	0.11	0.005	0.62	0.72	0.21
Hip height, cm						
Mean	126.0	126.4	0.27	0.30	<0.01	0.43
Initial	120.3	119.9	0.99	0.75		
Final	131.9	131.5	0.78	0.56		
Change, cm/d	0.10	0.10	0.004	0.98	<0.01	0.44
Body length, cm						
Mean	114.9	114.6	0.73	0.76	<0.01	0.84
Initial	106.1	105.6	1.4	0.99		
Final	125.0	124.9	0.98	0.82		
Change, cm/d	0.16	0.16	0.013	0.86	0.05	0.68
Heart girth, cm						
Mean	145.6	145.2	0.53	0.64	<0.01	0.68
Initial	135.4	134.2	1.00	0.67		
Final	156.7	154.6	1.03	0.36		
Change, cm/d	0.18	0.17	0.008	0.48	<0.01	0.52
Hip width, cm						
Mean	38.0	38.2	0.28	0.47	<0.01	0.82
Initial	34.3	34.3	0.28	0.84		
Final	41.5	41.8	0.48	0.15		
Change, cm/d	0.06	0.06	0.003	0.44	0.33	0.67
BCS[1]						
Mean	3.0	3.0	0.01	0.47	<0.01	0.82
Initial	3.0	2.9	0.03	0.05		
Final	3.0	3.1	0.04	0.89		

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

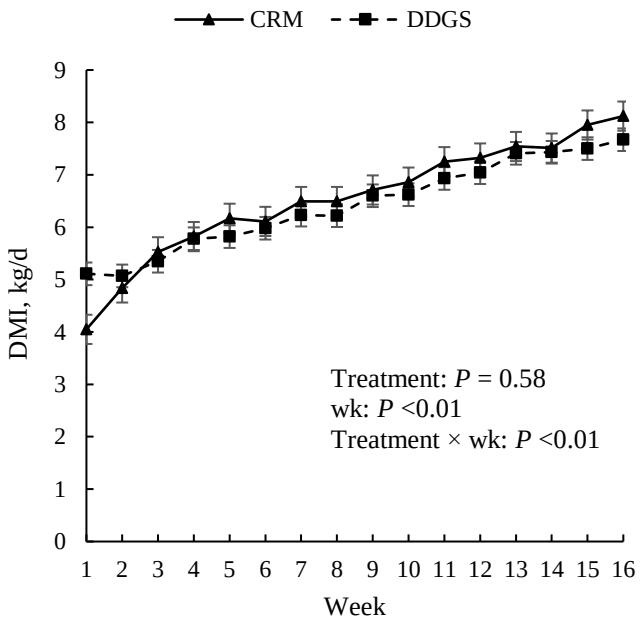
Table 3.6. Rumen fermentation characteristics of growing Holstein heifers fed diets with 10% carinata meal (CRM), or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Item	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
pH	7.0	6.9	0.07	0.19	0.44	0.15
NH_3-N, mg/dL	17.6	15.7	0.99	0.18	0.67	0.20
Total VFA, m*M*	85.5	86.9	4.24	0.81	0.24	0.11
Acetate, mmol/100mmol	67.2	65.8	0.27	<0.01	0.74	0.99
Propionate, mmol/100mmol	21.3	21.6	0.33	0.55	0.06	0.49
Isobutyrate, mmol/100mmol	0.65	0.62	0.106	0.82	<0.01	0.75
Butyrate, mmol/100mmol	8.6	9.8	0.21	<0.01	0.09	0.93
Isovalerate, mmol/100mmol	1.3	1.2	0.05	0.13	0.54	<0.01
Valerate, mmol/100mmol	0.89	0.94	0.028	0.18	0.37	0.30
Acetate:Propionate	3.18	3.08	0.058	0.22	0.15	0.59

Table 3.7. Total tract digestion of nutrients for growing Holstein heifers fed diets with 10% of carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values
Item, % digested	CRM	DDGS	SEM	Treatment
DM	67.4	69.8	2.21	<0.05
OM	70.2	72.5	1.93	0.05
CP	74.9	75.6	0.80	0.54
NDF	60.8	64.5	1.78	<0.01
ADF	68.4	70.9	1.42	<0.05

Figure 3.1. Dry matter intakes (DMI) of growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS) over 16 wk. Error bars represent SEM=0.16



CHAPTER 4. EVALUATION OF CARINATA MEAL AS A FEEDSTUFF FOR GROWING DAIRY HEIFERS: EFFECTS ON METABOLIC PROFILE AND ONSET OF PUBERTY.

ABSTRACT

Carinata meal is a new feedstuff, co-product of the oil extraction of carinata oilseeds. Our objective was to determine the effects of feeding cold-pressed carinata meal on metabolic profile, thyroid hormones, and onset of puberty in peripubertal dairy heifers compared with distillers dried grains with solubles. A 16-week randomized block design experiment with 24 Holstein heifers [6.6 ± 0.7 mo and 218 ± 27 kg of body weight (**BW**)] was conducted. Treatments diets were: 1) 10% cold-pressed carinata meal (**CRM**), and 2) 10% distillers dried grains with solubles (**DDGS**) on a dry matter basis (**DM**). The remainder of the diets consisted of grass hay, ground corn, soybean meal and mineral mix; diets were formulated to be isonitrogenous and isocaloric. Heifers were individually fed using a Calan gate feeding system, and the rations were limit-fed at 2.65% of BW on DM basis. Heifers were weighed every 2 wk and the ration amount offered was adjusted accordingly. Jugular blood samples were collected 3.5 h post-feeding on 2 consecutive d during wk 0, 4, 8, 12, and 16 for metabolite and metabolic hormone analyses. Plasma fatty acid (**FA**) were measured on samples from wk 4 and 16. Throughout the study, coccygeal vein blood samples were taken twice per week for progesterone analysis to estimate onset of puberty. Major FA on CRM diet were C18:2 and C22:1 (0.55 and 0.43% of the DM, respectively) and on DDGS diet C18:2 and C18:1

(0.63 and 0.28% of the DM, respectively). Intake of FA for heifers fed CRM was higher compared with heifers fed DDGS (171.9 vs. 122.9 g/d; $P < 0.01$). Major plasma FA were C5:0, C16:0, C18:0, C18:1 and C18:2 for heifers on both treatments. Glucose, plasma urea nitrogen, insulin, and thyroxine concentrations were similar among treatments ($P > 0.05$). Plasma triiodothyronine (140.2 vs 154.7 ng/dL; $P = 0.068$) tended to be less on CRM compared with DDGS heifers. Insulin-like growth factor-1 tended to be greater in CRM heifers (89.9 vs. 78.2; $P = 0.09$). Cholesterol concentration was greater in heifers fed CRM than in DDGS (89.9 vs. 78.2 d; $P < 0.01$). Age (329 and 319; SEM = 3.1) and weight (319 and 306 kg; SEM = 3.1) at puberty were similar ($P >0.05$) between heifers fed CRM and DDGS. These results demonstrate that growing heifers can be limit-fed diets with 10% carinata meal without negative effects on thyroid hormones, metabolic profile, and onset of puberty.

Keywords: dairy heifer, brassica carinata, glucosinolates, erucic acid, puberty

Introduction

Cost of raising heifers is the second or third largest expense in dairy farms and feeding accounts for near to 73% of rearing expenses (Tozer and Heinrichs, 2001; Heinrichs et al., 2013). One strategy to reduce feeding cost and promote heifer growth is the use of by-product feedstuffs (Clark et al., 1984). Carinata meal is a new feedstuff co-product of the oil extraction of carinata oilseeds (*Brassica carinata*) which has high protein content. Carinata is a non-food oilseed with a high oil content rich in very long-chain fatty acids such as erucic acid (C22:1) useful to produce renewable, non-fossil

biofuels, polymers, plastics, pharmaceutical and nutraceutical oils (Cardone et al., 2003; Zhu et al., 2016). Carinata is receiving considerable interest in North America for its ability to adapt to drought and low fertility soils, being promising for portions of the Great Plains and U.S. Pacific Northwest which currently have limited oilseed cultivation (Marillia et al., 2014; Zhu et al., 2016). In addition, carinata meal is rich in essential sulfur-containing amino acids has low fiber and higher protein content compared with canola meal. The total digestibility of its protein is similar to soybean meal and linseed meal and better than canola meal and distillers dried grains with solubles (Xin and Yu, 2014; Ban et al., 2017; Lawrence and Anderson, 2018). One drawback is that carinata meal, as other meals co-product of oilseed crops (i.e., canola, rapeseed, and camelina), has glucosinolates and erucic acid which may affect animal performance. Glucosinolates are innocuous but their hydrolysis originates secondary products which may cause bitter taste and have antithyroid effects that could impact animal growth (Tripathi and Mishra, 2007; Björkman et al., 2011; Marillia et al., 2014). Erucic acid is associated with abnormal accumulation of lipids on the heart (FSANZ, 2003). However, no growth or metabolic issues were observed on Holstein heifers limit-fed diets of cold-pressed camelina meal at 10% of the diet DM which also contains glucosinolates and erucic acid (Lawrence et al., 2016).

The objective of this research was to conduct an initial study to determine the effects of feeding cold-pressed carinata meal on metabolic profile, thyroid hormones, and onset of puberty of dairy heifers. To determine if carinata meal could be comparable as a feedstuff for dairy heifers, distillers dried grains with solubles was chosen as control as it

has been shown to be a replacement for corn and soybean meal in dairy heifer diets without causing changes in ADG or negative long-term performance (Anderson et al., 2015a, Anderson et al., 2015c). We hypothesized that as carinata meal has high crude protein content and quality, its inclusion in the diet at 10% (on a DM basis) for peripubertal dairy heifers limit-fed at 2.65% of BW, will maintain metabolic profile, thyroid hormone concentrations, and onset of puberty compared with the control diet.

Materials and Methods

Samples for this study were taken during the feeding experiment described by Rodriguez-Hernandez and Anderson (2018; Chapter 3); this companion article contains details on diets, feeding protocols, animal care, heifer growth performance, rumen fermentation, and total-tract digestibility of nutrients. All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 15-060A. The institutional Animal Welfare assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Experimental Design

Twenty-four Holstein heifers (6.6 ± 0.7 mo of age; BW 218 ± 27 kg) were used in a 16-wk randomized complete block design feeding study with 2 treatment diets. Heifers were blocked in groups of 2 based on birth date. Heifers were randomly assigned to treatment after assignment to block. Heifers were started on the study in groups of 6 at

different times based on age and availability. The 2 treatment diets (**Table 4.1**) were limit-fed at 2.65% of body weight. Treatments were 1) cold-pressed carinata meal (CRM), and 2) distillers dried grains with solubles (DDGS) both at 10% of the diet on a dry matter basis. The dietary inclusion of 10% as CRM is the limit established by the FDA for rapeseed meals (Benz, 2010). Diets were formulated (NRC, 2001) to provide similar protein and energy intakes when fed to a 250-kg BW Holstein heifer. Heifers were housed in pens in groups of 6 and fed individually using the Calan gate feeding system (American Calan Inc., Northwood, NH).

Sample Collection and Analysis

During wk 0, 4, 8, 12 and 16 of the feeding study blood samples from the jugular vein were taken on 2 consecutive days. Blood samples were taken approximately 3.5 h after feeding (1000 h) via venipuncture of the jugular vein into vacutainer tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing NaFl and potassium oxalate ($C_2K_2O_4$) for glucose analysis (cat. No. 367925) or K_2EDTA for all other analyses (cat. No. 366643). After collection, samples were immediately placed on ice and then brought to the laboratory within 3 h for processing. Blood collection tubes were centrifuged (1,000 x g) for 20 minutes at 4°C (CR412, Jouan Inc., Winchester, VA). Serum (from NaFl and $C_2K_2O_4$ tubes) or plasma (from K_2EDTA tubes) was transferred to polystyrene tubes (Falcon, cat. 352052, Corning Science S.A de C.V., Mexico) and frozen at -20°C until further processing and analysis.

To determine onset of puberty, additional blood samples were taken for progesterone analysis. Sampling began on wk 1 of the feeding trial and continued until presence of a corpus luteum was confirmed via ultrasonography (Agroscan AL, Echo Control Medical, Angoulême, France). During wk 8 of the feeding study, ultrasonography began and was performed once weekly independent of blood sampling until a corpus luteum was identified, at which time ultrasonography and blood sampling ceased. Blood samples were taken via coccygeal venipuncture into vacutainer tubes containing K_2EDTA twice weekly (Tuesday and Friday) approximately 3.5 h post feeding. Plasma was harvested as previously described.

Samples of the second day of sampling were analyzed for glucose, plasma urea nitrogen (**PUN**), cholesterol, triglycerides (**TG**), using commercially available enzymatic or colorimetric assay on a microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA). Serum glucose was analyzed using glucose oxidase as described by Trinder (1969; Pointe Scientific Inc., Canton, MI). Plasma total cholesterol was analyzed using cholesterol esterase and oxidase (Pointe Scientific Inc.) as described by Allain et al. (1974). Plasma urea nitrogen was analyzed using diacetyl monoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Plasma TG concentration was analyzed using glycerol phosphate oxidase after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) that paired the reaction with the classic Trinder (1969) reaction.

For metabolic hormones including insulin, insulin-like growth factor 1 (**IGF-1**), triiodothyronine (**T3**), and thyroxine (**T4**) plasma samples of the second day of sampling

were analyzed by RIA. Serum concentrations of insulin were determined in duplicate according to manufacturer's protocol using a Porcine Insulin RIA kit (PI-12K, EMD Millipore Corporation, St. Charles, MO). The antibody contained in the kit cross reacts with bovine insulin 90%. Sensitivity of the assay was 1.13 mU/mL. Intra-assay CV was 12.6% and interassay CV was 16.2%. Plasma concentrations of IGF-I were determined in duplicate by RIA as described by Echternkamp et al. (1990) and Funston et al. (1995). To extract the IGF binding proteins from the plasma, samples were first incubated overnight with a ratio of 1:17 sample to acidified ethanol (12.5% 2 N HCl: 87.5% absolute ethanol; Daughaday et al., 1980). Samples were centrifuged (12,000 x g at 4°C for 10 min) and an aliquot of the supernatant was removed and neutralized with 0.855 M Tris base. Samples were then incubated overnight again at 4°C and were centrifuged at the same speed and temperature to remove any residual IGF binding proteins. Inhibition curves of the neutralized extracted plasma (range 25-50 μL) and the standard curve were parallel. The radioiodinated antigen and standard used was recombinant human IGF-I (GF-050, Austral Biological, San Ramon, CA). Antisera AFP4892898 (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) was used at a dilution of 1:50,000. Sensitivity was 14.06 ng/dL, intra-assay coefficient of variation was 9.0% and interassay coefficient of variation was 9.9%.

For thyroid hormones, total T3 and total T4 were analyzed in duplicate according to 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, 4.6 ng/dL, 4.4 and 4.3% for T3, and 1.19 μg/dL, 14.5 and 14.0% for T4.

Samples of the first day of sampling of wk 4 and 16 were used for plasma fatty acid determination; lipid extractions were performed as described by Bligh and Dyer (1959). Extracted lipids were then prepared for fatty acid analysis using butylation methods as described by Sukhija and Palmquist (1988) with adaptations by Abdelqader et al. (2009). Feed samples for fatty acid analysis were collected, and 5- or 6-mo composites of DDGS, CRM, grain mixes, and grass hay were analyzed for fatty acid profiles via direct butylation techniques (Abdelqader et al., 2009). All prepared fatty acid samples were analyzed via GC (Hewlett Packard 6890, Palo Alto, CA) as described by Abdelqader et al. (2009).

Plasma concentrations of progesterone were determined in duplicate in all blood samples by RIA. Progesterone (P0130; Sigma Life Science; St. Louis, MO) was the standard and radioiodinated progesterone (#07-170126; MP Biomedicals, Solon, OH) was used as the tracer. Antisera (#111.2C7.3; Enzo Life Sciences, Farmingdale, NY) was used at a dilution of 1:700,000. Inhibition curves of increasing amounts of sample were parallel to standard curves. Inter- and intra-assay coefficients of variation were 11.3% and 10.2%, respectively. Sensitivity of the assay was 3.42 pg/tube. Heifers were determined to have reached puberty when progesterone concentrations were greater than 1 ng/mL, indicating that ovulation had occurred and a corpus luteum had formed.

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Feed fatty acid analysis data were compiled for the 5- or 6-mo feed composite analysis, and standard errors were calculated using the MEANS procedure in SAS. Dietary fatty acid values were calculated based on analysis of the grass hay and grain mixes (CRM and DDGS) for each treatment over the course of the study. Metabolites, hormones (insulin, IGF-1, T3, and T4), and plasma fatty acids data were analyzed as a randomized complete block design with week as the repeated measure and heifer (block) as the subject using PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, and treatment x week interactions. 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. Least squares means are reported for each treatment in the tables. The slice option was used to determine if differences between treatments were significant at each week or time point of measurements. Puberty data were analyzed as binomial data (cycling or not cycling) by age or weight. Puberty data were also analyzed using repeated measures by 10-d and 10-kg intervals of age and BW. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

Results and Discussion

Fatty Acids

Fatty acid profile of total FA (mg/ 100 mg of FA) and composition (g/ kg of DM) of grass hay, carinata meal, distillers dried grains with solubles, and grain mixes used on CRM and DDGS diets are shown on **Table 4.2** and **Table 4.3**. Major FA in carinata meal were C22:1 (36.5%), C:18 n-3 (11.2%), C18:2 *cis*-9, *cis*-12 (17.8%), and C18:1 *cis*-9 (9.5%). The FA relative percentages differ in the meal compared with those reported for carinata oil cold-press extracted (Zhao et al., 2015), where 59% was C18:1 n-5 ad 35% C22:1. Although cold-press extraction does not require external heat, the process generates internal heat (up to 120˚C) because of friction (Sackey, 2015). Xin et al. (2014) found that while heat does not change the ether extract content of moist-heat treated carinata seeds compared with raw carinata seeds, the contents of total FA and some individual FA changed significantly (specifically 18:3 n-3, C20:1, and C22:1) which could explain the differences between carinata meal and carinata oil FA profiles. Major FA in distillers dried grains with solubles where C18:2 *cis*-9, *cis*-12 (51.3%), C18:1 *cis*-9 (22.2%), and C16:0 (11.3%) which coincide with FA profile observed by Manthey et al. (2017), who also reported similar values for grass hay. Grain mixes of the experimental diets had C16:0, C18:1 *cis*-9, C18:2 *cis*-9, *cis*-12, C18:3 n-3 and C22:1 as main FA but in different proportions; however, C18:2 *cis*-9, cis-12 was the predominant FA for both grain mixes.

Table 4.4 and **Table 4.5** show the FA composition (g/kg of DM) and proportions (g/100 g of FA) of the experimental diets (63.53% of grass hay and 36.47% of grain mix). Total FA content was consistent with the ether extract values for CRM and DDGS diets shown in **Table 4.1**. The FA profile of each diet was equivalent to its corresponding grain mix. Overall, CRM diet had more monounsaturated fatty acids (MUFA), less saturated fatty acids (SFA), and similar polyunsaturated fatty acids (PUFA) compared with the DDGS diet.

Fatty acid intake (g/d) is in **Table 4.6**. Heifers fed CRM diet ate 104.4 mg/kg of BW of C22:1 which represents 7% of the observed dose to cause myocardial lipidosis in rats and 12% of the dose for nursing pigs (FSANZ, 2003). Although the difference of FA content between the experimental diets was close to 34%, total FA intake was only 8.6% more for CRM-fed heifers compared with DDGS-fed heifers ($P < 0.01$), which could be explained by the absence of difference on DM intake between heifers fed CRM and DDGS (6.55 vs 6.42 kg/d, respectively; SEM = 0.152; $P = 0.58$; Rodriguez-Hernandez and Anderson, 2018; Chapter 3). Our results are consistent with previous research with limit-fed heifers of similar age where fat contents of the diets were different (Manthey et al., 2017).

Plasma FA proportions (mg/ 100 mg FA) (**Table 4.7**) was different between heifers in both treatments. Overall CRM-fed heifers had greater proportions of MUFA and smaller of SFA than DDGS-fed heifers. Our results can be explained by the differences of fat on the diet and agreed with those observed by Manthey et al. (2017)

where MUFA plasma proportions tended to increase and SFA tended to decrease linearly as fat in diet increased. No differences on the proportion of plasma PUFA between heifers fed CRM or DDGS were observed. Overall, heifers of both treatments had less plasma FA concentrations (μg/ mL of plasma) (**Table 4.8**) on wk 4 compared with wk 16. Plasma total FA concentrations tended ($P = 0.10$) to be higher in CRM-fed heifers compared with DDGS-fed heifers, MUFA concentrations were greater ($P < 0.01$) in heifers fed CRM than those fed DDGS. This difference was due to C18:1 *cis*-9 which also was different ($P < 0.01$). No differences were observed for PUFA and SFA concentrations ($P > 0.05$) which was expected as rumen lipid hydrolysis and biohydrogenation can reduce 70-90% of the PUFA and transform them to SFA or trans isomers of MUFA (Chilliard, 1993). However, our results differ from those reported by Manthey et al. (2017) where the linear increase of the inclusion of DDGS in the diet had a quadratic effect on plasma PUFA concentrations and no differences in MUFA and SFA were observed. This difference in findings may be because in the current experiment diets with different FA proportions are compared whereas in the Manthey et al. study the diets had the same FA profile as all included DDGS and only the inclusion rate changed. Therefore, as intakes of C18:1 cis-9 were not different between treatment heifers, the greater amount of this FA on plasma of CRM-fed heifers may be caused mostly by rumen biohydrogenation and breakdown of the C22:1 from the diet.

Metabolites and Metabolic Hormones

Metabolites and metabolic hormones concentrations in blood are in **Table 4.9**; the values observed are consistent with values reported for heifers of the same age and under a limit-feeding program (Anderson et al., 2015b; Lawrence et al., 2016; Manthey et al., 2017; Manthey and Anderson, 2018). There was a treatment by wk interaction ($P = 0.04$) for plasma triglyceride concentrations, where the proportions of triglycerides between treatments switched on wk 4 and wk 8 (**Figure 4.1**) which could be explained by the wk 0 concentrations which tended ($P = 0.06$) to be greater for heifers on the DDGS diet and no effect of wk or treatment were observed. As triglycerides are composed of FA chains and a glycerol backbone, the lack of difference for triglycerides between treatments is not surprising as only a tendency for greater plasma total FA concentrations between heifers fed CRM was observed. Concentration of triglycerides were consistent with the values reported for limit-fed heifers of similar age (Manthey et al., 2017; Manthey and Anderson, 2018).

No treatment by wk interactions were found for any of the remaining metabolites or metabolic hormones measured. There was an effect of wk for all other metabolites and hormones where their concentrations increased over time which was expected since heifers were growing. **Figures 4.2** to **4.8** show the profiles for the metabolites and metabolic hormones measured. Plasma concentrations of glucose, IGF-1, and insulin decreased from wk 0 to 4, then increased for the rest of the study for heifers on both treatments. Prior to the start of the study, heifers were fed grass hay ad libitum and

grower pellets (between 3 and 4 kg/d) which had greater starch (23% DM basis) than the experimental diets. It is also possible that the change to a limit-fed program also contributed to the decreased glucose, insulin, and IGF-1. A similar IGF-1 profile was observed by Manthey et al. (2017). The increase over time of glucose in both treatments after wk 4 (**Figure 4.2**) could be explained as dietary fat can serve as oxidative substrate sparing glucose oxidation (Chilliard, 1993). Additionally, this sparing effect on glucose oxidation may explain why insulin concentrations did not increase until the end of the experiment, as the increase of glucose in plasma could be more related to an internal homeostatic mechanism. Moreover, the increase of insulin concentrations at the end of the study (**Figure 4.6**) could be more related to the fat in the diets as Thomas et al. (1997) observed delayed increase of insulin after 7 wk of feeding fat-supplemented diets.

No effect of treatment was observed for glucose, PUN, insulin, and T4. There was a tendency ($P = 0.09$) for greater plasma concentrations of IGF-1 in the heifers fed CRM diet compared with heifers fed DDGS diet. Although, Garcia et al. (2003) found that peripubertal beef heifers fed high fat diets had greater plasma IGF-1 concentrations, the difference of dietary fat was two-fold greater between treatments. Therefore, in the present study the difference in dietary fat between diets may not have been enough to cause a significant difference.

A tendency ($P = 0.07$) to have low plasma concentrations of T3 was observed in the CRM-fed heifers. This tendency was probably due to the concentrations observed during wk 4, after this wk, plasma concentrations of T3 kept increasing over time in a

similar fashion to DDGS-fed heifers (**Figure 4.7**). Plasma concentrations of T4 also decreased in a similar way but slighter than T3 (**Figure 4.8**). Richards et al. (1995) observed a decrease of T3 and T4 concentrations in restricted-fed cows, and after cows returned to a maintenance diet, concentrations of both thyroid hormones increased. Additionally, plasma concentrations of T3 and T4 were above concentrations reported for hypothyroid beef heifers (Thrift et al., 1999).

Effect of treatment was observed for plasma cholesterol concentrations, where CRM-fed heifers had greater concentrations than DDGS-fed heifers (89.9 vs. 78.2 mg/dL, respectively). This difference probably is related to the differences in fat intake and the greater amount of PUFA on CRM diet. Anderson et al. (2015b) and Manthey and Anderson (2018) observed greater cholesterol plasma concentrations on heifers limit-fed diets with higher fat content. Additionally, the increase on plasma cholesterol concentrations also has been observed in heifers fed high fat diets with sunflower seeds (Park et al., 1983). Moreover, the profile of plasma cholesterol concentrations (**Figure 4.4**) after wk 8 of the experiment is similar to that observed by Anderson et al. (2015b) on the heifers fed high-fat distillers grains. This increase over time of plasma cholesterol concentrations was also observed by Thomas et al. (1997) who fed beef heifers isoenergetic diets rich on PUFA but no on diets rich on SFA.

Puberty

No effect of treatment was observed for average age and BW at the onset of puberty (**Table 4.10**). Although most of the metabolic profile of heifers between

treatments was similar, and no differences on growth performance were observed (Rodriguez-Hernandez and Anderson, 2018), the proportion of heifers cycling by the end of the study (**Table 4.11**; **Figure 4.9**) was less for CRM-fed heifers compared with DDGS-fed heifers. Additionally, less than 50% of heifers on both treatments were cycling by 300 kg of BW (**Table 4.11**; **Figure 4.10**). Heifers limit-fed diets containing 3% of fat showed a similar proportion of cyclicity by 300 kg of BW; however, more than 80% of the heifers fed the diet with 7% of fat were cycling by that BW (Anderson et al., 2015b). Changes on the diet are not reflected in reproduction in a sudden manner, as was shown by Gonzalez-Padilla et al. (1975) which restricted energy intake of prepubertal beef heifers after 15-mo of age and until 50 to 60 days after fed a high energy diet the heifers started cycling. In restricted-fed dairy heifers, the first pubertal ovulation occurred after approximately 47 d after switching them to a higher energy density diet (Chelikani et al.,2003). We do not believe this change was the result of the negative effects of the glucosinolates content in the CRM diet. When propylthiouracil a thyroid inhibitor was administered to prepubertal lambs, the onset of puberty was not affected (Wells et al., 2003). Additionally, propylthiouracil is a member of the same family of compounds as allyl thiocyanate and allyl isothiocyanate, both metabolites of sinigrin, the main glucosinolate in carinata meal (Kaneko, 1980; Tsao et al., 2000). It is more likely that the different proportion of heifers cycling between treatments is more related to the 1-kg of difference on DMI intake during the first week of the experiment between CRM-fed heifers and DDGS-fed heifers (Rodriguez-Hernandez and Anderson, 2018).

Conclusions

In agreement with our hypothesis, no negative effects of feeding carinata meal at 10% of the diet DM were observed. Heifers fed cold-pressed carinata meal had a similar metabolic profile compared to heifers fed DDGS, and since no difference in thyroid hormones between heifers on treatments were found, the difference of the proportion of heifers cycling at the end of the experiment between treatments may be caused for a decrease in the intake of DMI at the start of the study for CRM-fed heifers. Feeding cold-pressed carinata did not surpass the toxic doses of erucic acid for animals, as intake of C22:1 was below the toxic doses reported for rats and pigs. Overall, this research supports that cold-pressed carinata meal is a good protein source for growing dairy heifers and is comparable to distillers dried grains with solubles.

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Table 4.1. Ingredient and nutrient composition of diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS) fed to growing Holstein heifers[1]

	Diet[1]			
	CRM		DDGS	
Item	Mean	SEM	Mean	SEM
Ingredient[2]				
Grass hay	63.53	-	63.53	-
Carinata meal	10.00	-	-	-
DDGS	-	-	10.00	-
Ground corn	14.51	-	13.01	-
Soybean meal	10.51	-	12.01	-
Vitamin and mineral pre-mix[3]	0.65	-	0.65	-
Calcium carbonate	0.40	-	0.40	-
Salt	0.40	-	0.40	-
Nutrient[2]				
DM[4], %	86.9	0.68	86.8	0.65
Ash[4]	8.5	0.21	8.5	0.22
OM[4]	91.5	0.21	91.5	0.22
CP[4]	15.5	0.15	15.3	0.15
ADF[4]	26.0	0.27	25.7	0.27
NDF[4]	46.0	0.35	46.9	0.38
EE[4,5]	3.5	0.05	2.4	0.06
NFC[4,6]	26.5	0.45	27.0	0.37
Forage NDF[4]	41.7	0.37	41.7	0.37
Nonforage NDF[4]	4.2	0.16	5.2	0.10
Glucosinolate, mg/g[5]	2.06	-	-	-
ME[7], Mcal/kg of DM	2.38	-	2.34	-
NEg[7], Mcal/kg of DM	0.87	-	0.85	-

[1]Formulated (NRC, 2001).
[2]% of DM, unless otherwise indicated.
[3]Contained: Ca 18.9%, NaCl 24.5%, Mg 1.6%, K 0.5%, Cu 880 mg/kg, I 50 mg/kg, Se 25 mg/kg,Zn 3,880 mg/kg, vitamin A 551,146 UI/kg, vitamin D_3 110,229 UI/kg, and vitamin E 4,189 UI/kg (HeiferSmart No Phos, Purina Animal Nutrition LLC, Shoreview, MN).
[4]Results from monthly composite samples.
[5]EE = Ether extract.
[6]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).
[7]Values are calculated based on glucosinolate analysis (Table 5) and inclusion rate (10%) of CRM.
[8]Values are calculated based on inputting sample nutrient analysis into ration formulations in the Dairy NRC computer program (2001, Washington, DC).

Table 4.2. Fatty acid proportions of main ingredients including carinata meal, distillers dried grains with solubles, grass hay and grain mixes used in diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS) fed to growing Holstein heifers

	Carinata meal		Distillers dried grains with solubles		Grass hay		CRM grain mix		DDGS grain mix	
Fatty acid[1], mg/100 mg	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C10:0	0.10	0.008	0.39	0.031	2.17	0.127	0.29	0.034	0.43	0.123
C12:0	0.06	0.004	0.24	0.039	0.38	0.018	0.05	0.016	0.00	0.000
C12:1	0.96	0.071	0.75	0.061	15.80	0.435	3.23	0.501	1.70	0.759
C14:0	0.07	0.006	4.96	0.058	3.01	0.399	0.37	0.113	3.28	0.082
C14:1	0.04	0.010	0.09	0.007	1.37	0.288	0.07	0.018	0.13	0.034
C16:0	3.53	0.009	11.32	0.030	7.53	0.217	6.39	0.377	11.45	0.161
C16:1 *trans*	0.02	0.005	0.03	0.005	5.16	0.638	0.88	0.118	0.61	0.189
C16:1	0.12	0.001	0.14	0.003	0.76	0.027	0.45	0.065	0.30	0.001
C18:0	0.92	0.006	1.36	0.005	0.83	0.019	1.31	0.056	1.94	0.116
C18:1 *cis-9*	9.50	0.070	22.20	0.052	2.24	0.189	13.48	0.328	21.63	0.402
C18:1 *cis-11*	1.37	0.023	1.50	0.020	0.36	0.042	1.40	0.047	1.56	0.019
C18:2 *cis-9, cis-12*	17.77	0.049	51.25	0.129	6.54	0.172	27.30	0.865	49.33	0.402
C18:2 CLA *trans-9, trans-11*	1.65	0.011	0.03	0.022	0.00	0.000	1.00	0.043	0.03	0.033
C18:3 n-6	0.03	0.006	0.02	0.008	0.25	0.040	0.03	0.019	0.07	0.012
C18:3 n-3	11.15	0.073	1.78	0.005	10.43	0.165	7.27	0.443	2.48	0.005
C20:0	0.78	0.016	0.33	0.002	0.49	0.004	0.60	0.012	0.32	0.006
C20:1, 8	0.65	0.015	1.51	0.046	5.91	0.347	1.17	0.122	1.48	0.441
C20:1 *cis*	7.48	0.015	0.45	0.056	2.76	0.081	4.9	0.156	0.41	0.066
C22:1	36.47	0.108	0.02	0.003	0.00	0.000	23.20	1.065	0.04	0.008
Others[2]	6.36	0.207	1.63	0.030	22.39	1.013	6.02	0.582	2.54	0.016

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1, *cis*- 9, C20:1, 5, C18:2, *cis*- 9, *trans*- 11, C20:2, 11, 14, C20:3 homo ɤ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C22:3, C22:4, C24:1, C22:5, C22:6, and unidentified fatty acids.

Table 4.3. Fatty acid composition of main ingredients including carinata meal, distillers dried grains with solubles, grass hay and grain mixes used in diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS) fed to growing Holstein heifers

	Carinata meal		Distillers dried grains with solubles		Grass hay		CRM grain mix		DDGS grain mix	
Fatty acid[1], g/kg DM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C10:0	0.13	0.015	0.28	0.022	0.27	0.014	0.15	0.024	0.14	0.056
C12:0	0.08	0.006	0.17	0.032	0.05	0.002	0.02	0.008	0.00	0.000
C12:1	1.28	0.127	0.53	0.060	1.94	0.041	1.62	0.279	0.57	0.308
C14:0	0.10	0.004	3.51	0.125	0.37	0.049	0.18	0.050	1.04	0.101
C14:1	0.06	0.013	0.06	0.004	0.17	0.036	0.04	0.010	0.04	0.016
C16:0	4.70	0.186	8.01	0.275	0.93	0.034	3.17	0.092	3.65	0.390
C16:1 *trans*	0.03	0.006	0.02	0.004	0.63	0.072	0.44	0.073	0.19	0.037
C16:1	0.15	0.007	0.10	0.005	0.09	0.003	0.23	0.036	0.10	0.012
C18:0	1.22	0.054	0.96	0.035	0.10	0.003	0.65	0.013	0.61	0.038
C18:1 *cis-9*	12.61	0.487	15.70	0.553	0.28	0.025	6.71	0.180	6.89	0.705
C18:1 *cis-11*	1.82	0.091	1.07	0.051	0.05	0.006	0.69	0.016	0.50	0.066
C18:2 *cis-9, cis-12*	23.60	0.954	36.24	1.254	0.80	0.019	13.58	0.299	15.72	1.761
C18:2 *trans-9, trans-11*	2.20	0.078	0.02	0.016	0.00	0.000	0.50	0.041	0.01	0.009
C18:3 n-6	0.04	0.010	0.02	0.006	0.03	0.005	0.02	0.009	0.02	0.001
C18:3 n-3	14.82	0.650	1.26	0.043	1.28	0.023	3.66	0.375	0.88	0.108
C20:0	1.04	0.059	0.23	0.008	0.06	0.001	0.30	0.011	0.10	0.011
C20:1, 8	0.86	0.044	1.06	0.045	1.96	0.041	0.59	0.094	0.49	0.198
C20:1 *cis*	9.94	0.394	0.32	0.047	0.34	0.012	2.50	0.193	0.13	0.037
C22:1	48.46	1.946	0.02	0.003	0.00	0.000	11.63	0.921	0.01	0.004
Others[2]	8.45	0.466	1.15	0.038	2.76	0.148	3.05	0.457	0.81	0.093
Total	132.87	5.455	70.73	2.531	12.30	0.137	49.98	2.556	31.91	3.853

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1, *cis*- 9, C20:1, 5, C18:2, *cis*- 9, *trans*- 11, C20:2, 11, 14, C20:3 homo ϒ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C22:3, C22:4, C24:1, C22:5, C22:6, and unidentified fatty acids.

Table 4.4. Fatty acid composition of diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS) fed to growing Holstein heifers

	Diet			
Fatty acid[1], g/ kg of DM	CRM	SEM	DDGS	SEM
C10:0	0.22	0.016	0.22	0.041
C12:0	0.04	0.003	0.03	0.001
C12:1	1.82	0.101	1.40	0.146
C14:0	0.30	0.025	0.59	0.026
C14:1	0.12	0.023	0.12	0.049
C16:0	0.76	0.047	1.97	0.147
C16:1 *trans*	0.56	0.060	0.39	0.007
C16:1	0.14	0.012	0.09	0.002
C18:0	0.31	0.005	0.29	0.014
C18:1 *cis-9*	2.66	0.055	2.75	0.255
C18:1 *cis-11*	0.29	0.005	0.22	0.025
C18:2 *cis-9, cis-12*	5.53	0.111	6.31	0.654
C18:2 *trans-9, trans-11*	0.19	0.015	0.00	0.003
C18:3 n-6	0.03	0.026	0.03	0.003
C18:3 n-3	2.16	0.143	1.12	0.050
C20:0	0.15	0.004	0.08	0.005
C20:1, 8	1.45	0.043	1.43	0.133
C20:1 *cis*	1.14	0.070	0.27	0.001
C22:1	4.30	0.341	0.01	0.001
Others[2]	2.87	0.107	2.19	0.012
Total	26.24	0.870	19.64	1.435

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1, *cis*- 9, C20:1, 5, C18:2, *cis*- 9, *trans*- 11, C20:2, 11, 14, C20:3 homo ɤ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C22:3, C22:4, C24:1, C22:5, C22:6, and unidentified fatty acids.

Table 4.5. Fatty acid proportions in diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS) fed to growing Holstein heifers

	Diet			
Fatty acid[1], g/ 100 g of FA	CRM	SEM	DDGS	SEM
C10:0	0.85	0.042	1.11	0.129
C12:0	0.15	0.013	0.14	0.007
C12:1	6.95	0.299	7.09	0.226
C14:0	1.15	0.125	3.04	0.355
C14:1	0.46	0.097	0.62	0.293
C16:0	6.72	0.340	10.02	0.019
C16:1 *trans*	2.13	0.154	2.01	0.180
C16:1	0.55	0.038	0.47	0.022
C18:0	1.17	0.039	1.50	0.038
C18:1 *cis-9*	10.14	0.156	13.97	0.277
C18:1 *cis-11*	1.09	0.034	1.10	0.045
C18:2 *cis-9, cis-12*	21.11	0.328	32.03	0.987
C18:2 *trans-9, trans-11*	0.71	0.038	0.02	0.018
C18:3 n-6	0.01	0.012	0.17	0.005
C18:3 n-3	8.22	0.332	5.72	0.161
C20:0	0.57	0.009	0.39	0.003
C20:1, 8	5.54	0.101	7.26	0.147
C20:1 *cis*	4.33	0.140	1.35	0.038
C22:1	16.33	0.887	0.03	0.006
Others[2]	13.93	0.319	11.18	0.753

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1, *cis*- 9, C20:1, 5, C18:2, *cis*- 9, *trans*- 11, C20:2, 11, 14, C20:3 homo ɤ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C22:3, C22:4, C24:1, C22:5, C22:6, and unidentified fatty acids.

Table 4.6. Mean fatty acid intake for growing Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Fatty acid[1], g/d	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
C10:0	1.46	1.38	0.037	<0.01	<0.01	<0.01
C12:0	0.25	0.17	0.006	<0.01	<0.01	<0.01
C12:1	11.95	8.74	0.295	<0.01	<0.01	<0.01
C14:0	1.96	3.77	0.059	<0.01	<0.01	<0.01
C14:1	0.78	0.74	0.020	<0.01	<0.01	<0.01
C16:0	11.51	12.43	0.300	<0.01	<0.01	<0.01
C16:1 *trans*	3.69	2.45	0.090	<0.01	<0.01	<0.01
C16:1	0.94	0.58	0.022	<0.01	<0.01	<0.01
C18:0	2.00	1.85	0.050	<0.01	<0.01	<0.01
C18:1 *cis-9*	17.41	17.32	0.447	0.49	<0.01	<0.01
C18:1 *cis-11*	1.87	1.36	0.046	<0.01	<0.01	<0.01
C18:2 *cis-9, cis-12*	36.24	39.82	0.947	<0.01	<0.01	<0.01
C18:2 *trans-9, trans-11*	1.22	0.00	0.028	<0.01	<0.01	<0.01
C18:3 n-6	0.17	0.21	0.005	<0.01	<0.01	<0.01
C18:3 n-3	14.16	6.94	0.338	<0.01	<0.01	<0.01
C20:0	0.98	0.47	0.023	<0.01	<0.01	<0.01
C20:1, 8	9.51	8.99	0.242	<0.01	<0.01	<0.01
C20:1 *cis*	7.46	1.57	0.173	<0.01	<0.01	<0.01
C22:1	28.18	0.00	0.640	<0.01	<0.01	<0.01
Others[2]	18.78	13.68	0.463	<0.01	<0.01	<0.01
Total	171.94	122.90	4.229	<0.01	<0.01	<0.01

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1, *cis*- 9, C20:1, 5, C18:2, *cis*- 9, *trans*- 11, C20:2, 11, 14, C20:3 homo ɤ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C22:3, C22:4, C24:1, C22:5, C22:6, and unidentified fatty acids.

Table 4.7. Plasma fatty acid proportions from wk 4 and 16 of the feeding period for growing Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Fatty acid[1], mg/100 mg	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
C4:0						
Mean	7.527	9.739	0.4975	<0.01	<0.01	0.02
Wk 4	12.516	16.367	0.6954			
Wk 16	2.537	3.111	0.6954			
C5:0						
Mean	0.376	0.412	0.0575	0.67	<0.01	0.91
Wk 4	0.041	0.667	0.0874			
Wk 16	0.712	0.758	0.0874			
C6:0						
Mean	8.868	11.047	0.5251	<0.01	<0.01	0.81
Wk 4	7.026	9.370	0.7162			
Wk 16	10.711	12.724	0.7162			
C7:0						
Mean	0.355	0.463	0.0573	0.20	<0.01	0.20
Wk 4	0.000	0.000	0.0846			
Wk 16	0.709	0.926	0.0846			
C14:0						
Mean	0.861	0.846	0.1017	0.92	<0.01	0.75
Wk 4	0.603	0.544	0.1421			
Wk 16	1.120	1.148	0.1421			
C14:1						
Mean	0.231	0.196	0.0259	0.35	<0.01	0.30
Wk 4	0.064	0.000	0.0328			
Wk 16	0.398	0.393	0.0328			
C15:0						
Mean	0.756	0.745	0.0618	0.90	0.04	0.52
Wk 4	0.808	0.843	0.0800			
Wk 16	0.704	0.648	0.0800			
C15:1						
Mean	0.484	0.544	0.0558	0.45	0.75	0.45
Wk 4	0.462	0.598	0.0908			
Wk 16	0.507	0.490	0.0908			
C16:0						
Mean	13.996	13.981	0.3558	0.98	<0.01	0.40
Wk 4	15.126	14.782	0.4526			
Wk 16	12.866	13.181	0.4526			
C16:1 *cis-9*						
Mean	0.869	0.993	0.1352	0.51	0.18	0.15
Wk 4	0.855	1.305	0.2067			
Wk 16	0.883	0.682	0.2067			
C17:0						
Mean	0.780	0.793	0.0244	0.71	0.02	0.23
Wk 4	0.842	0.814	0.0339			
Wk 16	0.718	0.772	0.0339			
C17:1						
Mean	1.103	0.942	0.1101	0.30	0.97	0.34

	Treatment			*P*-values		
Fatty acid[1], mg/100 mg	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Wk 4	0.971	1.064	0.2186			
Wk 16	1.234	0.820	0.2186			
C18:0						
Mean	18.858	18.527	0.5299	0.66	<0.01	0.17
Wk 4	20.185	19.044	0.6735			
Wk 16	17.531	18.010	0.6735			
C18:1 *trans-9*						
Mean	0.558	0.944	0.1007	0.01	<0.01	0.11
Wk 4	0.127	0.265	0.1476			
Wk 16	0.988	1.624	0.1476			
C18:1 *trans-10*						
Mean	0.642	0.909	0.0500	<0.01	0.18	0.15
Wk 4	0.718	0.906	0.6273			
Wk 16	0.566	0.913	0.6273			
C18:1 *cis-9*						
Mean	12.250	9.193	0.4784	<0.01	<0.01	0.13
Wk 4	13.915	9.832	0.6744			
Wk 16	10.585	8.554	0.6744			
C18:2 *cis-9, cis-12*						
Mean	14.412	15.498	0.6916	0.27	<0.01	0.03
Wk 4	13.306	12.198	0.9814			
Wk 16	15.518	18.797	0.9814			
C18:2 CLA *cis-9, trans-11*						
Mean	1.075	0.760	0.2572	0.39	0.26	0.33
Wk 4	0.602	0.727	0.4093			
Wk 16	1.548	0.793	0.4093			
C18:3 n-6						
Mean	1.350	1.131	0.1945	0.43	0.06	0.38
Wk 4	0.827	0.929	0.3238			
Wk 16	1.872	1.334	0.3238			
C20:3 homo γ						
Mean	0.780	0.994	0.0513	<0.01	<0.01	<0.01
Wk 4	0.000	0.000	0.0725			
Wk 16	1.560	1.989	0.0725			
C20:3 *cis-11,14,17*						
Mean	2.286	2.153	0.2703	0.73	0.45	0.89
Wk 4	2.436	2.257	0.3621			
Wk 16	2.136	2.049	0.3621			
C20:4						
Mean	2.070	1.822	0.3323	0.60	<0.01	0.59
Wk 4	1.106	1.113	0.4694			
Wk 16	3.034	2.532	0.4694			
C22:0						
Mean	0.937	0.829	0.1402	0.58	0.23	0.42
Wk 4	0.748	0.792	0.1928			
Wk 16	1.126	0.866	0.1928			
C22:1 *cis*-13						
Mean	0.960	0.416	0.2492	0.13	<0.01	0.39
Wk 4	0.261	0.016	0.3514			

	Treatment			*P*-values		
Fatty acid[1], mg/100 mg	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Wk 16	1.659	0.815	0.3514			
Others[2]						
Mean	3.908	3.529	0.4138	0.52	0.15	0.30
Wk 4	4.046	4.360	0.6248			
Wk 16	3.770	2.699	0.6248			
LCFA[3]						
Mean	80.537	75.985	1.0294	<0.01	<0.01	0.22
Wk 4	78.482	72.212	1.4289			
Wk 16	82.592	79.758	1.4289			
SCFA[3]						
Mean	19.461	24.009	1.0305	<0.01	<0.01	0.22
Wk 4	21.519	20.230	1.4292			
Wk 16	17.404	27.789	1.4292			
MUFA[3]						
Mean	19.764	16.630	0.5914	<0.01	0.85	0.42
Wk 4	20.075	16.434	0.7379			
Wk 16	19.452	16.825	0.7379			
PUFA[3]						
Mean	27.532	26.658	1.2750	0.63	<0.01	0.89
Wk 4	21.582	20.474	1.7300			
Wk 16	33.482	32.841	1.7300			
SFA[3]						
Mean	54.773	58.529	1.1823	0.03	<0.01	0.54
Wk 4	59.450	64.205	1.6555			
Wk 16	50.096	52.854	1.6555			

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C10:0, C12:0, C16:1 *trans*, C16:1 *cis*, C18:1 *trans-6*, C18:1 *trans-11*, C20:0, C20:1 *cis-8*, C18:2 CLA *trans-10, cis-12*, C20:2, C20:5, C22:3. C24:0, C24:1, C22:6, and unidentified fatty acids.

[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 4.8. Plasma fatty acid concentrations from wk 4 and 16 of the feeding period for growing Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Fatty acid[1], μg/mL plasma	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
C4:0						
Mean	16.223	16.908	0.3004	0.11	<0.01	0.68
Wk 4	18.475	19.313	0.4032			
Wk 16	13.970	14.502	0.4032			
C5:0						
Mean	1.917	1.723	0.2420	0.57	<0.01	0.54
Wk 4	0.060	0.075	0.3443			
Wk 16	3.775	3.370	0.3443			
C6:0						
Mean	34.621	35.439	1.2054	0.63	<0.01	0.93
Wk 4	10.395	11.054	1.7086			
Wk 16	58.847	59.823	1.7086			
C7:0						
Mean	1.846	2.194	0.2608	0.35	<0.01	0.35
Wk 4	0.000	0.000	0.3689			
Wk 16	3.693	4.389	0.3689			
C14:0						
Mean	3.701	3.071	0.3638	0.24	<0.01	0.52
Wk 4	0.911	0.620	0.5360			
Wk 16	6.492	5.522	0.5360			
C14:1						
Mean	1.228	0.967	0.1580	0.25	<0.01	0.45
Wk 4	0.103	0.000	0.2160			
Wk 16	2.353	1.934	0.2160			
C15:0						
Mean	2.685	2.116	0.2487	0.11	<0.01	0.23
Wk 4	1.194	1.034	0.3463			
Wk 16	4.176	3.198	0.3463			
C15:1						
Mean	1.786	1.589	0.2458	0.57	<0.01	0.68
Wk 4	0.692	0.655	0.3721			
Wk 16	2.880	2.523	0.3721			
C16:0						
Mean	48.438	41.724	3.2594	0.15	<0.01	0.57
Wk 4	23.178	18.601	4.2027			
Wk 16	73.698	64.848	4.2027			
C16:1 *cis-9*						
Mean	3.414	2.493	0.5560	0.25	<0.01	0.19
Wk 4	1.262	1.448	0.8104			
Wk 16	5.565	3.539	0.8104			
C17:0						
Mean	2.683	2.431	0.2002	0.37	<0.01	0.94
Wk 4	1.299	1.032	0.2477			
Wk 16	4.067	3.829	0.2477			
C17:1						
Mean	4.828	2.730	0.9350	0.12	<0.01	0.22

Fatty acid[1], µg/mL plasma	Treatment			*P*-values		
	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Wk 4	1.453	1.197	1.4109			
Wk 16	8.201	4.264	1.4109			
C18:0						
Mean	65.013	56.591	4.5952	0.20	<0.01	0.75
Wk 4	30.689	56.591	5.9115			
Wk 16	99.336	89.235	5.9115			
C18:1 *trans-9*						
Mean	2.919	3.807	0.3396	0.07	<0.01	0.11
Wk 4	0.167	0.269	0.4792			
Wk 16	5.671	7.346	0.4792			
C18:1 *trans-10*						
Mean	2.168	2.877	0.2697	0.07	<0.01	<0.05
Wk 4	1.085	1.161	0.3476			
Wk 16	3.251	4.594	0.3476			
C18:1 *cis-9*						
Mean	40.932	27.636	3.0453	<0.01	<0.01	0.20
Wk 4	21.449	12.443	3.8320			
Wk 16	60.415	42.829	3.8320			
C18:2 *cis-9, cis-12*						
Mean	53.423	54.414	4.7001	0.88	<0.01	0.29
Wk 4	20.688	15.917	6.0730			
Wk 16	86.158	92.911	6.0730			
C18:3 n-6						
Mean	7.135	3.974	1.6869	0.19	<0.01	0.24
Wk 4	1.243	1.046	2.4513			
Wk 16	13.026	6.901	2.4513			
C18:2 CLA *cis-9, trans-11*						
Mean	6.380	2.552	2.0901	0.20	0.03	0.23
Wk 4	0.927	0.830	3.0219			
Wk 16	11.834	4.274	3.0219			
C20:3 homo γ						
Mean	4.338	4.941	0.4578	0.35	<0.01	0.35
Wk 4	0.000	0.000	0.6474			
Wk 16	8.677	9.882	0.6474			
C20:4						
Mean	11.207	7.633	2.4943	0.31	<0.01	0.34
Wk 4	1.730	1.493	3.5177			
Wk 16	20.684	13.774	3.5177			
C20:3 *cis-11,14,17*						
Mean	7.930	6.119	1.1712	0.28	<0.01	0.54
Wk 4	3.766	2.954	1.6535			
Wk 16	12.093	9.284	1.6535			
C22:0						
Mean	3.647	2.570	0.2778	0.01	<0.01	0.02
Wk 4	1.146	0.927	0.3686			
Wk 16	6.148	4.213	0.3686			
C22:1 *cis*-13						
Mean	6.324	2.180	2.0039	0.15	<0.01	0.19
Wk 4	0.375	0.015	2.8378			

Fatty acid[1], μg/mL plasma	Treatment			*P*-values		
	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Wk 16	12.272	4.346	2.8378			
C22:5						
Mean	6.507	2.782	2.0860	0.24	0.01	0.21
Wk 4	0.476	0.652	3.1068			
Wk 16	12.537	4.911	3.1068			
Others[2]						
Mean	15.018	8.967	2.3656	0.08	<0.01	0.15
Wk 4	5.550	4.635	3.4619			
Wk 16	24.535	13.299	3.4619			
LCFA[3]						
Mean	304.43	244.12	24.000	0.08	<0.01	0.33
Wk 4	120.45	90.92	32.809			
Wk 16	488.42	397.33	32.809			
SCFA[3]						
Mean	64.03	64.09	2.000	0.98	<0.01	0.76
Wk 4	31.83	32.75	2.833			
Wk 16	96.23	95.72	2.833			
MUFA[3]						
Mean	73.72	52.20	6.422	0.02	<0.01	0.20
Wk 4	30.75	20.28	8.834			
Wk 16	116.69	84.12	8.834			
PUFA[3]						
Mean	120.00	96.54	13.773	0.23	<0.01	0.40
Wk 4	34.32	27.02	19.295			
Wk 16	205.69	116.06	19.295			
SFA[3]						
Mean	185.95	167.10	9.770	0.18	<0.01	0.50
Wk 4	88.94	77.86	12.754			
Wk 16	282.95	256.34	12.754			
Total						
Mean	368.47	308.25	25.4475	0.10	<0.01	0.34
Wk 4	152.28	123.67	34.6638			
Wk 16	584.67	492.83	34.6638			

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C10:0, C12:0, C16:1 *trans*, C16:1 *cis*, C18:1 *trans-6*, C18:1 *trans-11*, C20:0, C20:1 *cis-8*, C18:2 CLA *trans-10, cis-12*, C20:2, C20:5, C22:3. C24:0, C24:1, C22:6, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 4.9. Plasma metabolites and metabolic hormones concentrations for growing Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Item	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Glucose, mg/dL	84.0	82.3	1.25	0.33	<0.01	0.92
Plasma urea nitrogen, mg/dL	19.8	19.8	0.48	0.98	0.06	0.79
Cholesterol, mg/ dL	89.9	78.2	2.58	<0.01	<0.01	0.26
Triglycerides, mg/dL	20.91	20.35	1.23	0.76	0.26	0.04
Insulin, μU/dL	11.9	12.3	1.05	0.78	<0.01	0.90
IGF-I, ng/mL	117.1	105.0	4.93	0.09	<0.01	0.79
Triiodothyronine, ng/dL	140.2	154.7	5.28	0.07	<0.01	0.90
Thyroxine, μg/dL	6.4	6.6	0.24	0.40	0.02	0.51

Table 4.10. Mean age and body weight (BW) at puberty for growing Holstein heifers fed diets with 10% carinata meal (CRM) or distillers dried grains with solubles (DDGS)

	Treatment			*P*-values		
Item	CRM	DDGS	SEM	Treatment	Week	Treatment × Week
Age, d	329	321	3.1	0.54	<0.01	0.92
BW, kg	319	306	3.1	0.20	<0.01	0.81

Figure 4.1. Plasma concentrations of triglycerides for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

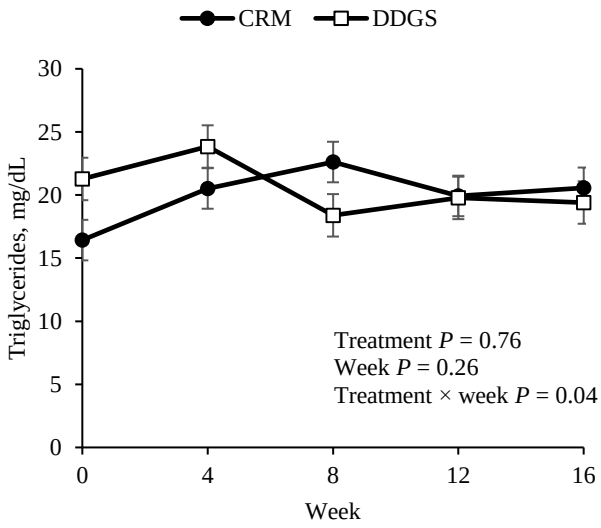


Figure 4.2. Plasma concentrations of glucose for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

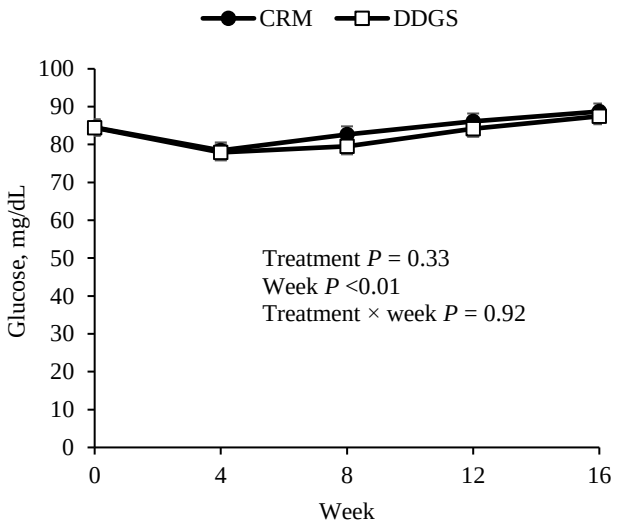


Figure 4.3. Plasma urea nitrogen (PUN) concentrations for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

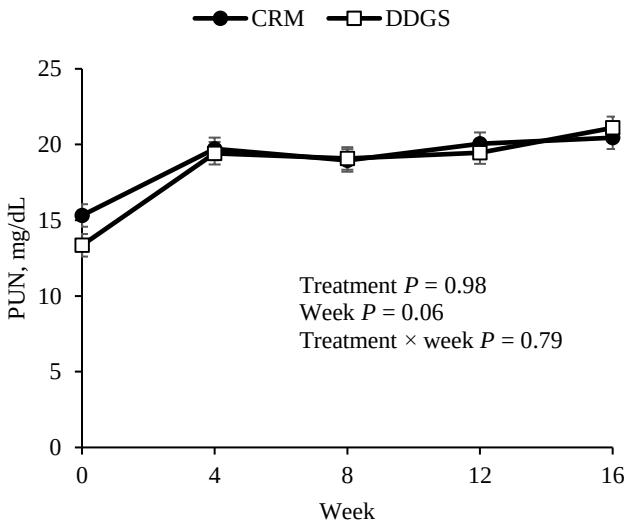


Figure 4.4. Plasma concentrations of cholesterol for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

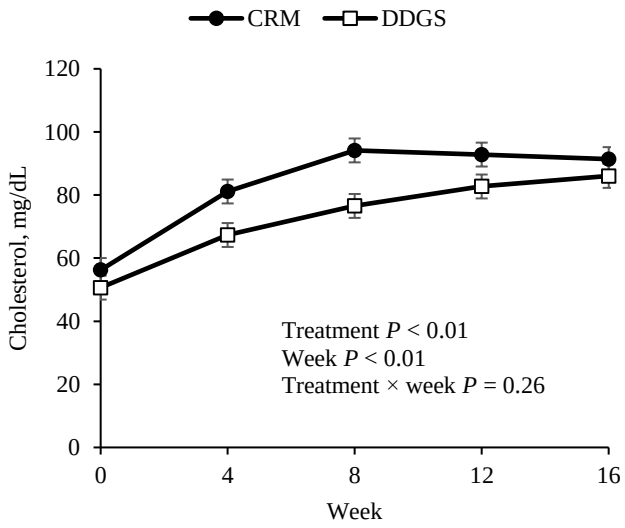


Figure 4.5. Plasma concentrations of IGF-I for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

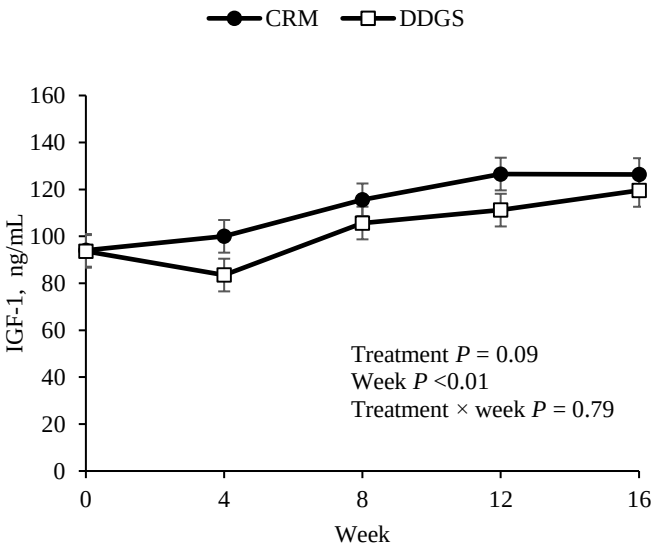


Figure 4.6. Plasma concentrations of insulin for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Error bars represent SEM)

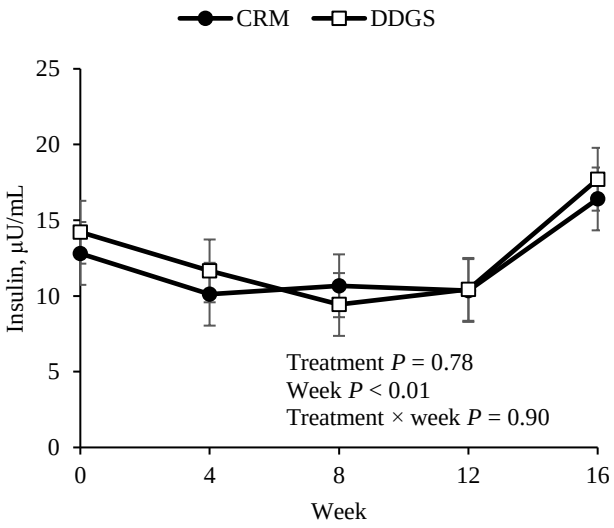


Figure 4.7. Plasma concentrations of triiodothyronine (T3) for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

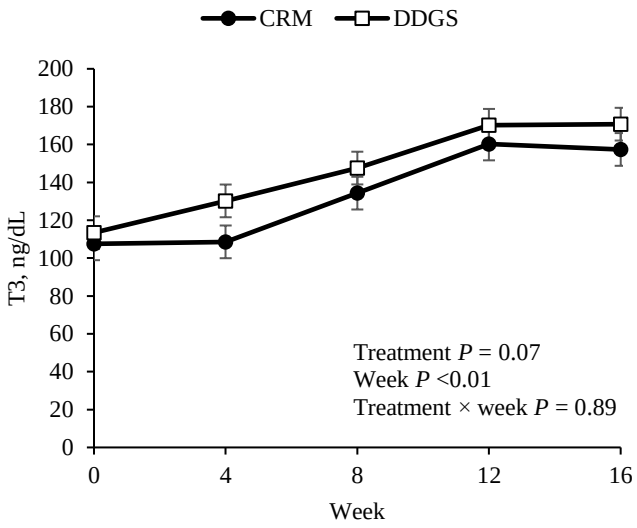


Figure 4.8. Plasma concentrations of thyroxine (T4) for growing Holstein heifers fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Values of wk 0 where used as a covariable. Error bars represent SEM)

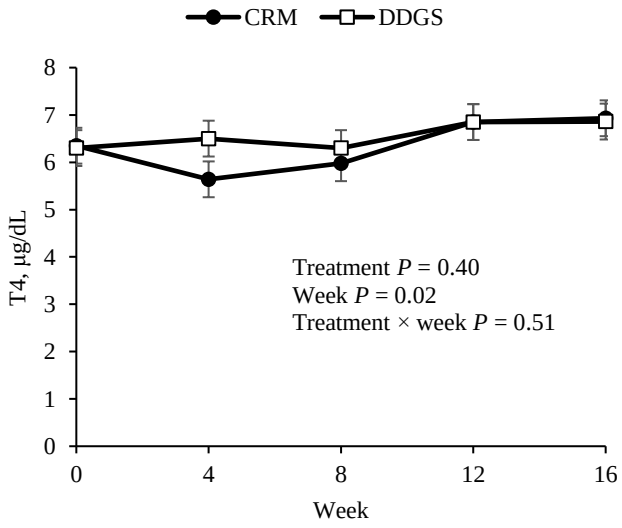


Figure 4.9. Percentage of Holstein heifers pubertal (cycling) by age that were fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Error bars represent SEM)

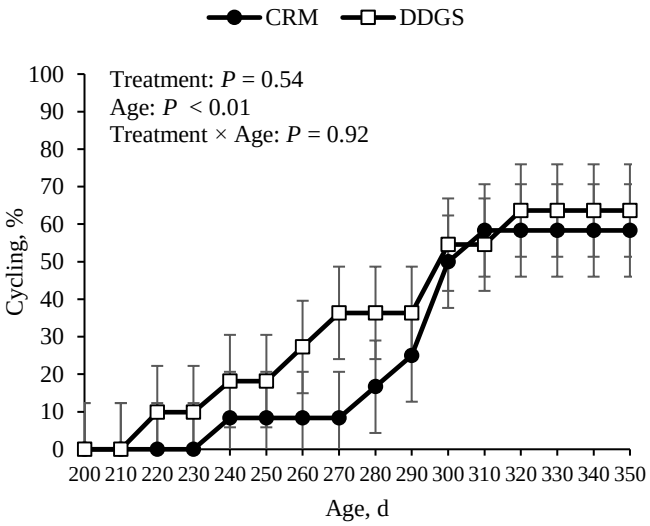
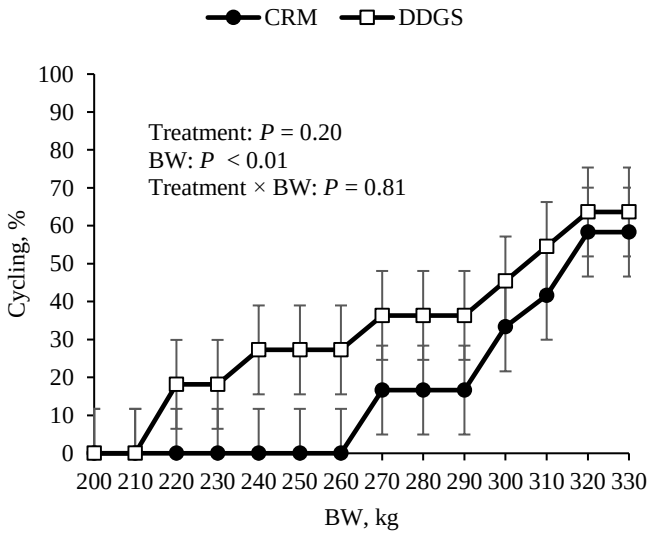


Figure 4.10. Percentage of Holstein heifers pubertal (cycling) by body weight that were fed diets containing 10% (DM basis) carinata meal (CRM) or distillers dried grains with solubles (DDGS). (Error bars represent SEM)



CHAPTER 5. SOLVENT-EXTRACTED CARINATA MEAL COMPARED WITH CANOLA MEAL OR SOYBEAN PRODUCTS IN DIETS FOR GROWING DAIRY HEIFERS: EFFECTS ON GROWTH PERFORMANCE, RUMEN FERMENTATION, AND TOTAL TRACT DIGESTIBILITY OF NUTRIENTS

ABSTRACT

Our objective was to compare growth performance, rumen fermentation, and nutrient utilization of prepubertal dairy heifers fed solvent-extracted carinata meal compared with canola meal and a control diet with soybean products. A 16-week randomized block design experiment with 36 Holstein heifers (6.3±0.1 mo of age, and 207±3 kg of body weight) was conducted. The three treatment diets were limit-fed at 2.40% of body weight on dry matter (**DM**) basis to target a 0.8 kg/d average daily gain. Treatments were: 1) solvent-extracted carinata meal (**CRM**), 2) solvent-extracted canola meal (**CAN**), both at 10% of diet DM; and 3) control diet (**CON**) where most of the protein was provided from soybean meal. The remainder of the diets were comprised of grass hay, ground corn, distillers dried grains with solubles, soybean meal, soybean hulls and mineral mix to meet nutrient requirements. Diets were formulated to be isonitrogenous and isocaloric. Heifers were individually fed using a Calan gate feeding system. Heifers were weighed every 2 wk and the ration amount offered was adjusted accordingly. Frame sizes, body weight, and body condition scores were measured 2 d every 2 wk throughout the study. During week 12 and 16, rumen fluid samples were collected via esophageal tubing for pH, ammonia N, and volatile fatty acid analyses. In

week 16, fecal grab samples were collected for apparent total tract digestibility estimation. There were no differences among treatments for dry matter intake, growth performance, and body condition scores. Rumen fermentation profiles, rumen ammonia N, and total volatile fatty acid concentrations were not different among treatments. Finally, there were no differences in total tract digestibility of nutrients. Overall, limit-fed diets that include solvent-extracted carinata meal fed at 10% of the diet DM fed to growing dairy heifers were comparable to other protein sources such as canola and soybean meal. Therefore, solvent-extracted carinata meal is a viable supplement and shows great potential as a new feedstuff for growing dairy heifers.

Keywords: dairy heifer, carinata meal, canola meal, soybean meal, growth performance

Introduction

Interest in developing alternative renewable fuels as biodiesel has been increasing because of the environmental impact of burning fossil fuels and a desire to decrease fossil oil imports into the United States (Hristov et al., 2011; U.S. Department of Energy, 2015). Oilseeds with high content of very-long-chain fatty acids (**VLCFA**) are used as feedstocks for biodiesel and for environmentally safe oil products as lubricants, surfactants, and cutting fluids along with other industrial uses (Brown et al., 1998). *Brassica carinata* is a new and promising feedstock because its oil has high concentrations of VLCFA, in particular C22:1, which can be used to produce biofuels and bio-oils more efficiently compared to oil from other oilseeds (Cardone et al., 2003; Enjalbert et al., 2013). Additionally, carinata is a crop well adapted to dry climates and

can grow in fallow lands or areas where other crops such as corn and soybeans cannot adapt (Marillia et al., 2014) making this crop of interest for use in the Great Plains area.

However, the economic success of a biofuel feedstock also depends on the use of the co-product meal as animal feed (Van Dyne and Raymer, 1992). Carinata meal has high content of rumen degradable protein, with a total protein digestibility comparable to that of soybean and linseed meals (Lawrence and Anderson, 2018). Additionally, results of a previous research study (Rodriguez-Hernandez and Anderson, 2018) indicate that dairy heifers fed diets with cold-pressed carinata meal at 10% of the diet DM basis have the same growth performance compared with heifers feed distillers dried grains with solubles. As carinata meal is a promising feedstuff for use in the Great Plains area and potential competitor with the imported canola meal, the objective of this research was to determine the effects of feeding solvent-extracted carinata meal with solvent-extracted canola meal and a control diet that contain soybean products on growth performance, rumen fermentation, and nutrient utilization of peripubertal dairy heifers. We hypothesized that as carinata meal has high crude protein content and digestibility, its inclusion in the diet at 10% (on a DM basis) will maintain or enhance the growth performance of dairy heifers compared with canola meal and a control diet contain soybean products.

Materials and Methods

All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 16-079E. The

institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Experimental Design

Thirty-six Holstein heifers [6.3±0.1 mo of age, and 207±3 kg of body weight (BW)] were used in a randomized complete block design with three treatment diets. Heifers were blocked in groups of three based on birth date. Heifers were randomly assigned to treatment within block. Heifers were added on the study in groups of 6 or 2 blocks at different times based on age and availability with a target starting age of 6.3 mo. The feeding study was completed in 9 mo from December 2016 to August 2017 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). Heifers were adapted to the research barns and feeding system for approximately 2 wk, followed by an experimental feeding period of 16 wk.

The three treatment diets were limit-fed at 2.40% of body weight. Treatments were: 1) solvent-extracted carinata meal (**CRM**), 2) solvent-extracted canola meal (**CAN**), both at 10% of diet dry matter (DM); and 3) control diet (**CON**) where most of the protein was provided from soybean meal. The remainder of the diets were comprised of grass hay, ground corn, distillers dried grains with solubles (**DDGS**), soybean meal, soybean hulls and mineral mix to meet nutrient requirements and formulated to allow for similar intakes of protein and energy among treatments (**Table 5.1**). The dietary inclusion of carinata meal as 10% of the diet was used as is described in the tentative status definition by the FDA-AAFCO (AAFCO, 2018).

Animal Care and Feeding

Heifers were observed daily for any injury or disease problems and treated according to normal farm management protocols at the Dairy Research and Training Facility. Heifers were housed in pens in groups of 6. Each pen had an inside roofed area (7 m x 4 m) and an outside exercise lot (7 m x 23.5 m). The inside areas of the pens were manure pack bedded with straw. Pens were bedded only once per week to discourage consumption of straw. Fresh water was available at all times. Feeding occurred once daily at approximately 0600 h using the Calan gate feeding systems (American Calan Inc., Northwood, NH) so that individual intakes could be measured. Rations were formulated using the NRC (2001) to be limit-fed to 2.4% of BW (dry basis) in an effort to meet requirements of a heifer weighing 250 kg and to target 0.8 kg/d of average daily gain (**ADG**) as recommended by Hoffman (1997) and Zanton and Heinrichs (2005). The 250 kg of BW was a pre-estimated average BW for heifers during the study based on age and previous herd data. Rations were adjusted every 2 wk based on BW and DM of feeds. At each feeding, coarsely ground brome grass hay and grain mix were individually weighed for each heifer into a large tub, hand mixed, and then placed in the Calan boxes. Since rations were limit-fed, heifers consumed the majority of the feed offered on most days during the feeding period and sorting was not an issue. Any orts were weighed and recorded in the morning before feeding to determine daily intakes. Each week, samples of grass hay and grain mixes were taken. Each month, samples of individual concentrate mix ingredients (corn, soybean meal, soybean hulls, DDGS, carinata meal, and canola

meal) were also taken. All feed samples were stored at -20°C until processing and analysis could be completed as described under laboratory analysis.

Animal Measurements and Sampling

Body growth measurements including BW, withers height, hip height, hip width, heart girth, paunch girth, and body length were taken on 2 consecutive days at the beginning of the study and then every 2 wk during the study at 4 h post-feeding. Body length was measured from the top point of the withers to the end of the ischium (Hoffman, 1997). Body condition scores were recorded every 2 wk by 4 independent observers based on a quarter-point scale with 1 being emaciated and 5 being obese (Wildman et al., 1982). Rumen fluid was collected via esophageal tubing during wk 12 and 16 on 2 consecutive days, at 4 h post feeding at the same time as body measurements were being taken. After discharging the first 200 mL of fluid to minimize saliva contamination, approximately 50 mL of rumen fluid were collected. The pH of the samples was immediately measured using a pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots of 10 mL were acidified with either 200 μL of 50% (vol/vol) sulfuric acid or 2 mL of 25% (wt/vol) meta-phosphoric acid and stored at -20°C until later analysis of ammonia N (**NH_3-N**) and volatile fatty acids (**VFA**). During wk 16 of the feeding period, on 3 consecutive days, fecal grab and ort samples were collected for analysis of total tract digestibility of nutrients using acid detergent insoluble ash (**ADIA**) as an internal marker. Fecal sampling time points were scheduled

so that the samples represented every 3 h in a 24-h feeding cycle. Samples were stored at -20°C until processing and analysis.

Laboratory Analysis

Total dietary nutrient concentrations were calculated based on analyses of grass hay and grain mix for each treatment. Every 2 wk throughout the study an aliquot of feed samples was dried for 24 h at 105°C for DM analysis to adjust dietary ingredient inclusion amounts and determine dry matter intakes (**DMI**). Monthly feeds samples and grain mix weekly samples were thawed and samples from 4 consecutive weeks were composited on as-fed basis by weight. Composite samples were dried in duplicate for 48 h at 55°C in a Dispatch oven (Style V-23, Dispatch Oven Co., Minneapolis, MN), ground to 4-mm particle size with a Wiley Mill (model 3, Arthur H. Thomas Co., Philadelphia, PA), and further ground to 1-mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To correct nutrient analyses to 100% DM, 1-g aliquots of ground feed samples were dried for 4 h in a 105°C oven (Model 28, Precision Scientific Co., Chicago, IL). Ash content (AOAC International, 2002 method 942.05) was determined by incinerating a 1-g sample for 8 h at 450°C in a muffle furnace (Model F1730, Thermolyne Corp., Dubuque, IA; temperature controller Model Wheelco 293, Barber-Colman Co., Rockford, IL). Organic matter was calculated as **OM** = (100 - % ash). Samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC International, 2002, method 968.06), on a rapid N Cube (Elementar Analysensysteme, GmbH, Hanau, Germany). Nitrogen content was then multiplied by

6.25 to calculate crude protein (**CP**). Neutral detergent fiber (**NDF**) (Van Soest et al., 1991) and acid detergent fiber (**ADF**) (Robertson and Van Soest, 1981; AOAC International, 2002, method 973.18) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable α-amylase and sodium sulfite were used (AOAC International, 2002, method 2002.04). Petroleum ether was used to determine ether extract (**EE**; AOAC International, 2002, method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Nonfibrous carbohydrates were calculated as % **NFC** = 100 – (% ash + % CP + % NDF + % EE) according to the NRC (2001).

Dried and ground samples of individual ingredients (grass hay, soybean hulls, ground corn, DDGS, soybean meal, canola meal, and carinata meal), and CRM, CAN, and CON grain mixes were further composited into 5 or 4 mo composites and sent to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for analysis of starch, minerals (Ca, Cl, Mg, P, K, Na, S, Fe, Mn, Mo, Cu and Zn) and dietary cation-anion difference (**DCAD**). Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC International, 2002). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY). Glucosinolate analysis and quantitation in the carinata meal was performed by a commercial laboratory POS Bio-Sciences, Saskatoon, Canada, according to the official method of the Canadian Grain Commission (Daun and McGregor, 1981).

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 x *g* for 20 min at 4°C (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for NH_3-N using a colorimetric assay performed on a microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA) according to Chaney and Marbach (1962). Rumen fluid samples that were preserved with 25% meta-phosphoric acid were thawed and centrifuged at 30,000 x *g* for 20 min at 4°C and analyzed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations using an automated GC (model 6890, Hewlett-Packard Co., Palo Alto, CA) using a flame ionization detector. Volatile fatty acids were separated on a capillary column (15 m x 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethylbutyrate as an internal standard. The split ratio of 100:1 in the injector port was at a temperature of 250°C with flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140 and 250°C, respectively.

Fecal samples from all collection time points were composited on an as-is basis by volume for each heifer. Aliquots of 100 mL of fecal samples were taken from each time point and composited. Orts (if any were left) were collected three days during the fecal collection period. Orts were composited based on proportions of weight from each day for any heifers that had orts on multiple days. Fecal and orts composites were then dried and ground and analyzed for DM, ash, CP, NDF, and ADF as previously described for feed composites. Analysis of ADIA consisted of determination ADF content (Robertson and Van Soest, 1981) and then analysis of the ash content using a modified procedure of AOAC method 935.29 (AOAC International, 2002) for all feed composites,

fecal and orts samples. Apparent total tract digestibility calculations for DM, OM, CP, NDF, and ADF were determined according to Merchen (1988).

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The MEANS procedure of SAS was used to estimate the means and standard errors of the nutrients from analysis of the monthly feed composites. To determine ADG for body weight and change per day for body frame measurements the difference was found between each data collection time point and the previous time point and then divided by the number of days in the time period [i.e. (wk 2-wk 0)/14 d]. Gain to feed ratio was calculated as the ratio of ADG of body weights to total DMI for each heifer during each 2-week time interval between weight and frame measurements.

Intakes, gain:feed, growth data, ADG, and rumen fermentation data were analyzed as a randomized complete block design with week as the repeated measure and heifer (block) as the subject using PROC MIXED procedures of SAS (Littell et al., 2006). Initial (week 0) body weights and frame measurements were used as covariate terms for each respective variable. The model included treatment, week, and treatment x week interactions. 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 $0.05 < P \leq 0.10$. Least squares means are reported for each treatment in the tables. The slice option was used to determine if differences among treatments were significant at individual weeks or time points of measurements.

The MIXED procedures of SAS were used for the analysis of total tract digestibility of nutrients. As total tract digestibility was analyzed only during wk 16, the model only included treatment with block included as a random variable.

Results and Discussion

Feed Analysis

Inclusion amounts of soybean meal and soybean hulls were slightly different to balance the diets to be isonitrogenous and isoenergetic (**Table 5.1**) and to create the CON diet. The nutrient composition of the grain mixes and grass hay (**Table 5.2**) was consistent during the study. One exception was the slight variation on DM of grass hay during the study, which was due to changes in season and humidity; however, as the dietary inclusion amount of grass hay was similar among diets these DM changes did not affect our interpretation of treatment effects. Generally, nutrient content analyzed in diets (**Table 5.3**) was consistent with the formulated diets; however, CP of all three diets was 1% greater than formulated because CP of the hay was slightly higher during the study than values used for initial diet formulations. When the nutrient composition of the ingredients based on analysis was reentered into the NRC (2001) software to calculate energy values, the values of the analyzed diets were consistent with the original formulations among treatments.

Heifer Growth Performance

Growth performance and intake results are in **Table 5.4**. There was no effect of treatment for any frame growth measurements. No treatment by week interactions were found for any of the frame growth measurements. There was no effect of treatment for DMI, ADG and BCS. There was an increase over time for frame growth measurements, DMI (**Figure 5.1**), ADG and BCS as expected for growing animals. There were no effects of treatment in change per day for growth variables measured except for heart girth. Heifers fed CRM had a lesser change in heart girth than heifers fed CAN or CON diets; however, the differences among treatments were numerically small and of questionable biological significance. It should be noted that ADG was high for heifers of this age for all treatments; although the diets were formulated to achieve 0.8 kg/d the target recommendation for heifers at this age (Zanton and Heinrichs, 2005), the ADG observed was 0.2 kg greater than the target regardless of treatment. The same phenomenon was observed by Anderson et al. (2015), Manthey et al. (2016) and Manthey and Anderson (2018), where it was suggested that the NRC (2001) model overestimates the energy requirements of growing dairy heifers or underestimates energy provided by distillers dried grains with solubles, which was included at a comparable amount across treatments (**Table 5.1**). Considering that in our previous experiment comparing CRM with distillers dried grains with solubles the target ADG was reached, but the percentage of the BW used to limit-fed was 2.65%, and in the present experiment where we utilized DDGS but 2.4% of BW was used to limit-fed, the target ADG was surpassed, it is more

probable the NRC (2001) overestimates the energy requirements of growing dairy heifers depending of the percentage of the BW used for limit-feeding.

The significant week effect on DMI was expected as the heifers were growing and consuming more (**Figure 5.1**). In our previous research study (Rodriguez-Hernandez and Anderson, 2018) there was a significant treatment by week interaction where heifers consuming cold-pressed CRM ate less in the first two weeks compared to the DDGS diet. In the current study with solvent-extracted CRM there was no interaction of treatment by week which allows us to suggest that there were no issues with the flavor of the solvent-extracted meal. It has been reported that when plants of the Brassicaceae family are chewed or cut, the enzyme myrosinase hydrolyzes glucosinolates and some of the degradation products could cause bitterness (Duncan and Milne, 1993). The glucosinolates content of solvent-extracted CRM diet in the present research was almost ten times less (1.9 vs. 16.6 μM/g of CRM diet) than in the research of Rodriguez-Hernandez and Anderson (2018), where cold-press extracted carinata meal was used. The solvent extraction process of the oil requires heat and it has been observed that heat induces decomposition of glucosinolates in the absence of myrosinase (Bones and Rossiter, 2006).

Overall, growth performance, DMI and feed efficiency were normal and comparable to other feeding studies by our research group with heifers in this age range (Anderson et al., 2015; Lawrence et al., 2016; Manthey et al., 2016; Manthey and Anderson, 2018; Rodriguez-Hernandez and Anderson, 2018).

Rumen Fermentation Characteristics

Collection of rumen samples via esophageal tubing at a single time point in a day is not an optimal or ideal method of collection; however, we considered it valuable to determine at a preliminary level if rumen fermentation was affected compared with our previous experiment in which we evaluated cold-pressed carinata meal (Rodriguez-Hernandez and Anderson, 2018). Rumen fluid fermentation profiles (**Table 5.5**) were not different among treatments. Total VFA concentrations in wk 12 tended ($P = 0.07$) to be less than wk 16 (90.0 vs. 97.0 mM, SEM = 3.44). Similarly, acetate concentrations tended ($P = 0.09$) to be less in wk 12 compared with wk 16 (56.5 vs. 60.6 mM, SEM = 2.26). Butyrate (8.8 vs. 9.6 mM, SEM = 0.39), isovalerate (1.4 vs. 1.6 mM, SEM = 0.07), and valerate (1.0 vs. 1.1 mM, SEM = 0.06) concentrations were less ($P < 0.05$) in week 12 compared with week 16. No significant treatment by week interactions were observed. Rumen fermentation characteristics were normal and comparable to other previous research from our group with heifers in this age range using the same methodology (Lawrence et al., 2016; Manthey et al., 2016; Manthey and Anderson, 2018; Rodriguez-Hernandez and Anderson, 2018). There was concern that the glucosinolates in the CRM may alter fermentation, but results indicated it was not an issue. Because of sampling methodology, these results should be regarded with caution and more research is warranted with cannulated heifers or cows to substantiate that feeding CRM solvent extracted at 10% of diet DM does not negatively alter rumen fermentation.

Apparent Total Tract Digestion of Nutrients

Total-tract nutrient digestibility is in **Table 5.6**. Digestibilities of DM, OM, NDF, and ADF were similar among diets. The apparent total-tract digestibilities of the DM and OM were similar to other research from our group. However, the apparent total-tract digestibility of the NDF and ADF, was 5% greater in the present study compared with past research (Anderson et al., 2015; Lawrence et al., 2016; Manthey et al., 2016; Manthey and Anderson, 2018), but comparable with our previous study with CRM (Rodriguez-Hernandez and Anderson, 2018). This difference could be due to variation in forage quality among growing seasons or differences in nutrient density in the various research diets. Additionally, the aforementioned studies also had variable rates of limit-feeding or fed ad libitum diets, which could impact passage rates through the gastrointestinal tract and digestibilities.

Conclusions

Overall, the present research demonstrates that dairy heifers limit-fed a diet containing solvent-extracted carinata meal at 10% (DM basis) have comparable intakes, growth performance, rumen characteristics, and apparent total-tract digestibility of nutrients, with heifers fed the control or canola meal diets. Additionally, to our knowledge, this is the second study that demonstrates that carinata meal can be fed and have similar growth performance compared with commonly used feedstuffs in the dairy industry. Therefore, carinata meal is a suitable competitor and can effectively replace canola meal or a portion of the protein provided from soybean meal in rations for

growing dairy heifers when limited-feeding is used as feeding strategy. More research on feeding CRM is warranted using other feeding strategies such as ad libitum TMR as feed for lactating cows, but this research lays the foundation that it is a viable feedstuff for use in the dairy feed industry. With its positive attributes both as a crop and as a biofuels feedstock, it is speculated that carinata meal will become more widely available and prominent feed in the future.

Acknowledgments

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Table 5.1. Ingredient composition of diets with 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN), and control (CON) fed to growing Holstein heifers[1]

	Diet		
Ingredients, % of DM	CRM	CAN	CON
Grass hay	60.0	60.0	60.0
Ground corn	5.0	5.0	5.0
DDGS	13.0	13.0	13.0
Soybean meal	4.5	6.0	13.0
Soybean hulls	5.5	4.0	7.0
Carinata meal	10.0	-	-
Canola meal	-	10.0	-
Vitamin and mineral pre-mix[2]	1.2	1.2	1.2
Calcium carbonate	0.4	0.4	0.4
Salt	0.4	0.4	0.4

[1]Formulated (NRC, 2001).

[2]Contained: Ca 18.9%, NaCl 24.5%, Mg 1.6%, K 0.5%, Cu 880 mg/kg , I 50 mg/kg, Se 25 mg/kg,Zn 3,880 mg/kg, vitamin A 551,146 UI/kg, vitamin D_3 110,229 UI/kg, and vitamin E 4,189 UI/kg (HeiferSmart No Phos, Purina Animal Nutrition LLC, Shoreview, MN).

Table 5.2. Nutrient composition of the test feeds (solvent-extracted carinata meal and solvent-extracted canola meal) and ration components (grain mixes and forage) used to make the 10 % solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN), and control (CON) diets fed to growing Holstein heifers

	Test feeds				Concentrate grain mixes						Forage	
	Carinata meal		Canola meal		CRM		CAN		CON		Brome grass hay	
Item[1]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]
DM[2], %	91.1	0.49	87.6	0.72	89.7	0.09	89.1	0.16	88.7	0.16	85.1	1.58
Ash[2]	8.0	0.04	8.6	0.03	9.9	0.11	10.4	0.13	9.9	0.13	9.2	0.33
OM[2]	92.0	0.04	91.4	0.03	90.1	0.11	89.6	0.13	90.1	0.13	90.8	0.33
CP[2]	49.6	0.27	43.0	0.35	32.3	0.33	30.4	0.18	29.5	0.26	8.1	0.43
ADF[2]	6.6	1.18	17.2	0.51	13.6	0.43	13.5	0.58	12.3	0.84	23.3	0.45
NDF[2]	23.2	0.03	28.5	0.04	27.4	0.33	27.2	0.25	25.7	0.44	65.8	0.83
EE[2,3]	0.9	0.03	3.3	0.04	3.4	0.04	3.9	0.06	3.6	0.03	1.7	0.03
NFC[2,4]	18.3	0.28	16.7	0.24	59.3	0.36	58.5	0.32	60.7	0.43	23.3	0.83
Starch[5]	2.3	0.01	1.22	0.11	11.8	0.57	11.6	0.34	11.8	0.22	0.8	0.10
Ca[5]	0.52	0.005	0.85	0.845	1.26	0.025	1.54	0.190	1.23	0.110	0.33	0.010
P[5]	1.28	0.025	1.27	0.000	0.79	0.010	0.76	0.010	0.56	0.000	0.21	0.010
Mg[5]	0.56	0.020	0.56	0.010	0.46	0.005	0.45	0.020	0.36	0.000	0.18	0.010
K[5]	1.66	0.035	1.24	0.010	1.35	0.010	1.26	0.010	1.49	0.040	2.03	0.030
S[5]	1.70	0.025	0.76	0.000	0.87	0.020	0.54	0.000	0.43	0.005	0.14	0.005
Na[5]	0.02	0.000	0.05	0.005	0.73	0.030	0.75	0.035	0.78	0.000	0.03	0.005
Cl[5]	0.08	0.005	0.15	0.025	1.03	0.020	1.11	0.010	1.07	0.035	0.70	0.060
Mo[5], mg/kg	0.31	0.250	1.28	0.185	0.65	0.215	0.97	0.045	1.37	0.240	1.72	0.225
Mn[5], mg/kg	61.50	9.500	69.50	0.500	128.50	1.500	114.00	7.000	114.00	3.000	52.50	10.500
Zn[5], mg/kg	86.50	1.500	72.50	2.500	210.00	2.000	182.50	3.500	191.50	1.500	34.50	6.500
Cu[5], mg/kg	13.50	0.500	11.50	0.050	50.50	3.500	53.50	2.500	53.50	1.500	9.50	0.500
Fe[5], mg/kg	226.00	8.000	279.50	4.500	211.00	3.000	236.00	23.000	197.50	11.500	131.50	3.500
DCAD[6], mEq/100 g	-64.97	1.025	-17.83	0.225	-17.26	0.035	-0.75	2.060	15.34	2.545	2.34	2.335
Glucosinolates[7], µM/g	19.0	-	2.3	-	4.75	-	0.58	-	-	-	-	-

[1]% of DM, unless otherwise indicated.
[2]Results from monthly composite samples.
[3]EE = Ether extract, petroleum ether.
[4]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).

[5]Results calculated from the analysis of 5 or 4 mo composites of the ration components.
[6]DCAD = dietary cation-anion difference.
[7]Value of test feed from glucosinolate analysis; value for the CRM and CAN grain mixes were calculated from glucosinolates analysis and inclusion rate (10%) of the test feed in the diet. CON ingredients and brome grass hay do not contain glucosinolates.
[8]The MEANS procedure of SAS was used to estimate means and standards errors of nutrients of the monthly feed composites, and 5 or 4 mo composites of ration components.

Table 5.3. Overall nutrient composition of diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

	Diet					
	CRM		CAN		CON	
Item[1]	Mean[8]	SE[8]	Mean[8]	SE[8]	Mean[8]	SE[8]
DM[2], %	86.9	0.94	86.7	0.94	86.5	0.98
Ash[2]	9.5	0.22	9.7	0.22	9.5	0.19
OM[2]	90.5	0.22	90.3	0.22	90.5	0.19
CP[2]	17.8	0.21	17.0	0.29	16.7	0.27
ADF[2]	19.4	0.37	19.4	0.40	18.9	0.50
NDF[2]	50.5	0.58	50.4	0.52	49.8	0.51
EE[2,3]	2.4	0.03	2.6	0.01	2.5	0.02
NFC[2,4]	19.9	0.69	20.3	0.70	21.6	0.56
Starch[5]	5.2	0.29	5.1	0.19	5.2	0.03
Forage NDF[2]	39.5	0.50	39.5	0.50	39.5	0.50
Nonforage NDF[2]	10.9	0.13	10.9	0.10	10.3	0.18
Ca[5]	0.70	0.016	0.81	0.070	0.69	0.050
P[5]	0.44	0.010	0.43	0.002	0.35	0.006
Mg[5]	0.29	0.004	0.29	0.002	0.25	0.006
K[5]	1.76	0.022	1.72	0.022	1.81	0.002
S[5]	0.43	0.005	0.30	0.003	0.25	0.001
Na[5]	0.31	0.009	0.31	0.011	0.33	0.003
Cl[5]	0.83	0.028	0.86	0.040	0.85	0.022
Mo[5], mg/kg	1.29	0.221	1.42	0.153	1.58	0.039
Mn[5], mg/kg	82.90	6.900	77.10	9.100	77.10	7.500
Zn[5], mg/kg	104.70	3.100	93.70	2.500	97.30	3.300
Cu[5], mg/kg	25.90	1.100	27.10	1.300	27.10	0.300
Fe[5], mg/kg	163.30	0.900	173.30	7.100	157.90	6.700
DCAD[5], mEq/100 g	7.96	1.387	14.56	2.23	20.99	0.383
Glucosinolate[6], µM/g	1.9	-	0.23	-	-	-
ME[7], Mcal/kg of DM	2.37	-	2.36	-	2.38	-
NEg[7], Mcal/kg of DM	0.90	-	0.89	-	0.91	-

[1]% of DM, unless otherwise indicated.
[2]Results from monthly composite samples.
[3]EE = Ether extract.
[4]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).
[5]Results calculated from the analysis of 5 and 4 mo composites of the ration components.
[6]Value calculated based on glucosinolate analysis (Table 2) and inclusion rate (10%) of the test feed on the CRM diet.
[7]Values are calculated based on inputting sample nutrient analysis into ration formulations in the Dairy NRC computer program (2001, Washington, DC).
[8]The MEANS procedure of SAS was used to estimate the means and standards errors of the nutrients of the monthly feed composites, and 5 and 4 mo composites of the ration components.

Table 5.4. Dry matter intake, gain:feed ratios, BW, ADG, frame size measurements and body condition score for growing Holstein heifers fed diets with 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or a control diet (CON)

	Treatment				*P*-value		
Item	CRM	CAN	CON	SEM	Treatment	Week	Treatment × Week
DMI, kg/d	6.31	6.16	6.05	0.114	0.29	<0.01	0.99
Gain:feed	0.174	0.182	0.183	0.0041	0.23	<0.01	0.97
BW							
Mean	279.2	276.8	276.7	1.24	0.96	<0.01	0.80
Initial	210.0	210.8	199.9	6.19	0.34		
Final	329.0	329.6	330	2.09	0.88		
ADG, kg/d	1.01	1.02	1.03	0.033	0.85	0.04	0.86
Hip Height, cm							
Mean	125.9	125.7	126.1	0.29	0.73	<0.01	0.17
Initial	119.1	119.1	118.0	0.84	0.59		
Final	130.6	131.2	131.2	0.36	0.43		
Change, cm/d	0.10	0.10	0.11	0.003	0.33	0.11	0.25
Wither Height, cm							
Mean	122.2	122.6	122.4	0.34	0.80	<0.01	0.50
Initial	114.6	114.3	113.5	0.90	0.70		
Final	128.4	128.0	128.0	0.53	0.70		
Change, cm/d	0.12	0.12	0.11	0.007	0.93	0.25	0.25
Heart Girth							
Mean	147.3	147.9	148.2	0.42	0.34	<0.01	0.80
Initial	136.2	136.8	134.4	1.30	0.42		
Final	156.2	156.9	157.8	0.52	0.10		
Change, cm/d	0.17	0.18	0.19	0.004	0.05	0.09	0.82
Paunch Girth, cm							
Mean	185.3	183.4	184.3	1.39	0.64	<0.01	0.98
Initial	164.5	164.7	160.2	2.21	0.27		
Final	197.2	194.6	196.1	1.64	0.53		
Change, cm/d	0.28	0.26	0.29	0.016	0.36	<0.01	0.86
Body Length, cm							
Mean	109.8	110.1	109.1	0.70	0.55	<0.01	0.99
Initial	101.5	99.8	99.7	1.18	0.49		
Final	118.9	119.8	118.4	0.98	0.60		
Change, cm/d	0.15	0.16	0.15	0.009	0.49	<0.01	0.90
Hip Width, cm							
Mean	38.2	38.2	38.7	0.38	0.64	<0.01	0.57
Initial	34.5	34.3	33.3	0.37	0.07		
Final	39.8	41.4	41.6	0.53	0.97		
Change, cm/d	0.03	0.03	0.4	0.02	0.99	<0.01	0.99
BCS[1]							
Mean	3.06	3.06	3.05	0.020	0.87	<0.01	0.95
Initial	2.90[b]	2.95[a]	2.90[b]	0.009	<0.01		
Final	3.09	3.09	3.05	0.031	0.64		
Change, score/d	0.001	0.001	0.001	0.0003	0.84	<0.01	0.99

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

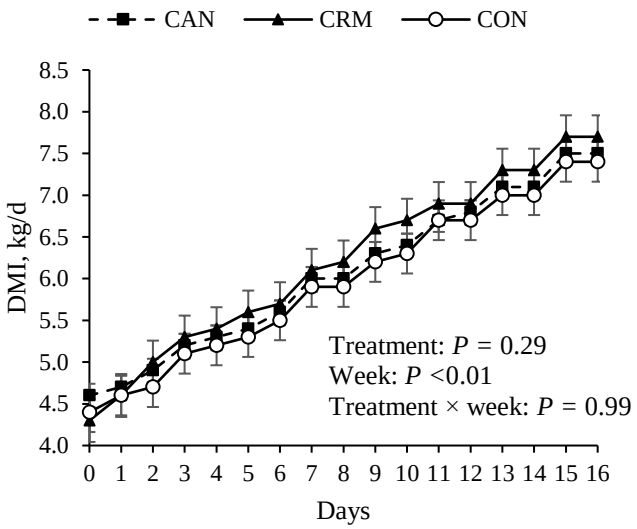
Table 5.5. Rumen fermentation characteristics of growing Holstein heifers fed diets with 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or a control diet (CON)

	Treatment				*P*-values		
Item	CRM	CAN	CON	SEM	Treatment	Week	Treatment × Week
pH	6.9	6.9	6.8	0.07	0.77	0.05	0.95
NH_3-N, mg/dL	12.8	12.3	13.7	1.39	0.77	< 0.01	0.82
Total VFA, m*M*	90.9	92.3	96.8	5.09	0.69	0.07	0.84
VFA, mmol/100mmol							
Acetate	56.6	58.1	61.0	3.32	0.64	0.09	0.87
Propionate	22.6	22.7	23.7	1.18	0.78	0.25	0.57
Isobutyrate	0.3	0.0	0.0	0.15	0.38	0.32	0.37
Butyrate	9.0	9.0	9.5	0.60	0.79	0.03	0.79
Isovalerate	1.5	1.5	1.6	0.11	0.49	<0.01	0.55
Valerate	1.0	1.0	1.1	0.09	0.65	0.02	0.84
Acetate:propionate	2.6	2.6	2.6	0.04	0.83	0.20	0.59

Table 5.6. Total tract digestion of nutrients for growing Holstein heifers fed diets with 10% of solvent-extracted carinata meal (CRM), 10 % solvent-extracted canola meal (CAN) or a control diet (CON)

	Treatment				*P*-values
Item, % digested	CRM	CAN	CON	SEM	Treatment
DM	71.6	71.6	73.7	2.30	0.42
OM	74.0	73.9	76.2	2.33	0.40
NDF	67.9	67.7	69.9	3.37	0.53
ADF	65.1	63.8	66.9	1.56	0.40
CP	81.8	79.8	81.6	1.98	0.50

Figure 5.1. Dry matter intakes (DMI) of growing Holstein heifers fed diets with 10% solvent-extracted carinata meal (CRM), 10 % solvent-extracted canola meal (CAN) or a control diet (CON) over 16 wk. Error bars represent SEM = 0.11



CHAPTER 6. SOLVENT-EXTRACTED CARINATA MEAL COMPARED WITH CANOLA MEAL OR SOYBEAN PRODUCTS IN DIETS FOR GROWING DAIRY HEIFERS: EFFECTS ON METABOLIC PROFILE, AND ONSET OF PUBERTY

ABSTRACT

Carinata meal is a developing oilseed meal that contains glucosinolates which may impair thyroid gland function and consequently metabolism and reproduction. Our objective was to compare the metabolic profile and onset of puberty of dairy heifers fed diets containing carinata meal, canola meal, or a control diet containing soybean products. A 16-wk randomized block design experiment with 36 Holstein heifers [6.3±0.1 mo of age, and 207±3 kg of body weight (**BW**)] was conducted. Heifers were blocked by age. Treatments were: 1) carinata meal (**CRM**), 2) canola meal (**CAN**) and 3) a control diet (**CON**) with most of the protein provided from soybean meal. Test meals were solvent extracted and included at 10% of diet DM. Diets were isocaloric and isonitrogenous and contained similar ingredients, other than the test feeds. Heifers were limit-fed rations at 2.4% of BW on DM basis using a Calan gate system. Jugular blood samples were collected 4 h post-feeding on 2 d during wk 0, 4, 8, 12, and 16 for metabolite and thyroid hormones analyses. To determine onset of puberty, blood samples were taken every 3 or 4 d for progesterone analysis. Data were analyzed using MIXED procedures with repeated measures in SAS 9.4. Puberty data were analyzed as binomial data (cycling or not cycling) and using repeated measures by 10-d and 10-kg intervals of

age and BW. Significance was declared at $P < 0.05$. Glucose, cholesterol, triglycerides, plasma urea nitrogen, triiodothyronine, and thyroxine concentrations were similar ($P > 0.05$). Age at puberty was similar among treatments. The proportion of heifers that were cycling by 270 kg of BW was greater for CRM and CON compared with CAN. Results show that growing heifers can be limit-fed diets with 10% CRM with no effects on thyroid hormones, metabolic status, and onset of puberty.

Keywords: dairy heifer, brassica carinata, glucosinolates, puberty

Introduction

The use of biofuel industry by-products as feedstuffs for heifers is a good option to reduce feed cost and promote growth in heifers (Clark et al., 1984). Carinata meal is a new feedstuff with a high protein content, co-product of the oil extraction of carinata oilseeds (*Brassica carinata*). Carinata is a non-food oilseed with a high oil content rich in very long-chain fatty acids such as erucic acid (C22:1) which is favorable for producing renewable, non-fossil biofuels, polymers, plastics, pharmaceutical and nutraceutical oils (Cardone et al., 2003; Zhu et al., 2016). Carinata is receiving considerable interest in North America for its ability to adapt to drought and low fertility soils, showing promise for portions of the Great Plains and U.S. Pacific Northwest that currently have limited oilseed cultivation (Marillia et al., 2014; Zhu et al., 2016). Additionally, carinata meal is rich in essential sulfur-containing amino acids, and has low fiber and higher protein content compared with canola meal. The total digestibility of its protein is similar to soybean meal and linseed meal and greater than canola meal and distillers dried grains

with solubles (Xin and Yu, 2014; Ban et al., 2017; Lawrence and Anderson, 2018). Chapter 3 and 4 presented results demonstrating that dairy heifers limit-fed diets with cold-pressed carinata meal at 10% of the diet DM basis have the same growth performance, and metabolic profile compared with heifers feed distillers dried grains with solubles. As carinata meal is a promising feedstuff for use in the Great Plains area and potential competitor with the imported canola meal the objective of this research was to conduct a study to determine the effects of feeding solvent-extracted carinata meal on metabolic profile, thyroid hormones, and onset of puberty of dairy heifers. We hypothesized that as solvent-extracted carinata meal has high crude protein content and quality, and its content of glucosinolates is less than cold-pressed meal, its inclusion in diets at 10% (on a DM basis) for peripubertal dairy heifers limit-fed at 2.4% of BW, will not affect metabolic profile, thyroid hormone concentrations, or onset of puberty compared with the canola meal or a soybean control diet.

Materials and Methods

Samples for this study were taken during the previously described feeding study from Chapter 5; this companion article contains details on the diets, protocols, animal care, heifer growth performance, rumen fermentation, and total-tract digestibility of nutrients. All animal procedures and uses were approved by the South Dakota State University Institutional Animal Care and Use Committee, protocol number 16-079E. The institutional Animal Welfare Assurance number filed with the Health Service Office for Protection from Research Risks is #A3958-01.

Experimental Design

Thirty-six Holstein heifers [6.3±0.1 mo of age, and 207±3 kg of body weight (BW)] were used in a randomized complete block design with three treatment diets. Heifers were blocked in groups of three based on birth date. Heifers were randomly assigned to treatment within block. Heifers were added on the study in groups of 6 or 2 blocks at different times based on age and availability with a target start age of 6.3 mo. The feeding study was completed in 9 mo from December 2016 to August 2017 at the South Dakota State University Dairy Research and Training Facility (Brookings, SD). Heifers were adapted to the research barns and feeding system for approximately 2 wk, followed by an experimental feeding period of 16 wk.

The three treatment diets were limit-fed at 2.40% of body weight. Treatments were: 1) solvent-extracted carinata meal (**CRM**), 2) solvent-extracted canola meal (**CAN**), both at 10% of diet dry matter (DM); and 3) control diet (**CON**) where most of the protein was provided from soybean meal. The remainder of the diets were comprised of grass hay, ground corn, distillers dried grains with solubles (**DDGS**), soybean meal, soybean hulls and mineral mix to meet nutrient requirements and were formulated with NRC (2001) software to allow for similar intakes of protein and energy among treatments (**Table 6.1**). The dietary inclusion of carinata meal at 10% of the diet was used as described in the tentative status definition by the FDA-AAFCO (AAFCO, 2018).

Sample Collection and Analysis

During wk 0, 4, 8, 12 and 16 of the feeding study blood samples from the jugular vein were taken on 2 consecutive days. Blood samples were taken approximately 3.5 h after feeding (1000 h) via venipuncture of the jugular vein into vacutainer tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) and potassium oxalate ($C_2K_2O_4$) for glucose analysis (cat. No. 367925) or K_2EDTA for all other analyses (cat. No. 366643). After collection, samples were immediately placed on ice and then brought to the laboratory within 3 h for processing. Blood collection tubes were centrifuged (1,000 x *g*) for 20 minutes at 4°C (CR412, Jouan Inc., Winchester, VA). Serum (from NaFl and $C_2K_2O_4$ tubes) or plasma (from K_2EDTA tubes) was transferred to polystyrene tubes (Falcon, cat. 352052, Corning Science S.A de C.V., Mexico) and frozen at -20°C until further processing and analysis.

To determine onset of puberty, additional blood samples were taken for progesterone analysis. Sampling began on week 1 of the feeding trial and continued during all the feeding period. Blood samples were taken via coccygeal venipuncture into vacutainer tubes containing K_2EDTA twice weekly (Tuesday and Friday) approximately 3.5 h post feeding. Plasma was harvested as previously described.

Serum or plasma samples of the second day of sampling were analyzed for glucose, plasma urea nitrogen (**PUN**), cholesterol, triglycerides (**TG**), using commercially available enzymatic or colorimetric assay on a microplate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA). Serum glucose was

analyzed using glucose oxidase as described by Trinder (1969; Pointe Scientific Inc., Canton, MI). Plasma total cholesterol was analyzed using cholesterol esterase and oxidase (Pointe Scientific Inc.) as described by Allain et al. (1974). Plasma urea nitrogen was analyzed using diacetyl monoxime (Procedure 0580; Stanbio Laboratory, Boerne, TX). Plasma TG concentration was analyzed using glycerol phosphate oxidase after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) that paired the reaction with the classic Trinder (1969) reaction.

For metabolic hormones triiodothyronine (**T3**), and thyroxine (**T4**) plasma samples of the second day of sampling were analyzed by RIA. Plasma concentrations of T3 were determined in duplicate using the T3 Solid Phase Component System according to the manufacturer's directions (06B-254216, MP Biomedicals, Solon, OH). Sensitivity of the assay was 5.44 ng/dL. Inter- and intra-assay CV of were 10.3% and 9.7%, respectively. Plasma concentrations of T4 were determined in duplicate using the T4 Mab Solid Phase Component System according to the manufacturer's directions (06B-254030, MP Biomedicals, Solon, OH). Sensitivity of the assay was 0.24 μg/dL. Inter- and intra-assay CV of were 7.7% and 8.4%, respectively.

Samples of the first day of sampling of wk 16 were used for plasma fatty acid determination; lipid extractions were performed as described by Bligh and Dyer (1959). Extracted lipids were then prepared for fatty acid analysis using butylation methods as described by Sukhija and Palmquist (1988) with adaptations by Abdelqader et al. (2009). Feed samples for fatty acid analysis were weekly collected, and 5- or 6-mo composites of

DDGS, CRM, grain mixes, and grass hay were analyzed for fatty acid profiles via direct butylation techniques (Abdelqader et al., 2009). All prepared fatty acid samples were analyzed via GC (Hewlett Packard 6890, Palo Alto, CA) as described by Abdelqader et al. (2009).

Plasma progesterone concentrations were determined using a validated RIA procedure as described by Engel et al. (2008). Inter- and intra-assay CV were 18.1 and 3.10%, respectively, and assay sensitivity was 0.394 ng/mL. Heifers were determined to have reached puberty when progesterone concentrations were greater than 1 ng/mL, indicating that ovulation had occurred and a corpus luteum had formed.

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Feed fatty acid means and standard errors were calculated using the MEANS procedure in SAS. Diet fatty acids values were calculated based on analysis of the grass hay and grain mixes (CRM and DDGS) for each treatment over the course of the study. Metabolites, hormones (T3, and T4), and plasma fatty acids data were analyzed as a randomized complete block design with week as the repeated measure and the term heifer (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, week, and treatment x week interactions. 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. Least squares means are reported for each treatment in the tables. The slice option was used to determine if differences among treatments were significant at each week or time point of measurements. Puberty data were analyzed as binomial data (cycling or not cycling) by age or weight using the LIFETEST procedure. Puberty data were also analyzed using repeated measures by 10-d and 10-kg intervals of age and BW. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

Results and Discussion

Fatty Acids

Fatty acid profile of total FA (mg/ 100 mg of FA) and composition (g/ kg of DM) of the grass hay, solvent-extracted carinata meal, solvent-extracted canola meal, soybean meal, and grain mixes used on CRM, CAN, and CON diets are shown on **Table 6.2 and Table 6.3**. Major FA in solvent-extracted carinata meal were C18:2 *cis*-9, *cis*-12 (25.87%), C16:0 (12.59%) and C16:1 (12.59%). Major FA in solvent-extracted canola meal were C18:1 *cis*-11 (39.09%) and C18:2 *cis*-9, *cis*-12 (27.05%). Major FA in soybean meal were C18:2 *cis*-9, *cis*-12 (49.21%) and C16:0 (13.28%). Hay FA proportions are similar to the observed on Chapter 4 and also coincide with FA profile observed by Manthey et al. (2017). The proportions of FA in the CRM, CAN and CON grain mixes were very similar in saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) but CAN grain mix had more monounsaturated fatty acids (MUFA) than

the other two grain mixes. It is also important to note that as all meals were solvent-extracted total FA were low.

Table 6.4 and **Table 6.5** show the FA composition (g/kg of DM) and profile (g/100 g of FA) of the experimental diets (60% of grass hay and 40% of grain mix). As distillers dried grains with solubles were included in all diets, the major FA in all diets was C18:2 which is the major fatty acid found in corn oil (Anderson et al., 2015b; Manthey et al., 2017). The FA proportions of each diet are equivalent to its corresponding grain mix.

Fatty acid intakes (g/d) are shown in **Table 6.6**. Heifers fed CRM diet eat 5.8 mg/kg of BW as C22:1 which represents 0.4% of the observed dose to cause myocardial lipidosis in rats and 0.6% of the dose for nursing pigs (FSANZ, 2003). Our results are consistent with our previous research with limit-fed heifers of similar age fed DDGS at 10% of the diet DM which had a similar EE content to the diets of the present study (Chapter 4).

Plasma FA proportions (mg/ 100 mg FA) (**Table 6.7**) were similar for most of the FA for the three treatments. Overall the proportions of SFA, MUFA, and PUFA were similar. Overall, heifers of the three treatments had similar week 16 plasma FA concentrations (μg/ mL of plasma) (**Table 6.8**). Additionally, the FA concentrations are consistent with the ones observed on wk 16 of our previous study (Chapter 4). Our results are consistent with the FA proportions observed by Manthey et al. (2017) which probably is because DDGS were used at the same inclusion rate (13% of DM) in all three

experimental diets. However, the FA total concentrations are slightly less than those observed in Manthey et al. (2017), this difference may be mostly from the difference in the DDGS inclusion rate between studies and decreased amounts of oil in the solvent-extracted meals.

Metabolites and Metabolic Hormones

Plasma metabolites and metabolic hormones concentrations are presented in **Table 6.9**; the values observed are consistent with values reported for heifers of the same age and under a limit-feeding program (Anderson et al., 2015b; Lawrence et al., 2016; Manthey et al., 2017; Manthey and Anderson, 2018). No treatment by wk interactions were found for any of the metabolites or metabolic hormones measured. **Figures 6.1** to **6.6** show the profiles for the metabolites and metabolic hormones measured. There was an effect of wk for PUN, cholesterol, and T3, as their concentrations increased over time, which was expected as the heifers were growing. There was a tendency ($P = 10$) for a wk effect on plasma glucose concentrations, which only changed slightly during the feeding period which may be related to the starch content of the diet and due to hormonal regulation by insulin, IGF-1, and glucagon (Allen and Bradford, 2012; Allen et al., 2017). There was no effect of wk for triglycerides, or T4. No change in triglycerides during the feeding period is consistent with previous studies where a limit-feeding strategy was used to control energy intakes and as fat intake was similar among treatments (Manthey et al., 2017). The lack of a wk effect on T4 concentrations allows us to suggest that there were no metabolic challenges for the heifers, although the profile of T4 is slightly below that

observed in our previous study. This could be related to differences in dietary fat between studies. Richards et al. (1995) observed a decrease on T3 and T4 concentrations in restricted-fed cows, and after returning the cows to a maintenance diet, concentrations of both thyroid hormones increased.

No effect of treatment was observed for any of the metabolites or metabolic hormones measured, which is consistent with the nutrient composition of the diets as they were isoenergetic and isonitrogenous. Therefore, metabolically the heifers fed CRM were comparable to heifers fed CAN and CON, despite the small amount of glucosinolates.

Puberty

No effect of treatment was observed for average age or BW at the onset of puberty (**Table 6.10**). Although most of the metabolic profile of the heifers among treatments were similar, no differences in growth performance were observed (Chapter 5), and no differences in the proportion of heifers cycling by the end of the study were detected (**Table 6.11**). However, there was a difference in the proportion of heifers cycling by 270 kg of BW (75% of CRM-fed, 58% of CAN-fed heifers, and 67% of CON-fed heifers; $P = 0.02$; **Figure 6.8**). The present results contrast with our previous results with heifers fed 10% of the diet DM with cold-pressed carinata meal or DDGS (Chapter 4) where less than 50% of the heifers in both treatments were cycling by 300 kg of BW. Anderson et al. (2015) observed a lower proportion of heifers cycling when limit-fed diets with 3% of EE content compared to the proportion of heifers cycling when limit-fed diets with 7% of EE were offered. In the present study, the percentage of fat in the diet

was very similar (2.6%), but more than 50% of the heifers in all treatments were cycling by 300 kg of BW. One possibility is that in contrast to the cholesterol profile of the heifers in the study in Chapter 4 where cholesterol concentrations on wk 0 were between 50-60 mg/dL, in the present study cholesterol concentrations on wk 0 were approximately of 90 mg/dL, and then decreased slightly in wk 4 and kept increasing slowly during the rest of the study. Additionally, the cholesterol profile of the heifers in this study is very similar to the observed by Anderson et al. (2015b) on the heifers fed the high-fat distillers grains. Manthey et al. (2017) observed precocious onset of puberty when heifers were fed diets with 30, 40 or 50% inclusion of DDGS and the cholesterol profile in that study increased faster than in our present study or Anderson et al. (2015b). Cholesterol conversion to pregnenolone is the rate-limiting step for the synthesis of progesterone (Talavera et al., 1985). Moreover, Garcia et al. (2003) observed an increase in plasma concentrations of cholesterol as heifers were near to puberty. Thus, it is speculated that a concentration of cholesterol is required as a metabolic signal to trigger onset of puberty.

Conclusions

In agreement with our hypothesis feeding solvent-extracted carinata meal at 10% of the diet DM resulted in heifers having similar metabolic profile to heifers fed the CAN and CON diets. Additionally, an increased proportion of heifers fed CRM or CON were cycling by 270 kg of BW compared with CON heifers. Intake of C22:1 was only 0.4 and 0.6% of the toxic dose reported for rats and piglets, respectivelly. Concentrations of thyroid hormones were comparable among treatments; therefore limit-fed diets

containing 10% solvent-extracted CRM do not have negative effects on thyroid function. Overall, these findings combined with our companion research (Chapter 5) support the hypothesis that solvent-extracted carinata meal is a good protein source for growing dairy heifers and comparable to canola meal and soybean products.

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Table 6.1. Ingredient and nutrient composition of diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

	Diet[1]					
	CRM		CAN		CON	
Item, % of DM	Mean[9]	SE[9]	Mean[9]	SE[9]	Mean[9]	SE[9]
Grass hay	60.0	-	60.0	-	60.0	-
Ground corn	5.0	-	5.0	-	5.0	-
DDGS	13.0	-	13.0	-	13.0	-
Soybean meal	4.5	-	6.0	-	13.0	-
Soybean hulls	5.5	-	4.0	-	7.0	-
Carinata meal	10.0	-	-	-	-	-
Canola meal	-	-	10.0	-	-	-
Vitamin and mineral pre-mix[9]	1.2	-	1.2	-	1.2	-
Calcium carbonate	0.4	-	0.4	-	0.4	-
Salt	0.4	-	0.4	-	0.4	-
DM[3], %	86.9	0.94	86.7	0.94	86.5	0.98
Ash[3]	9.5	0.22	9.7	0.22	9.5	0.19
OM[3]	90.5	0.22	90.3	0.22	90.5	0.19
CP[3]	17.8	0.21	17.0	0.29	16.7	0.27
ADF[3]	19.4	0.37	19.4	0.40	18.9	0.50
NDF[3]	50.5	0.58	50.4	0.52	49.8	0.51
EE[3,4]	2.4	0.03	2.6	0.01	2.5	0.02
NFC[3,5]	19.9	0.69	20.3	0.70	21.6	0.56
Starch[6]	5.2	0.29	5.1	0.19	5.2	0.03
Forage NDF[3]	39.5	0.50	39.5	0.50	39.5	0.50
Nonforage NDF[3]	10.9	0.13	10.9	0.10	10.3	0.18
Glucosinolate[7], μM/g	1.9	-	0.23	-	-	-
ME[8], Mcal/kg of DM	2.37	-	2.36	-	2.38	-
NEg[8], Mcal/kg of DM	0.90	-	0.89	-	0.91	-

[1]Formulated (NRC, 2001).

[2]Contained: Ca 18.9%, NaCl 24.5%, Mg 1.6%, K 0.5%, Cu 880 mg/kg , I 50 mg/kg, Se 25 mg/kg,Zn 3,880 mg/kg, vitamin A 551,146 UI/kg, vitamin D_3 110,229 UI/kg, and vitamin E 4,189 UI/kg (HeiferSmart No Phos, Purina Animal Nutrition LLC, Shoreview, MN).

[3]Results from monthly composite samples.

[4]EE = Ether extract using petroleum ether.

[5]% of NFC = 100 – (% ash + % CP + % NDF + % EE) (NRC, 2001).

[6]Results calculated from the analysis of 5 and 4 mo composites of the ration components.

[7]Value was calculated based on glucosinolate analysis and inclusion rate (10%) of the test feeds on the CRM and CAN diets.

[8]Values are calculated based on inputting sample nutrient analysis into ration formulations in the Dairy NRC computer program (2001, Washington, DC).

[9]The MEANS procedure of SAS was used to estimate the means and standards errors of the nutrients of the monthly feed composites, and 5 and 4 mo composites of the ration components.

Table 6.2. Fatty acid proportions of the main ingredients carinata meal, canola meal, soybean meal, grass hay and the grain mixes used on the diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

	Carinata meal		Canola meal		Soybean meal		Hay		CRM grain mix		CAN grain mix		CON grain mix	
Fatty acid[1], mg/100 mg	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C4:0	0.34	0.018	0.11	0.006	0.19	0.003	0.33	0.011	0.14	0.006	0.10	0.008	0.12	0.006
C5:0	0.82	0.009	0.54	0.008	0.38	0.016	1.18	0.048	0.35	0.018	0.35	0.016	0.32	0.005
C6:0	0.00	0.000	0.00	0.003	0.02	0.002	0.09	0.012	0.16	0.014	0.09	0.003	0.16	0.006
C8:0	0.03	0.009	0.00	0.000	0.00	0.000	1.88	0.053	0.04	0.001	0.03	0.002	0.04	0.001
C10:0	0.92	0.051	0.56	0.006	0.39	0.020	3.07	0.305	1.17	0.019	0.94	0.057	1.05	0.021
C12:0	0.39	0.035	0.08	0.004	0.23	0.047	2.19	0.275	0.02	0.023	0.07	0.006	0.08	0.009
C12:1	4.09	0.126	2.40	0.039	5.11	0.222	10.67	0.896	3.36	0.497	3.62	0.341	4.91	0.138
C14:0	0.89	0.020	0.31	0.306	0.33	0.011	1.52	0.068	5.01	0.029	4.10	0.056	4.75	0.060
C14:1	0.14	0.044	0.10	0.005	0.05	0.032	0.19	0.025	0.06	0.021	0.10	0.021	0.06	0.005
C16:0	12.59	0.149	7.75	0.039	13.28	0.343	10.21	0.323	14.85	0.103	12.97	0.095	14.67	0.078
C16:1 *trans*	0.62	0.065	0.57	0.026	1.96	0.168	8.29	0.339	0.27	0.023	0.43	0.067	0.50	0.014
C16:1 *cis*	0.53	0.024	0.32	0.010	0.24	0.080	1.13	0.024	0.44	0.030	0.40	0.031	0.45	0.008
C16:1	12.59	0.011	1.34	0.016	0.12	0.010	0.28	0.014	0.23	0.003	0.44	0.009	0.18	0.002
C18:0	2.33	0.033	1.49	0.010	3.59	0.083	1.10	0.065	2.99	0.084	2.74	0.042	3.02	0.095
C18:1 *cis-9*	2.55	0.023	7.34	0.045	1.12	0.647	0.00	0.000	4.24	0.038	5.03	0.027	4.07	0.033
C18:1 *cis-11*	6.55	0.084	39.09	0.078	3.95	0.105	0.98	0.036	4.92	0.080	12.53	0.353	4.48	0.028
C18:2 *cis-9, cis-12*	25.87	0.294	27.05	0.194	49.21	0.952	8.51	0.697	48.66	0.837	46.33	0.389	50.10	0.192
C18:2 *tran 10, trans-12*	3.28	0.028	0.65	0.035	0.00	0.000	0.00	0.000	0.87	0.066	0.51	0.015	0.44	0.012
C18:3 n-6	1.14	0.021	0.07	0.003	0.00	0.000	0.31	0.016	0.13	0.008	0.01	0.009	0.00	0.000
C18:3 n-3	12.49	0.177	5.17	0.048	7.89	0.169	13.07	0.525	4.20	0.102	3.62	0.072	3.79	0.045
C20:0	1.11	0.019	0.45	0.003	0.22	0.015	1.24	0.023	1.04	0.041	0.72	0.013	0.91	0.026
C20:1, 8	1.28	0.023	0.61	0.027	0.49	0.034	13.90	0.443	1.70	0.166	1.45	0.134	1.62	0.080
C20:3	0.75	0.097	0.36	0.005	0.42	0.008	1.03	0.034	0.33	0.034	0.29	0.020	0.30	0.011
C20:5	1.64	0.545	0.03	0.016	0.00	0.000	0.00	0.000	0.00	0.000	0.02	0.018	0.11	0.010
C22:1	7.84	0.057	0.02	0.006	0.00	0.000	0.00	0.000	1.01	0.035	0.03	0.006	0.03	0.001
C22:6	1.41	0.014	0.71	0.012	0.44	0.008	1.89	0.072	0.72	0.003	0.62	0.008	0.59	0.006
Others[2]	10.14	0.333	2.91	0.086	10.40	2.643	17.04	0.290	3.16	0.253	0.31	0.311	3.29	0.047
SCFA[3]	9.86	0.269	5.35	0.071	11.22	0.400	29.46	0.641	11.69	0.569	10.75	0.440	13.42	0.164
LCFA[3]	90.14	0.269	94.65	0.071	88.78	0.400	70.54	0.641	88.31	0.569	89.25	0.440	86.58	0.164
SFA[3]	22.36	0.155	12.32	0.057	22.66	0.608	31.33	0.520	27.10	0.229	23.24	0.068	26.80	0.091

	Carinata meal		Canola meal		Soybean meal		Hay		CRM grain mix		CAN grain mix		CON grain mix	
Fatty acid[1], mg/100 mg	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
MUFA[3]	27.80	0.151	53.34	0.063	19.17	1.748	39.49	0.597	17.77	0.666	25.26	0.256	17.71	0.205
PUFA[3]	49.84	0.274	34.34	0.120	58.16	1.142	29.18	0.325	55.13	0.799	51.50	0.305	55.50	0.127

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C7:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C20:1, 5, C20:1 *cis*, CLA C18:2, C20:2, C20:3, C20:4, C22:0, C22:2, C22:4, C22:5, C23:0C24:0, C24:1, and unidentified fatty acids.

[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.3. Fatty acid composition of the main ingredients carinata meal, canola meal, soybean meal, grass hay and the grain mixes used in the diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

	Carinata meal		Canola meal		Soybean meal		Hay		CRM grain mix		CAN grain mix		CON grain mix	
Fatty acid[1], g/kg DM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
C4:0	0.09	0.007	0.08	0.006	0.09	0.001	0.11	0.003	0.09	0.006	0.08	0.007	0.09	0.004
C5:0	0.23	0.007	0.39	0.011	0.18	0.007	0.40	0.020	0.22	0.016	0.29	0.021	0.24	0.004
C6:0	0.00	0.000	0.00	0.002	0.01	0.001	0.03	0.003	0.10	0.007	0.07	0.004	0.12	0.006
C8:0	0.01	0.002	0.00	0.000	0.00	0.000	0.63	0.016	0.03	0.000	0.02	0.003	0.03	0.001
C10:0	0.26	0.015	0.41	0.010	0.18	0.010	1.03	0.100	0.74	0.030	0.77	0.068	0.78	0.009
C12:0	0.11	0.012	0.06	0.003	0.10	0.021	0.74	0.101	0.01	0.014	0.05	0.006	0.06	0.006
C12:1	1.14	0.049	1.74	0.054	2.31	0.104	3.62	0.364	2.13	0.319	2.96	0.350	3.61	0.108
C14:0	0.25	0.010	0.22	0.005	0.15	0.006	0.51	0.014	3.18	0.115	3.33	0.089	3.50	0.064
C14:1	0.04	0.014	0.07	0.003	0.02	0.014	0.06	0.008	0.04	0.014	0.08	0.018	0.04	0.004
C16:0	3.50	0.049	5.62	0.109	6.01	0.171	3.44	0.050	9.42	0.240	10.55	0.266	10.80	0.270
C16:1 *trans*	0.17	0.016	0.41	0.018	0.89	0.071	2.80	0.120	0.17	0.011	0.35	0.049	0.37	0.018
C16:1 *cis*	0.15	0.006	0.23	0.005	011	0.037	0.38	0.002	0.28	0.023	0.33	0.034	0.33	0.009
C16:1	0.15	0.003	0.97	0.010	0.06	0.004	0.10	0.003	0.15	0.007	0.36	0.013	0.13	0.003
C18:0	0.65	0.008	1.08	0.026	1.62	0.042	0.37	0.015	1.89	0.044	2.24	0.094	2.23	0.104
C18:1 *cis-9*	1.71	0.011	5.33	0.106	0.51	0.293	0.00	0.000	2.69	0.065	4.09	0.266	3.00	0.089
C18:1 *cis-11*	1.82	0.023	28.34	0.493	1.79	0.050	0.33	0.018	3.12	0.090	10.18	0.107	3.30	0.093
C18:2 *cis-9, cis-12*	7.20	0.101	19.61	0.246	2.26	0.512	2.86	0.193	30.87	1.011	37.68	0.912	36.91	0.907
C18:2 CLA *trans-10, 12*	0.91	0.016	0.48	0.034	0.00	0.000	0.00	0.000	0.56	0.055	0.42	0.023	0.33	0.015
C18:3 n-6	0.32	0.004	0.05	0.003	0.00	0.000	0.10	0.004	0.08	0.006	0.01	0.007	0.00	0.000
C18:3 n-3	3.47	0.054	3.75	0.095	3.57	0.094	4.42	0.225	2.67	0.150	2.95	0.134	2.79	0.041
C20:0	0.31	0.005	0.33	0.005	0.10	0.006	0.42	0.002	0.66	0.022	0.58	0.028	0.67	0.029
C20:1, 8	0.36	0.009	0.44	0.027	0.22	0.016	4.69	0.148	1.09	0.143	1.18	0.113	1.19	0.041
C20:3	0.21	0.032	0.26	0.008	0.19	0.004	0.35	0.006	0.21	0.020	0.24	0.019	0.22	0.008
C20:5	0.46	0.153	0.02	0.011	0.00	0.000	0.00	0.000	0.00	0.000	0.01	0.014	0.08	0.007
C22:1	2.18	0.051	0.01	0.005	0.00	0.000	0.00	0.000	0.65	0.043	0.03	0.005	0.02	0.001
C22:6	0.39	0.009	0.51	0.012	0.20	0.004	0.64	0.035	0.46	0.013	0.50	0.014	0.44	0.013
Others[2]	2.83	0.156	2.11	0.088	4.70	1.190	5.75	0.188	2.01	0.196	2.08	0.301	2.42	0.045
Total	27.83	0.603	72.52	1.286	45.22	0.388	33.75	0.636	63.46	2.044	81.38	2.533	73.66	1.694
SCFA[3]	2.75	0.121	3.88	0.106	5.07	0.195	9.95	0.376	7.42	0.459	8.78	0.605	9.88	0.168
LCFA[3]	25.08	0.497	68.64	1.189	40.14	0.348	23.80	0.325	55.04	1.784	72.60	1.946	63.78	1.555
SFA[3]	6.22	0.155	8.94	0.184	10.25	0.303	10.57	0.070	17.19	0.478	18.91	0.639	19.74	0.466
MUFA[3]	7.74	0.160	38.68	0.712	8.67	0.777	13.33	0.413	11.29	0.634	20.57	0.817	13.04	0.291
PUFA[3]	13.87	0.307	24.90	0.401	26.31	0.619	9.85	0.246	34.98	1.200	41.89	1.091	40.88	0.982

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C7:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C20:1, 5, C20:1 *cis*, CLA C18:2, C20:2, C20:3, C20:4, C22:0, C22:2, C22:4, C22:5, C23:0C24:0, C24:1, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.4. Fatty acid composition of the diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

Fatty acid[1], g/kg of DM	Diet					
	CRM	SEM	CAN	SEM	CON	SEM
C4:0	0.10	0.003	0.10	0.002	0.10	0.001
C5:0	0.33	0.007	0.36	0.012	0.33	0.014
C6:0	0.06	0.002	0.05	0.003	0.07	0.001
C8:0	0.39	0.010	0.40	0.010	0.39	0.010
C10:0	0.92	0.060	0.93	0.043	0.93	0.061
C12:0	0.45	0.065	0.47	0.062	0.47	0.063
C12:1	3.02	0.304	3.36	0.331	3.62	0.245
C14:0	1.58	0.052	1.64	0.029	1.71	0.283
C14:1	0.05	0.008	0.07	0.007	0.05	0.006
C16:0	5.83	0.004	6.28	0.090	6.39	0.097
C16:1 *trans*	1.74	0.071	1.82	0.076	1.82	0.078
C16:1 *cis*	0.34	0.008	0.36	0.012	0.36	0.004
C16:1	0.12	0.004	0.20	0.005	0.11	0.002
C18:0	0.98	0.020	1.12	0.038	1.11	0.043
C18:1 *cis-9*	1.08	0.026	1.64	0.042	1.20	0.035
C18:1 *cis-11*	1.45	0.028	4.27	0.102	1.52	0.045
C18:2 *cis-9, cis-12*	14.06	0.505	16.79	0.296	16.48	0.354
C18:2 *trans-10, trans-12*	0.22	0.022	0.17	0.009	0.13	0.006
C18:3 n-6	0.10	0.004	0.07	0.003	0.06	0.003
C18:3 n-3	3.72	0.094	3.83	0.136	3.77	0.147
C20:0	0.51	0.009	0.48	0.012	0.52	0.012
C20:1, *cis-8*	3.25	0.074	3.28	0.080	3.29	0.083
C20:3	0.29	0.009	0.30	0.008	0.30	0.006
C20:5	0.00	0.000	0.01	0.006	0.03	0.003
C22:1	0.26	0.017	0.01	0.002	0.01	0.000
C22:6	0.57	0.018	0.59	0.023	0.56	0.025
Others[2]	4.26	0.147	4.28	0.208	4.42	0.114
Total	45.64	0.509	52.80	1.133	49.71	0.990
SCFA[3]	8.94	0.290	9.48	0.400	9.92	0.258
LCFA[3]	36.69	0.560	43.32	0.784	39.79	0.806
SFA[3]	13.22	0.173	13.91	0.243	9.92	0.180
MUFA[3]	12.51	0.310	16.23	0.454	13.22	0.346
PUFA[3]	19.90	0.336	22.67	0.452	22.26	0.470

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C7:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C20:1, 5, C20:1 *cis*, CLA C18:2, C20:2, C20:3, C20:4, C22:0, C22:2, C22:4, C22:5, C23:0C24:0, C24:1, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.5. Fatty acid proportions per 100 g of fatty acids of the diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) fed to growing Holstein heifers

Fatty acid[1], g/100 g of FA	Diet					
	CRM	SEM	CAN	SEM	CON	SEM
C4:0	0.22	0.007	0.19	0.006	0.20	0.002
C5:0	0.72	0.022	0.67	0.021	0.67	0.201
C6:0	0.12	0.004	0.09	0.005	0.13	0.002
C8:0	0.86	0.024	0.74	0.018	0.79	0.027
C10:0	2.01	0.141	1.76	0.111	1.87	0.121
C12:0	0.99	0.147	0.88	0.108	0.94	0.124
C12:1	6.63	0.683	6.33	0.518	7.26	0.411
C14:0	3.46	0.082	3.11	0.012	3.44	0.068
C14:1	0.12	0.018	0.13	0.015	0.11	0.012
C16:0	12.77	0.159	11.90	0.134	12.85	0.098
C16:1 *trans*	3.82	0.147	3.44	0.098	3.67	0.131
C16:1 *cis*	0.74	0.012	0.68	0.009	0.73	0.011
C16:1	0.25	0.007	0.38	0.008	0.22	0.007
C18:0	2.15	0.026	2.11	0.048	2.24	0.075
C18:1 *cis-9*	2.36	0.037	3.10	0.031	2.41	0.027
C18:1 *cis-11*	3.17	0.043	8.09	0.213	3.06	0.032
C18:2 *cis-9, cis-12*	30.81	0.964	31.80	0.328	33.16	0.452
C18:2 *trans-10, trans-12*	0.49	0.043	0.32	0.012	0.26	0.007
C18:3 n-6	0.21	0.007	0.13	0.006	0.13	0.006
C18:3 n-3	8.15	0.250	7.25	0.221	7.58	0.275
C20:0	1.12	0.018	0.92	0.009	1.04	0.010
C20:1, 8	7.12	0.160	6.23	0.242	6.62	0.130
C20:3	0.64	0.018	0.58	0.014	0.60	0.017
C20:5	0.00	0.000	0.01	0.011	0.07	0.005
C22:1	0.57	0.031	0.02	0.003	0.02	0.001
C22:6	1.24	0.044	1.11	0.026	1.12	0.029
Others[2]	9.33	0.321	8.10	0.235	8.89	0.125
SCFA[3]	19.60	0.657	17.94	0.437	19.96	0.332
LCFA[3]	80.40	0.657	82.06	0.437	80.04	0.332
SFA[3]	28.96	0.185	26.34	0.163	28.65	0.211
MUFA[3]	27.42	0.623	30.72	0.230	26.58	0.186
PUFA[3]	43.62	0.545	42.93	0.113	44.78	0.088

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C7:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C20:1, 5, C20:1 *cis*, CLA C18:2, C20:2, C20:3, C20:4, C22:0, C22:2, C22:4, C22:5, C23:0C24:0, C24:1, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.6. Mean fatty acid intakes for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment				*P*- value		
Fatty acid[1], g/d	CRM	CAN	CON	SEM	Treatment	Week	Treatment × Week
C4:0	0.63	0.61	0.60	0.011	< 0.01	< 0.01	0.99
C5:0	2.07	2.20	2.02	0.037	< 0.01	< 0.01	0.99
C6:0	0.36	0.29	0.40	0.006	< 0.01	< 0.01	< 0.01
C8:0	2.46	2.42	2.37	0.042	< 0.01	< 0.01	0.99
C10:0	5.76	5.76	5.62	0.100	< 0.01	< 0.01	0.99
C12:0	2.83	2.90	2.83	0.050	< 0.01	< 0.01	0.99
C12:1	20.26	20.82	21.83	0.367	< 0.01	< 0.01	0.99
C14:0	9.91	10.18	10.30	0.177	< 0.01	< 0.01	0.99
C14:1	0.34	0.43	0.33	0.006	< 0.01	< 0.01	< 0.01
C16:0	36.56	38.98	38.56	0.667	< 0.01	< 0.01	0.99
C16:1 *trans*	10.94	11.27	11.01	0.194	< 0.01	< 0.01	0.99
C16:1 *cis*	2.13	2.24	2.19	0.383	< 0.01	< 0.01	0.99
C16:1	0.73	1.24	0.66	0.016	< 0.01	< 0.01	< 0.01
C18:0	6.14	6.92	6.72	0.116	< 0.01	< 0.01	0.99
C18:1 *cis-9*	6.75	10.15	7.25	0.143	< 0.01	< 0.01	< 0.01
C18:1 *cis-11*	9.08	26.49	9.18	0.278	< 0.01	< 0.01	< 0.01
C18:2 *cis-9, cis-12*	88.21	104.16	99.52	1.709	< 0.01	< 0.01	0.99
C18:2 *trans-10, trans-12*	1.40	1.039	0.79	0.019	< 0.01	< 0.01	< 0.01
C18:3 n-6	0.61	0.42	0.38	0.008	< 0.01	< 0.01	< 0.01
C18:3 n-3	23.32	23.76	22.74	0.408	< 0.01	< 0.01	0.99
C20:0	3.21	3.00	3.14	0.055	< 0.01	< 0.01	0.99
C20:1, *cis-8*	20.36	20.38	19.86	0.354	< 0.01	< 0.01	0.99
C20:3	1.91	1.39	1.80	0.030	< 0.01	< 0.01	< 0.01
C20:5	0.00	0.04	0.20	0.002	< 0.01	< 0.01	< 0.01
C22:1	1.62	0.06	0.05	0.013	< 0.01	< 0.01	< 0.01
C22:6	3.55	3.63	3.37	0.062	< 0.01	< 0.01	0.99
Others[2]	26.70	26.58	25.50	0.460	< 0.01	< 0.01	0.99
Total	286.19	327.63	300.20	5.355	< 0.01	< 0.01	0.99
SCFA[3]	56.08	58.85	59.92	1.021	< 0.01	< 0.01	0.99
LCFA[3]	230.12	268.79	240.28	4.335	< 0.01	< 0.01	0.99
SFA[3]	82.88	86.28	85.96	1.490	< 0.01	< 0.01	0.99
MUFA[3]	78.48	100.70	79.81	1.526	< 0.01	< 0.01	0.21
PUFA[3]	124.83	140.65	134.44	2.34	< 0.01	< 0.01	0.99

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C7:0, C9:0, C11:0, C11:1, C13:0, C13:1, C15:0, C15:1, C17:0, C17:1, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C20:1, 5, C20:1 *cis*, CLA C18:2, C20:2, C20:3, C20:4, C22:0, C22:2, C22:4, C22:5, C23:0C24:0, C24:1, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.7. Plasma fatty acid proportions from wk 16 of the feeding period for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment				
Fatty acid[1], mg/100 mg	CRM	CAN	CON	SEM	*P* - value
C4:0	0.92	0.92	0.96	0.065	0.66
C5:0	2.85	2.89	3.00	0.122	0.67
C6:0	0.07	0.08	0.08	0.015	0.66
C14:0	0.41	0.40	0.47	0.066	0.46
C14:1	0.63	0.61	0.67	0.031	< 0.01
C15:0	0.89	0.90	0.94	0.028	0.14
C15:1	0.32	0.36	0.35	0.037	0.32
C16:0	12.36	12.32	12.27	0.143	0.91
C16:1 *cis-9*	0.71	0.68	0.78	0.042	0.25
C18:0	20.66	20.97	20.36	0.781	0.54
C18:1 *trans-10*	0.82	0.99	0.90	0.171	0.53
C18:1 *cis-9*	10.28	10.73	9.76	0.328	0.13
C18:1 *cis-11*	0.88	1.01	0.86	0.048	< 0.01
C18:1 *trans*-11	0.34	0.31	0.33	0.027	0.40
C18:2 *cis-9, cis-12*	29.86	28.92	30.53	1.228	0.33
C18:3 n-6	0.99	1.11	1.18	0.130	0.18
C18:3 n-3	0.14	0.17	0.19	0.022	0.34
C20:0	3.81	3.26	3.33	0.361	0.14
C20:2	0.36	0.39	0.28	0.056	0.39
C20:3 *cis-11,14,17*	2.21	2.25	2.37	0.094	0.45
C20:4	4.63	4.91	4.72	0.183	0.25
C20:5	0.49	0.47	0.46	0.022	0.45
C22:0	0.28	0.31	0.18	0.203	0.60
C22:1 *cis*-13	0.21	0.18	0.20	0.302	0.35
C22:3	0.18	0.18	0.18	0.011	0.65
C22:5	0.18	0.18	0.18	0.011	0.65
C22:3	0.18	0.18	0.18	0.011	0.65
Others[2]	3.59	3.63	3.70	0.083	0.62
SCFA[3]	6.23	6.29	6.61	0.394	0.29
LCFA[3]	93.77	93.71	93.39	0.394	0.29
SFA[3]	43.14	43.01	42.54	0.798	0.64
MUFA[3]	16.95	17.60	16.67	0.857	0.28
PUFA[3]	40.16	39.64	41.05	1.134	0.34

[1]Represented as number of carbons:number of double bonds.
[2]Sum of C10:0, C12:0, C16:1 *trans*, C16:1 *cis*, C18:1 *trans-6*, C18:1 trans-9, C18:2 CLA cis-9, trans-11, C20:0, C20:1 *cis-8*, C18:2 CLA *trans-10, cis-12*, C24:0, C24:1, and unidentified fatty acids.
[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.8. Plasma fatty acid concentrations from wk 16 of the feeding period for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment				
Fatty acid[1], μg/mL plasma	CRM	CAN	CON	SEM	*P* - value
C4:0	12.13	12.10	12.13	0.180	0.97
C5:0	38.39	38.63	38.75	0.798	0.88
C6:0	0.97	1.05	1.08	0.154	0.72
C14:0	5.92	5.90	6.33	0.994	0.80
C14:1	8.63	8.45	8.91	0.649	0.70
C15:0	12.00	12.23	12.11	0.491	0.95
C15:1	4.24	4.85	4.60	0.329	0.43
C16:0	163.32	164.55	156.74	10.216	0.58
C16:1 *cis-9*	9.02	8.62	9.51	0.634	0.61
C18:0	266.14	274.09	253.67	26.920	0.47
C18:1 *trans-10*	12.12	14.73	12.51	2.687	0.46
C18:1 *cis-9*	135.76	143.84	125.05	11.187	0.10
C18:1 *cis-11*	11.42	13.40	10.83	0.543	< 0.01
C18:1 *trans-11*	4.60	4.30	4.31	0.247	0.63
C18:2 *cis-9, cis-12*	406.61	395.60	398.07	32.884	0.91
C18:3 n-6	14.02	15.55	15.91	1.772	0.41
C18:3 n-3	1.81	2.12	2.29	0.290	0.50
C20:0	53.15	46.32	44.68	3.612	0.23
C20:2	4.51	4.76	3.46	0.571	0.25
C20:3 *cis-11,14,17*	30.50	31.10	31.66	2.978	0.89
C20:4	62.55	66.12	61.72	3.471	0.35
C20:5	6.41	6.09	5.67	0.862	0.41
C22:0	3.96	4.01	2.73	1.945	0.58
C22:1 *cis*-13	2.89	2.49	2.63	0.223	0.45
C22:3	2.42	2.37	2.40	0.141	0.91
C22:5	12.33	12.43	11.79	0.521	0.66
C22:3	2.76	2.51	3.48	0.661	0.18
Others[2]	46.89	48.20	46.69	3.767	0.83
Total	1338.86	1349.80	1293.09	87.005	0.69
SCFA[3]	82.09	83.06	83.72	2.190	0.68
LCFA[3]	1256.26	1266.24	1208.86	85.814	0.67
SFA[3]	569.69	573.74	542.52	40.586	0.49
MUFA[3]	216.31	228.48	205.32	9.299	0.23
PUFA[3]	544.73	539.47	537.13	39.297	0.97

[1]Represented as number of carbons:number of double bonds.

[2]Sum of C10:0, C12:0, C16:1 *trans*, C16:1 *cis*, C18:1 *trans-6*, C18:1 trans-9, C18:2 CLA cis-9, trans-11, C20:0, C20:1 *cis-8*, C18:2 CLA *trans-10, cis-12*, C24:0, C24:1, and unidentified fatty acids.

[3]SCFA = short-chain fatty acids; LCFA = long-chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6.9. Plasma metabolites and metabolic hormones concentrations for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment				*P* - value		
Item	CRM	CAN	CON	SEM	Treatment	Week	Treatment x Week
Glucose, mg/dL	75.9	75.8	77.1	1.65	0.82	0.10	0.22
Plasma urea N, mg/dL	18.4	18.0	17.2	0.41	0.15	< 0.01	0.76
Cholesterol, mg/dL	81.7	81.2	79.0	2.95	0.78	< 0.01	0.16
Triglycerides, mg/dL	23.4	23.3	21.3	1.13	0.34	0.78	0.60
Triiodothyronine, ng/dL	135.4	140.7	141.6	5.73	0.71	< 0.01	0.50
Thyroxine, μg/dL	4.7	4.3	4.6	0.17	0.30	0.22	0.24

Table 6.10. Mean age and body weight (BW) at puberty for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment				*P* - value		
Item	CRM	CAN	CON	SEM	Treatment	Week	Treatment x Week
Age, d	289.6	297.4	294.1	3.21	0.74	< 0.01	0.99
BW, kg	317.7	328.8	317.4	3.45	0.60	< 0.01	0.44

Table 6.11. Binomial analysis for age and body weight (BW) at puberty for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON)

	Treatment			*P* – value[1]	
Item	CRM	CAN	CON	Log-Rank	Wilcoxon
Cycling, %		Age, d		0.18	0.27
25	270	280	270		
(C.I. 95%)	*(260-280)*	*(260-290)*	*(260-280)*		
50	300	310	300		
(C.I. 95%)	*(290-310)*	*(300-320)*	*(290-310)*		
75	320	330	330		
(C.I. 95%)	*(310-330)*	*(320-330)*	*(320-330)*		
		BW, kg		0.05	0.02
25	290	310	290		
(C.I. 95%)	*(280-300)*	*(300-320)*	*(280-300)*		
50	330	340	330		
(C.I. 95%)	*(310-330)*	*(330-350)*	*(320-340)*		
75	350	360	350		
(C.I. 95%)	*(340-360)*	*(350-360)*	*(350-360)*		

[1]Test of equality over strata (LIFETEST PROC, SAS)

Figure 6.1. Plasma concentrations of triglycerides for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

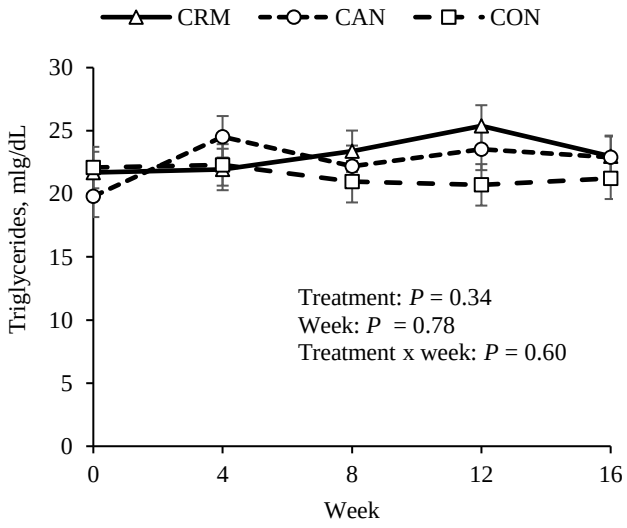


Figure 6.2. Plasma concentrations of glucose for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

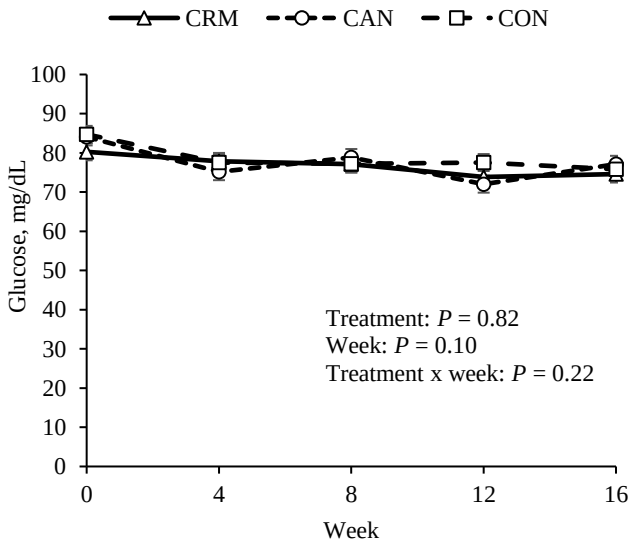


Figure 6.3. Plasma urea nitrogen (PUN) concentrations for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

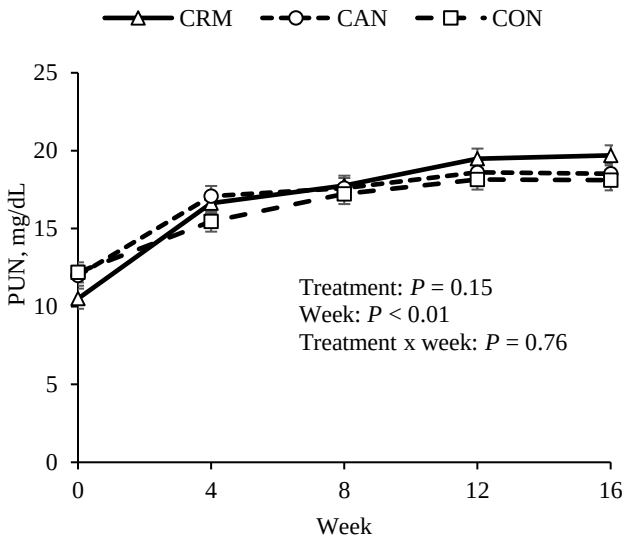


Figure 6.4. Plasma concentrations of cholesterol for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

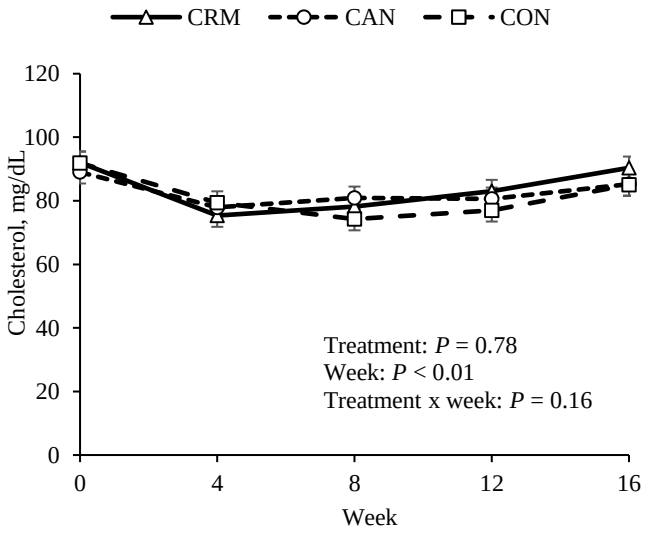


Figure 6.5. Plasma concentrations of triiodothyronine (T3) for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

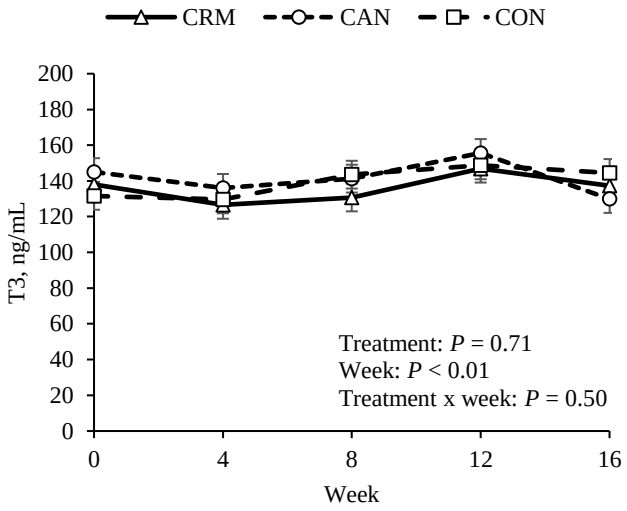


Figure 6.6. Plasma concentrations of thyroxine (T4) for growing Holstein heifers fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Values of wk 0 where used as covariable. Error bars represent SEM)

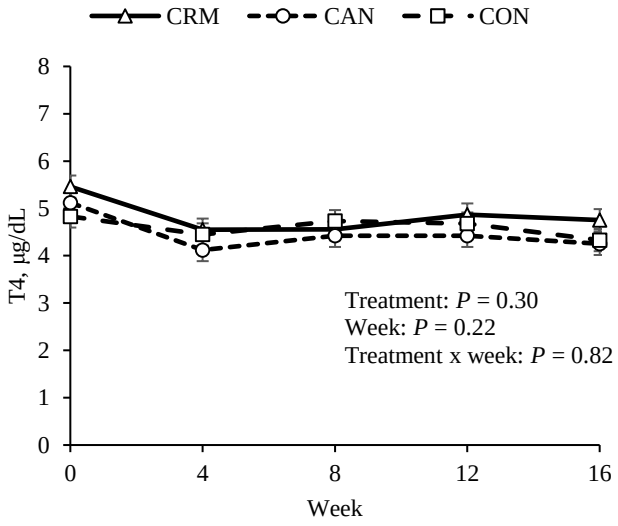


Figure 6.7. Percentage of Holstein heifers pubertal (cycling) by age that were fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Error bars represent SEM)

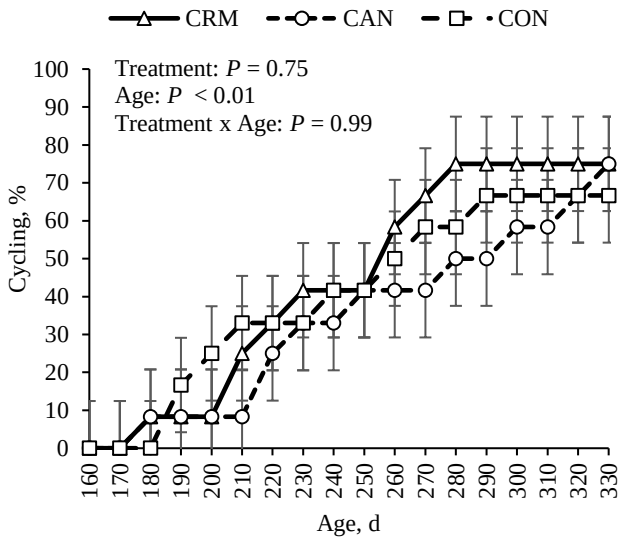
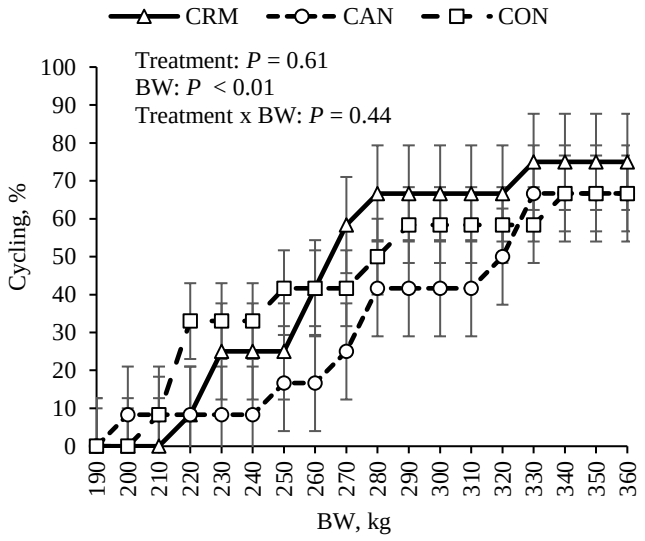


Figure 6.8. Percentage of Holstein heifers pubertal (cycling) by body weight that were fed diets containing 10% solvent-extracted carinata meal (CRM), 10% solvent-extracted canola meal (CAN) or control (CON) (Error bars represent SEM)



CHAPTER 7. ENSILING CARINATA MEAL WITH FORAGES TO DECREASE GLUCOSINOLATES: EVALUATION OF THE EFFECTS ON FERMENTATION CHARACTERISTICS, GLUCOSINOLATES CONTENT, AND IN SITU DEGRADABILITY AND IN VITRO DIGESTIBILITY OF THE PROTEIN

ABSTRACT

Carinata meal (**CRM**) has high quality protein, but it also has high concentration of sinigrin, a glucosinolate, which limits its use as a feedstuff. Additionally, since solvent extraction (**SLV**) or cold pressing (**CPR**) are methods used to extract oil from carinata seeds; different residual oil content in the meals may affect the fermentation when it is blended with forage for ensiling. Our first objective was to determine if ensiling CRM with alfalfa haylage (**AH**) and with corn silage (**CS**) would decrease sinigrin concentration without compromising fermentation. For both types of forages a micro-silo experiment was conducted with three blends of CRM to forage were made 0:100, 25:75, and 50:50 on a DM basis. Nutritive value of the resulting ensilages from d 60 were evaluated by in situ rumen degradation and in vitro digestibility. Our second objective was to determine the effect of ensiling CS with CRM cold-pressed or solvent-extracted on the fermentation characteristics of the silage and effect on glucosinolate content. A micro-silo experiment was conducted with three treatments: 1) corn forage (CS); 2) CS and solvent-extracted CRM blend (**CS:SLV**); and 3) CS and cold-pressed CRM blend (**CS:CPR**). Both blends of CRM to forage were 25:75 on a DM basis. Data were analyzed using MIXED procedures of SAS 9.4. The model included treatment (Trt), day

(d), and treatment by day (Trt × d) interaction with significance declared at $P < 0.05$. Experiment one results showed sinigrin was greatest ($P < 0.01$) in the 50:50 and decreased over time ($P < 0.01$) in the 25:75 and 50:50 in both trials. There was no treatment by d interaction for AH blends, but there was a treatment by d interaction for the CS blends. The pH decreased in all blends over time but was greater in the 50:50 compared to the other blends. Acetic acid and Lactic acid increased over time in all blends. Acetic acid was less in the AH blends with increased CRM. There was no treatment effect on acetic acid for the CS blends. Lactic acid was less in both trials with increased inclusion of CRM. In both trials, CP increased with the inclusion of CRM. The CP was similar over d of ensiling in AH blends but tended ($P = 0.05$) to decrease over d in CS blends. In both trials, NDF was less with the addition of the CRM, and there was a treatment by d interaction ($P < 0.01$) in CS trial, and a tendency ($P = 0.08$) for interaction in the AH trial. Ensiling CRM with forage decreases sinigrin concentration, without major detriment to silage fermentation. From the in situ degradability study, it was found that inclusion of CRM to the blends of CS increased the rumen solubility of the DM, where in the blends with AH increased the amount of potentially degradable DM. In experiment two is was found that sinigrin content of CRM before blending was 15.3 vs. 16.2 mg/g, for CPR and SLV meals, respectively. On d 0, within hours after mixing, the sinigrin content was reduced 94.8% in the CS:CPR, but not in the CS:SLV blend. Compared with the original meal, by d 60 sinigrin content decreased 99.7% in CS:CPR, and 99.4% in CS:SLV. Sinigrin was greater ($P < 0.01$) in CS:SLV compared to CS:CPR over time. Fat content as determined by ether extract (**EE**) was greater ($P < 0.01$) in the

CS:CPR than in CS:SLV and CS. The pH decreased in all treatments over time but was greater in the blends. Acetic and lactic acids increased over time in all treatments. Acetic acid was less in the CS, compared to the blends. Acetic acid was greater ($P < 0.01$) in CS:CPR than CS:SLV, whereas lactic acid was less in CS:CPR. The CP was greater in both blends with CRM. Despite different fat contents, ensiling cold-pressed or solvent extracted CRM with corn forage decreased sinigrin concentration, without major detriment to silage fermentation.

Keywords: carinata meal, glucosinolates, corn silage, haylage

Introduction

Carinata (*Brassica carinata*) is a new oilseed that shows great potential for the Midwest region as has been developed to have high amounts of oils suitable for biofuels production. Its agronomic characteristics also allow it to establish in areas where other crops cannot adapt (Marillia et al., 2014). Additionally, after the oil extraction carinata meal (**CRM**) has high protein (40-48% of crude protein) which is highly degradable in the rumen and is a comparable protein source to soybean meal and linseed meal for total digestibility (Lawrence and Anderson, 2018). One of the weaknesses of carinata meal, as other oilseed derived meals from brassicas plants such as canola, camelina, and rapeseed, is that it contains glucosinolates, mainly sinigrin. Glucosinolates are compounds that by themselves are non-harmful. When degraded during chewing and digestion, however, they form substances that cause a bitter taste which affects the preference for the meal, therefore, potentially decreasing the intake of oilseed meals. In some instances, and when

fed at high amounts, they may result in health problems such as hypothyroidism, potentially affecting animal growth, which limits its potential use for livestock (Tripathi and Mishra, 2007). Additionally, FDA regulations limit the inclusion of carinata meal to 10% of the diet dry matter of the diet for cattle (AAFCO, 2018). Therefore, finding options to decrease the glucosinolates content would allow more flexibility in the use of CRM for cattle. Acids, heat and certain enzymes can break down glucosinolates (Duncan and Milne, 1993; Tsao et al., 2000, Bones and Rositer, 2006). In 1987, Fales et al., ensiled in bags rapeseed forage (*Brassica napus*). In this experiment, the ensiled bags were maintained at 22°C for 30 days and frozen until samples were analyzed. Fermentation quality, pH, and dry matter content loss were evaluated. Even though the DM loss of resulting ensilages was near to 20%, the pH was 4, and obtained a decrease of 90% of glucosinolates content after the ensiling process. This research indicates that ensiling carinata meal with forage may decrease glucosinolates content.

Additionally, oil extraction of oilseeds used as feedstocks in the biofuels industry can be done by using solvents or by mechanical processes such as the cold-pressing which is a lower cost method that may be feasible for on-farm extraction. However, the oil yield of cold press is in a range of 70-89% of solvent extraction, depending on oilseed species and operating conditions. Therefore, the amount of fat retained in the resulting oilseed meal could affect the nutritive value of the meal such as CP digestibility (Sackey, 2015). Furthermore, the amount of fat in the CRM cold-press extracted could affect the fermentation when the meal is blended with forage for ensiling because during early

fermentation fatty acids are oxidized (Han and Zhou, 2013) and potentially decrease the hydrolysis of glucosinolates.

We hypothesized: 1) that ensiling CRM with forages could decrease the glucosinolate content and increase the nutritive quality of the silage without affect fermentation; 2) as during ensiling fermentation process fatty acids are oxidized, the quality of fermentation and the amount of glucosinolates may decrease in corn forage ensiled with CRM from cold-press extraction compared with solvent-extracted CRM, and if affected, the amount of glucosinolates degraded will be reduced.

To test our hypothesis, we conducted two experiments. In the first, as CRM has high crude protein, we wanted to evaluate if ensiling it with corn forage would increase the nutritive value of the corn silage, and since producers can only ensile corn forage one time per year, we also wanted to evaluate ensiling CRM with alfalfa forage which is harvested several times per year as another option, and determine if the ensiling process reduced the glucosinolates content of carinata meal without affecting the fermentation profile of corn or alfalfa forage. And finally, determine in situ the nutritional quality of carinata meal ensiled with corn or alfalfa forage. For the second experiment, to determine the effect of fat content of CRM on fermentation quality and ability to decrease glucosinolates content, we used a blend 25:75 on dry matter basis of CRM ensiled with corn forage.

Materials and Methods

Micro-silo experiment 1

To test our hypothesis two trials were conducted with micro-silos in summer 2014, one on ensiling carinata meal with alfalfa haylage (AH), and one with corn silage (CS). For both trials, three blends of CRM to forage (0:100, 25:27 and 50:50) were made on a DM basis. After preparing the blends quadruplicate samples of each blend were taken as fermentation period 0 and stored at -20°C until analysis could be completed. Ensiled feedstuffs were placed in micro-silos of polyvinyl chloride pipes (10 cm of diameter and 30 cm in height with an approximate volume of 2,394 cm^3) with rubber stoppers sealed with O-rings and a one-way valve. Approximately 637 g of DM were packed into each micro-silo with a bulk density of 256 kg/m^3. Four micro-silos were filled per blend (0:100, 25:75, and 50:50) and treatment (AH and CS) per ensiling period (7, 21 and 60 d). At the end of each ensiling period, micro-silos were opened, and all contents were stored at -20°C until processing and analyses could be completed as described under the laboratory analysis section.

Micro-silo experiment 2

To test our second hypothesis a micro-silo experiment was conducted one year later (summer 2015) with three treatments: 1) corn forage (**CS**); 2) CS and solvent-extracted CRM blend (**CS:SLV**); and 3) CS and cold-pressed CRM blend (**CS:CPR**). Both blends of CRM to forage were 25:75 on a DM basis. The same micro-silos from

experiment 1 were used after being thoroughly washed. Approximately 637 g of DM were packed into each micro-silo with a bulk density of 256 kg/m^3 in triplicate for 0, 7, 21 and 60 days of ensiling. At the end of each ensiling period, micro-silos were opened, and all content was stored at -20°C until processing and analyses could be completed.

Laboratory analysis

A frozen sub-sample of feed from each micro-silo was sent to Dairyland Laboratory (Arcadia, WI) for analysis of fermentation profile including pH (Orion Research, 1977), ethanol, VFA, and ammonia. The ethanol and VFA analysis was conducted using HPLC (Siegfried et al., 1984) in a modified method described by Muck and Dickerson (1988). Ammonia-N was analyzed using method 920.03, Nitrogen (Ammoniacal) in Fertilizers: Magnesium Oxide Method of the AOAC (2002).

Another sub-sample of feed from each micro-silo was processed for nutrient analysis. Dry matter content of samples and CRM was determined by drying a 250 g sample at 55°C per 48 h in duplicate in a Dispatch oven (Style V-23, Dispatch Oven Co., Minneapolis, MN). Dried samples were ground sequentially to pass through a 2-mm screen with a Wiley mill (model 3; Arthur Thomas Co., Philadelphia, PA), and in a 1-mm screen in an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). To correct analysis to 100% DM, 1-g aliquots were dried for 3 h in a 105°C oven. Ash content was analyzed by incinerating a 1-g sample for 8 h at 450°C in a muffle furnace (AOAC International, 2002; method 942.05). Organic matter was calculated as OM = (100 - %Ash). Samples were analyzed for nitrogen content via Dumas combustion analysis

(AOAC International, 2002; method 968.06), on a Rapid N cube (Elementar Analysensysteme, GmbH, Hanau Germany). Nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (Van Soest et al., 1991) and acid detergent fiber (Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Macedon, NY). For the NDF, heat-stable α-amylase and sodium sulfite were used. Ether extracts were analyzed using petroleum ether (AOAC International, 2002; method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Macedon, NY). Nonfibrous carbohydrates were calculated as %NFC= 100 – (%Ash + %CP + %NDF + %EE) according to the NRC (2001).

Ruminal degradation and intestinal degradability (Experiment 1).

All procedures involving the use of animals were approved by the South Dakota State University Institutional Animal Care and Use Committee. The farm portion of the ruminal degradation was completed at the South Dakota State University Dairy Research and Training Facility.

In situ rumen degradation measurements were done on day 60 samples from experiment 1 using 3 multiparous, late lactation, ruminally cannulated Holstein cows (second lactation; 328 ± 17 DIM; 26.9 ± 7.3 kg/d of milk yield; BW 694.9 ± 61.8 kg; 2.9 ± 0.2 of body condition score; DMI 23.3±2.2 kg/d). Cows were fed a TMR that was being fed to the general herd at the time of the study (**Table 7.1**). Over the three days the in situ trial lasted, cows were fed for ad libitum intake and intakes were recorded.

Samples of the TMR fed to the lactating cows were dried and ground and sent to a commercial laboratory (Dairyland Laboratories Inc., Arcadia, WI) for analysis.

Blends of AH and CS of the d 60, carinata meal and soybean meal were evaluated. To prepare the samples for incubation in the rumen, the frozen sub-samples from each micro silo were thawed and composited by equal proportions. Composites were made on an as-fed basis and chopped using a commercial food processor (Oster, Sunbeam Products Inc., FL, USA) for 60 s, achieving a particle size of 1–4 mm in the 84.5 ± 4.2 percent of the sample. Particle size was checked by shaking through the bottom two pans of the Penn State Particle Separator (Kononoff et al., 2005). Samples of chopped silage or meals were weighed in triplicate on an as-fed basis to provide 5 grams of DM into 10 × 20 CRM nylon bags (50 µm porosity; Ankom Technology Corp., Macedon, NY) and ruminally incubated for 0, 2, 4, 8, 12, 24 and 48 h. Six extra bags of each sample were incubated for 12 h to have enough residue for in vitro intestinal digestibility analysis. Before incubation, the bags with sample were soaked in 39°C water for 20 min, placed into a larger nylon mesh bag (91 x 60 CRM), and incubated in the rumen for their respective times in decreasing order of incubation time. All bags were then removed from the rumen at the same time. After ruminal incubation, mesh bags were placed into 20-L buckets, gently agitated, and rinsed with cold tap water. The individual nylon bags were further rinsed with cold tap water until the water ran clear. Bags were allowed to drain and then stored at -20°C until further processing. The 0 h bags underwent the same soaking and washing procedure as described to estimate the amount of water-soluble CP. Three blank bags for each time exposure were incubated

with the samples to correct for microbial attachment (Poos-Floyd et al., 1985). Additionally, after thawing, to remove attached bacteria, the bags were suspended in a 0.1% methylcellulose solution (Spectrum Chemical MFG Corp., Gardena, CA), and incubated in a shaking bath at 37°C for 30 min (Gargallo et al., 2006). After the methylcellulose wash, the bags were washed gently, allowed to drain and oven-dried at 55°C for 48 h. Residues were composited by cow, treatment and time and then analyzed for CP as previously described.

As mentioned, six additional bags were incubated at 12 h for use in the determination of in vitro intestinal CP digestibility (Gargallo et al., 2006). Residues of the samples after ruminal incubation from the six bags were composited and then were reweighed into nylon bags (5 x 10 CRM with 50-micron porosity; Ankom Technology Corp., Macedon, NY) with 2.5 g of each sample. Bags were incubated in a Daisy II Incubator (Ankom Technology Corp., Macedon, NY) with pepsin and pancreatin solutions for 1 h and 24 h, respectively. Following pancreatin incubation, the bags were washed gently until the water ran clear and oven-dried at 55°C for 48 h. Residues were composited by cow and treatment and then analyzed for CP as previously described.

Glucosinolates quantitation

Dried ground silage samples were extracted with methanol and analyzed by LC-MS to confirm the glucosinolate composition and by RP-HPLC at 237 nm to determine concentrations.

Sample extraction. For HPLC analysis, between 0.25 g to 0.5g of ground hexane-defatted samples were placed in a capped vial with between 2-5 mL of methanol. The vials were sonicated for 15 minutes in a sonicate water bath then allowed to stand overnight. After another brief sonication, a portion of this extract was filtered through a 0.45-micron filter into an autosampler vial.

HPLC Analysis and Quantitation. For glucosinolate quantitation, a modification of the HPLC method developed by Betz and Fox (1994) was used. The extract was run on a Shimadzu (Columbia, MD) HPLC System (two LC 20AD pumps; SIL 20A autoinjector; DGU 20As degasser; SPD-20A UV-VIS detector; and a CBM-20A communication BUS module) running under the Shimadzu LCsolutions Version 1.25 software. The column a C18 Inertsil reverse phase column (250 mm X 4.6 mm; RP C-18, ODS-3, 5µ; with a Metaguard guard column; Varian, Torrance, CA). The glucosinolates were detected by monitoring at 237 nm. The initial mobile phase conditions were 12% methanol/88% aqueous 0.005M tetrabutylammonium bisulfate (TBS) at a flow rate of 1 mL/min. After injection of 15 µl of sample, the initial conditions were held for 2 min, and then up to 35% methanol for another 20 minutes, then to 50% methanol over another 20 minutes then up to 100% methanol over another 10 minutes.

Glucosinolate Standards Analysis. Freshly prepared sinigrin standard (Sigma, St. Louis, MO) was prepared on a molar concentration basis. A series of dilutions were prepared to make a standard curve and to determine lower detection limit. Concentrations in these standards were determined by sinigrin calibration curve on an nM/mAbs basis.

LC-ESI-MS Analysis of glucosinolates. To confirm the identity of the glucosinolates found in the seed extracts (as reported in Shuster and Friedt, 1998), aliquots were injected on a LTQ-XL Orbitrap MS. Samples were run on an Thermo Scientific Accela UHPLC system (auto-injector, PDA detector and a 1250 quaternary pump) and mass spectra were obtained on LTQ Orbitrap Discovery Mass Spectrometer (a linear ion trap (LTQ XL) MS, coupled to a high precision electrostatic ion trap (Orbitrap) MS with an Ion Max electrospray ionization (ESI) source), all running under Thermo Scientific Xcalibur 2.1.0.1140 LC-MS software. The MS was calibrated at least weekly with a standard calibration mixture recommended by Thermo Scientific, and the signal detection was optimized by running the autotune software feature as needed. The MS was run with the ESI probe in the negative mode. The source inlet temperature was 350 ˚C, the sheath gas rate was set at 10 arbitrary units, the auxiliary gas rate was set at 2 arbitrary units and the sweep gas rate was set at 2 arbitrary units. The maximal mass resolution was set at 30,000; the spray voltage was set at 3.0 kV, the tube lens was set at -100 V. Other parameters were determined and set by the calibration and tuning process.

The column used was an Inertsil ODS-3 reverse phase C-18 column (3 µ, 150 x 3 mm, with a Metaguard column, from Varian). The initial HPLC conditions were 15% methanol and 85% 0.25% formic acid in water, at a flow rate of 250 ul per minute, then the column was developed to 100% methanol over 50 minutes. The effluent was also monitored at 237 nm on the PDA. Sinigrin peaks were detected which had mass ions of 358.

Samples were run on a Thermo Electron LTQ Orbitrap Discovery Mass Spectrometer (a linear ion trap (LTQ XL) MS, coupled to a high precision electrostatic ion trap (Orbitrap) MS) with an Ion Max electrospray ionization (ESI) source, coupled to a Thermo Scientific ACCELA series HPLC system (ACCELA 1250 UHPLC pump, ACCELA1 HTC cool stack autoinjector, and a ACCELA 80 Hz PDA detector) all running under Thermo Scientific Xcalibur 2.1.0.1140 LC-MS software. The MS was calibrated at least weekly with a standard calibration mixture recommended by Thermo Scientific, and the signal detection was optimized by running the autotune software feature as needed. The MS was run with the ESI probe in the negative mode. The source inlet temperature was 300˚C, the sheath gas rate is set at 50 arbitrary units, the auxiliary gas rate was set at 3 arbitrary units and the sweep gas rate was set at 2 arbitrary units. The maximal mass resolution was set at 30,000; the spray voltage was set at 3.0 kV, the tube lens was set at -100 V. Other parameters were determined and set by the calibration and tuning process. The column was a 3 mm x 150 mm Inertsil reverse phase C-18, ODS 3, 3-µ column (Metachem, Torrance, CA). The initial solvent system was either 15% (or 40%) methanol and 80% (or 60%) water with 0.25% formic acid at a flow rate of 0.25 mL per minute. After injection (1 µl) the column was held at the initial conditions for 5 minutes then developed with a linear gradient to 100% methanol over an additional 60 min. The column effluent was monitored at 237 nm in the PDA detector.

Six mass spec “events” were programmed to run in sequence in the MS detection scheme: 1) LTQ(IT)-MS full scan m/z 150 to 2000; 2) LTQ(IT)-MS set to trap the most abundant ion above a threshold of 500 units and perform CID at 35% energy, with the

resulting ions being detected by the IT-MS; 3) FT-MS (Orbitrap) full scan m/z 150 to 2000; 4) Mass-dependent MS/MS on the most abundant ion trapped by the IT-MS in Event 1 and perform HCD at 25% energy with the resulting fragmentation ions being detected by the FT-MS; 5) Mass-dependent MS3 on the most abundant fragment ion generated from Event 2 and perform HCD at 25% energy with the resulting fragmentation ions being detected by FT-MS; and, 6) Mass-dependent MS3 on the most abundant fragmentation ion generated from Event 2 and perform CID at 35% energy with the resulting ions being detected by IT-MS.

If the major ion detected by the MS was a glucosinolate, MS event 4 would generate a 96.9 ion corresponding to a free SO4 ion. MS event 3 provided the accurate mass of the (M-H)- ion.

Statistical Analysis

Data were analyzed using MIXED procedures (Littell et al., 2006) of SAS 9.4 (SAS Institute Inc., Cary, NC). The model included treatment (Trt), day (d), and treatment by day (Trt × d) interaction. Treatments were compared as LS means and analyzed with Tukey's test with significance declared at $P < 0.05$.

Rumen degradation constants for DM and CP were estimated using the NLIN procedures in SAS 9.4 (SAS Institute Inc., Cary, NC) as described by Ørskov and McDonald (1979) and McDonald (1981), and the fractional passage rate was calculated as described in the NRC (2001). Intestinally digestible protein (IDP), intestinally absorbable dietary protein (IADP = ruminally undegradable protein (RUP) × IDP), and

total digestible protein (TDP = ruminally degradable protein (RDP) + IADP) were evaluated using MIXED procedures in SAS 9.4 (SAS Institute Inc., Cary, NC).

Results and Discussion

Experiment 1

Sinigrin was greatest ($P < 0.01$) in the 50:50 blends and decreased over time ($P < 0.01$) in the 25:75 and 50:50 blends in both trials (**Figures 7.1** and **7.2**). Sinigrin concentration percentage of decrease was more in 25:75 (76 %) than in 50:50 (34 %) in alfalfa haylage blends. For the corn silage blends, the percentage of decrease was more in 50:50 (57 %) than in 25:75 (44 %).

Nutrient composition over the days of ensiling for the blends of CMR:AH are presented in **Table 7.2**, and for CMR:CS in **Table 7.3**. Regarding the nutritive quality of both blends of AH, as the rate of inclusion of CRM increased ($P < 0.05$) DM, OM and CP content increased compared to the 0:100 blend. Ash, NDF, ADF, and ether extract contents decreased ($P<0.05$) with the increased inclusion rate of CRM. In the blends of CS, as the level of inclusion of CRM increased ($P < 0.05$) DM, Ashes, and CP. Only ether extract increased ($P < 0.05$) in the blend 25:75. The OM, NDF and ADF content decreased ($P < 0.05$) with the increased inclusion rate of CRM (Table 7.2). The CP was significantly different in all blends of both types of forages, and increased with the addition of CRM. The percentage of CP increase on CS blends was considerably higher (150% for 25:75 and 300% for 50:50) compared with AH (20% for 25:75 and 32% for 50:50).

There was no loss of DM over days of ensiling for the blends of AH ($P = 0.32$), but the CS blends lost between 4 and 6% of DM ($P < 0.01$). In both trials, CP increased with inclusion of CRM. The CP was similar over days of ensiling in AH blends but tended ($P = 0.05$) to decrease over time in CS blends. In both trials, NDF was less with the addition of the CRM and there was a treatment by day interaction ($P < 0.01$) in CS trial, and a tendency ($P = 0.08$) for interaction in the AH trial. There was an increase in EE content for all AH blends, being greater in the 0:100 ($P < 0.01$). For the CS blends the increase of EE content was over time, but no effect of interaction was observed. Ash content increased over time on all AH blends ($P < 0.01$), and inversely OM content decreased ($P < 0.01$). In CS blends ash and OM content did not change over the time.

Overall, the changes on nutrient composition in AH blends were normal as a part of the fermentation process and the inclusion of CRM. Although, there were no differences over ensiling time in DM, during fermentation there are unavoidable losses of energy, such as OM and consequently carbohydrates being lost during fermentation and oxidation processes; therefore ash and EE proportions may increase (McDonald et al., 1991). For CS blends, the loss of DM over time was within the normal range, therefore, the changes in nutrient composition over the time are also associated to unavoidable losses (McDonald et al., 1991) more than undesirable fermentation pathways as the fermentation profiles on both AH and CS blends were within normal parameters (**Tables 7.4** and **7.5**). The observed differences in NDF, ADF, and organic acids over the time are related via changes in carbohydrate components during fermentation (Anderson et al., 2009).

For the blend 50:50 of both forages, the pH was greater compared to the other blends. Acetic acid was less in the AH blends as CRM increased. Lactic acid was less in both trials with increased inclusion of carinata meal. The pH in the 50:50 blends and the decrease in lactic acid probably occurred because adding protein with CRM increased the buffering capacity (McDonald et al., 1991; Kung et al., 2018). In both trials, the addition of CRM decreased the NDF content of the blend. As expected, in both trials CP increased with CRM inclusion. The CP was similar over days of ensiling in AH blends, but tended to decrease over days of ensiling in CS blends. Ammonia N increased over the time in both trials, without exceeding normal fermentation parameters; however, it was greater in the AH blends than in the CS blends. Iso-butyric, butyric, propanol, and butanol were tested but none were detected at any time point for the blends of AH. For CS blends the propionic, iso-butyric, butyric, propanol, and butanol, methanol, and propanediol were tested but none were detected at any time point.

Ruminal degradation and intestinal degradability

The pH of ruminal fluid averaged 6.0 ± 0.07 during the rumen incubation of samples. This pH was in the normal range for lactating dairy cows, indicating fermentation was also normal. Dry matter (DM) degradation of the blends and original feeds is on **Table 7.6**. Ruminally degradable DM (RDDM) and rate of DM (K_d) degradation in the rumen were greatest ($P < 0.05$) for the all the blends of CRM:CS, and did not differ from the 50:50 CRM:AH blend. This last blend, however, was similar to the 25:75 and 0:100 CRM:AH blends. The original CRM and soybean meal did not differ

from any of the CRM:AH blends, but CRM was similar and soybean meal was different when compared to the CRM:CS blends. The blends of 25:75 and 0:100 of CRM:AH had the slowest rate of rumen DM degradation. This may be related to the different content of starch in the forages used since corn forage has more starch than alfalfa. Starch combined with the protein could help increase the rumen degradation compared with AH blends. This is supported by the rate of disappearance of the soluble DM (fraction A) observed in all the CS blends which was greater than in AH blends. The AH blends had greater rate of disappearance of potentially degradable DM (fraction B) than the CS blends. Inclusion of CRM to the blends of CS did not increase the solubility of the DM, but as it has a good proportion of potentially degradable DM, its inclusion in the blends of CS and AH increased the amount of potentially degradable DM, and consequently the rumen degradable DM, increasing the nutritional value of the corn silage. The rate of degradation observed for CRM alone was faster than the observed by Lawrence and Anderson (2018). This difference could be due to utilization of different cows at different stages of lactation, with different DMI and passage rates on that study compared to the current study.

Table 7.7 presents the CP degradation results. The blends of CRM with CS or AH did not increase the rumen degradable protein. However, compared with CS and AH the content of soluble protein in CRM is low; therefore its inclusion to the blends decreased the disappearance of the A fraction and increased the B fraction. This is beneficial as the protein availability matches DM degradation, synchronizing protein and energy which may increase microbial crude protein synthesis (Herrera-Saldana et al., 1990).

Experiment 2

Sinigrin content of CRM before blending was 15.3 vs. 16.2 mg/g, for CPR and SLV meals, respectively. On d 0, within hours after mixing, the sinigrin content was reduced 94.8% in the CS:CPR, but not in the CS:SLV blend (**Figure 7.3**). Compared with the original meal, by d 60 sinigrin content decreased 99.7% in CS:CPR, and 99.4% in CS:SLV. Sinigrin was greater ($P < 0.01$) in CS:SLV compared to CS:CPR over time. During solvent- extraction the meal is heated, and two things may happen: 1) depending on the time and heat extent, Maillard reactions may occur negatively affecting the rate of hydrolysis of sinigrin making it less available for the action of the enzyme myrosinase; or, 2) the heat inactivates the myrosinase enzyme, then hydrolysis of sinigrin occurs slowly depending on the changes in water, pH, and heat (Peng et al., 2014; Martinez-Ballesta and Carbajal, 2015).

Nutrient composition over the days of ensiling for the CS and the blends of the two meals are in **Table 7.8**. Overall, DM and CP increased with the inclusion of CPR and SLV. The fat content, as determined by ether extract (EE), was greater ($P < 0.01$) in the CS:CPR than in CS:SLV and CS. The pH decreased in all treatments over time but was greater in the blends. Acetic and lactic acids increased over time in all treatments. Acetic acid was less in the CS, compared to the blends. Acetic acid was greater ($P < 0.01$) in CS:CPR than CS:SLV. Lactic acid was less in CS:CPR. The CP was greater in both blends with CRM but not different between them. Overall, fermentation profiles over time of CS and the CPR and SLV blends were within normal ranges. Compared with the

results of the experiment 1, CS had more DM and CP content and there were no DM losses over the time for any of the blends. The pH for CS and the blends with CPR and SLV decreased more compared with all the blends of CRM:CS in experiment 1, produced more lactic acid, less acetic acid, total acids (14 to 20% more), less ammonia N but when expressed as percentage of the CP were similar. In general, the fat added by CPR versus SLV appeared to have minimal impacts on silage fermentation.

Conclusions

In agreement with our hypothesis, ensiling carinata meal with forages decreased sinigrin concentrations, without major detriment to silage fermentation. This presents a potential opportunity for an on-farm treatment method of brassica meals to make dietary inclusion safer and at a potentially greater rate than 10%, as currently regulated. Additionally, the quality of silages was increased, with the most promising blend being 25:75 with CS as it increased the nutritive value of corn silage and increased RDDM by improving the availability of protein for rumen fermentation. Also, CRM with CS may provide a complementary combination of protein and energy sources. Despite different fat contents, ensiling cold-pressed or solvent extracted CRM with corn forage decreased sinigrin concentration, without major detriment to silage fermentation. Animal feeding trials with the ensiled blends are now needed to determine impacts of compounds left from glucosinolate breakdown on cattle intakes and performance.

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Table 7.1. Ingredients and chemical composition of the total mixed diet fed to cows during the in situ experiment to evaluate treatment blends (0:100, 25:75, and 50:50) of carinata meal with corn silage (CRM:CS) or with alfalfa haylage (CRM:AH)

Ingredient, % of DM	Diet
Corn silage	35.82
Alfalfa hay	14.07
Alfalfa haylage	3.99
Corn, high moisture	13.20
Soybean meal	7.34
Whole cottonseed	5.25
Liquid sugar supplement[1]	3.79
Grain Mix[2]	16.54
Chemical composition[3]	
DM, %	57.20
OM, % of DM	84.93
Ash, % of DM	15.07
CP, % of DM	18.06
NDF, % of DM	27.61
ADF, % of DM	18.48
EE[4], % of DM	5.33
NFC[4], % of DM	43.26
NE_L[6], Mcal/kg	1.63

[1]Dairy sugar (Quality Liquid Feeds, Dodgeville, WI).
[2]SDSU Milk Mix: Contains Ground Corn, DDGS, SoyBest, Limestone, Energy Booster, Sodium Bicarbonate, Salt, Yeast culture, Magnesium oxide, urea, vitamin premix, omnigen, phosphate, Vitamin E 2000 IU/lb, DTX binder, Biotin, and Rumensin.
[3]Laboratory analyses were performed at Dairyland Laboratories Inc. (Arcadia, WI).
[4]EE = ether extract, petroleum ether.
[5]% NFC (nonfibrous carbohydrates) = 100 − (% ash + % CP + % NDF + % ether extract) (NRC, 2001).
[6]Values are calculated based on inputting sample nutrient analysis into diet formulations in the Dairy NRC computer program (2001).

Table 7.2. Nutrient compositions over days of ensiling of treatment blends (0:100, 25:75, and 50:50) of carinata meal with alfalfa haylage (CRM:AH)

Item	Day of ensiling	CRM:AH Blend			Day mean	SEM	*P*-values[1]				
		0:100	25:75	50:50			Trt	d	Trt × d	Linear	Quadratic
DM, %	0	40.45	46.97	56.52	48.07	0.366	<0.01	0.32	0.05	<0.01	<0.01
	7	40.52	46.69	57.15	48.12	0.366					
	21	40.84	47.00	56.45	48.09	0.366					
	60	39.51	46.77	56.60	47.63	0.366					
	T mean	40.45^{c}	46.97^{b}	56.52^{a}		0.259					
Ash[2]	0	10.56	9.88	9.67	10.04^{z}	0.052	<0.01	<0.01	0.10	<0.01	<0.01
	7	10.69	10.11	9.71	10.17^{y}	0.052					
	21	10.80	10.17	9.68	10.22^{y}	0.052					
	60	10.95	10.18	9.88	10.34^{x}	0.052					
	T mean	10.75^{a}	10.08^{b}	9.74^{c}		0.026					
NDF[2]	0	40.48	35.23	31.85	35.85^{x}	0.493	<0.01	<0.01	0.08	<0.01	0.07
	7	37.68	34.45	31.52	34.55^{y}	0.493					
	21	37.19	34	31.26	34.14^{y}	0.493					
	60	38.43	34.51	32.54	35.16^{xy}	0.493					
	T mean	38.45^{a}	34.55^{b}	31.79^{c}		0.247					
ADF[2]	0	28.75	24.41	21.38	24.84^{xy}	0.446	<0.01	0.02	0.33	<0.01	0.30
	7	28.11	24.58	20.72	24.47^{xy}	0.446					
	21	27.72	24.28	20.82	24.27^{y}	0.446					
	60	28.41	24.94	22.79	25.38^{x}	0.446					
	T mean	28.25^{a}	24.55^{b}	21.43^{c}		0.223					
CP[2]	0	23.2	31.02	29.6	27.94	1.89	<0.01	0.2	0.09	<0.01	0.37
	7	23.14	28.45	33.76	28.44	1.89					
	21	26.11	25.15	33.61	28.29	1.89					
	60	25.83	33.81	33.32	30.98	1.89					
	T mean	24.57^{b}	29.61^{a}	32.57^{a}		0.945					
Ether extract[2]	0	1.95^{ij}	2.22^{hi}	1.94^{ij}	2.04^{y}	0.084	<0.01	<0.01	<0.01	<0.01	<0.01
	7	2.22^{hi}	2.15^{hi}	1.89^{i}	2.09^{y}	0.084					
	21	2.48^{h}	2.35^{hj}	2.13^{hi}	2.32^{x}	0.084					
	60	3.00^{g}	2.53^{h}	1.84^{i}	2.46^{x}	0.084					
	T mean	2.41^{a}	2.31^{a}	1.95^{b}		0.042					
Organic matter	0	89.43	90.11	90.33	89.96^{x}	0.052	<0.01	<0.01	0.10	<0.01	<0.01
	7	89.31	89.89	90.29	89.83^{y}	0.052					
	21	89.20	89.83	90.32	89.78^{y}	0.052					

Item	Day of ensiling	CRM:AH Blend 0:100	25:75	50:50	Day mean	SEM	*P*-values[1] Trt	d	Trt × d	Linear	Quadratic
	60	89.04	89.82	90.11	89.66[z]	0.052					
	T mean	89.25[c]	89.91[b]	90.26[a]		0.026					

[a-b]Means in the same row (T mean) with unlike letters differ ($P < 0.05$).
[g-m]For variables with significant treatment × day interactions, means across all treatments and time points with unlike letters differ ($P < 0.05$).
[x-z]Means in the same column (Day mean) with unlike letters differ ($P < 0.05$).
[1]Trt = treatment; d = day.
[2]% of DM.

Table 7.3. Nutrient compositions over days of ensiling of treatment blends (0:100, 25:75, and 50:50) of carinata meal with corn silage (CRM:CS)

Item	Day of ensiling	CRM:CS Blend			Day mean	SEM	*P*-values[1]				
		0:100	25:75	50:50			Trt	d	Trt × d	Linear	Quadratic
DM, %	0	32.36	38.92	48.50	39.93^{x}	0.576	< 0.01	< 0.01	0.48	<0.01	<0.01
	7	31.35	37.97	48.20	39.17^{xy}	0.576					
	21	31.63	37.57	48.26	39.15^{xy}	0.576					
	60	30.92	37.43	45.81	38.06^{y}	0.576					
	T mean	31.56^{c}	37.97^{b}	47.69^{a}		0.288					
Ash[2]	0	4.12	5.00	6.09	5.07	0.160	<0.01	0.73	0.10	<0.01	0.89
	7	3.86	5.00	6.13	5.00	0.160					
	21	3.51	4.98	6.34	4.94	0.160					
	60	4.02	4.93	5.87	4.94	0.160					
	T mean	3.88^{c}	4.98^{b}	6.11^{a}		0.080					
NDF[2]	0	35.18^{g}	31.06^{hi}	28.02^{k}	31.41^{x}	0.440	<0.01	<0.05	<0.01	<0.01	<0.01
	7	35.13^{g}	29.6^{ij}	28.86^{jk}	31.19^{xy}	0.440					
	21	33.17^{gh}	30.78^{hij}	28.74^{jk}	30.9^{xy}	0.440					
	60	32.2^{h}	30.08^{hijk}	28.99^{jk}	30.42^{y}	0.440					
	T mean	33.92^{a}	30.38^{b}	28.65^{c}		0.218					
ADF[2]	0	18.5^{gh}	16.80^{ghk}	13.69^{m}	16.33	0.512	<0.01	0.28	<0.01	<0.01	<0.01
	7	19.1^{g}	16.07^{hijl}	15.61^{jlm}	16.92	0.512					
	21	18.2^{gi}	14.85^{jlm}	15.76^{iklm}	16.27	0.512					
	60	18.04^{gh}	16.29^{gl}	16.22^{gml}	16.85	0.512					
	T mean	18.46^{a}	16.00^{b}	15.32^{c}		0.256					
CP[2]	0	6.24	15.54	25.28	15.68	0.560	<0.01	0.05	0.19	<0.01	0.83
	7	6.22	16.26	26.21	16.23	0.560					
	21	6.14	15.26	25.34	15.58	0.560					
	60	6.33	15.16	23.25	14.91	0.560					
	T mean	6.24^{c}	15.55^{b}	25.02^{a}		0.278					
Ether extract[2]	0	1.68	1.96	1.78	1.81^{y}	0.101	<0.05	<0.05	0.33	0.13	0.01
	7	1.79	1.77	1.86	1.81^{y}	0.101					
	21	1.70	2.17	1.95	1.94^{xy}	0.101					
	60	1.97	2.12	2.00	2.03^{x}	0.101					
	T mean	1.78^{b}	2.00^{a}	1.90^{ab}		0.051					
Organic matter	0	95.88	95.00	93.91	94.93	0.160	<0.01	0.73	0.10	<0.01	0.89
	7	96.14	95.00	93.87	95.00	0.160					
	21	96.49	95.01	93.66	95.05	0.160					

Item	Day of ensiling	CRM:CS Blend 0:100	25:75	50:50	Day mean	SEM	*P*-values[1] Trt	d	Trt × d	Linear	Quadratic
	60	95.98	95.07	94.13	95.06	0.160					
	T mean	96.12[a]	95.02[b]	93.89[c]		0.080					

[a-b]Means in the same row (T mean) with unlike letters differ ($P < 0.05$).
[g-m]For variables with significant treatment × day interactions, means across all treatments and time points with unlike letters differ ($P < 0.05$).
[x-z]Means in the same column (Day mean) with unlike letters differ ($P < 0.05$).
[1]Trt = treatment; d = day.
[2]% of DM.

Table 7.4. Fermentation profile over days of ensiling of treatment blends (0:100, 25:75, and 50:50) of carinata meal with alfalfa haylage (CRM:AH)

Item	Day of ensiling	CRM:AH Blend 0:100	CRM:AH Blend 25:75	CRM:AH Blend 50:50	Day mean	SEM	*P*-values[1] Trt	d	Trt × d	Linear	Quadratic
pH	0	6.02^{g}	5.75^{h}	5.70^{h}	5.82^{w}	0.047	<0.01	<0.01	<0.01	<0.01	<0.01
	7	4.67^{jk}	4.70^{j}	5.02^{i}	4.80^{w}	0.047					
	21	4.47^{jm}	4.45^{klm}	4.67^{jk}	4.53^{wx}	0.047					
	60	4.37^{lm}	4.40^{lm}	4.55^{jl}	4.44^{x}	0.047					
	T mean	4.89^{b}	4.82^{b}	4.99^{a}		0.023					
Lactic acid	0	0.03^{m}	0.03^{m}	0.02^{m}	0.03^{z}	0.133	<0.01	<0.01	<0.01	<0.01	<0.05
	7	3.69^{i}	2.63^{k}	1.46^{l}	2.59^{y}	0.133					
	21	5.12^{h}	3.63^{ij}	2.60^{k}	3.79^{x}	0.133					
	60	5.97^{g}	3.99^{i}	2.99^{jk}	4.32^{w}	0.133					
	T mean	3.70^{a}	2.57^{b}	1.77^{c}		0.066					
Acetic acid	0	0.23^{jk}	0.12^{k}	0.07^{k}	0.14^{z}	0.088	<0.01	<0.01	<0.01	<0.01	0.74
	7	1.34^{hi}	1.20^{hi}	0.59^{j}	1.04^{y}	0.088					
	21	1.57^{h}	1.35^{hi}	1.06^{i}	1.33^{x}	0.088					
	60	2.12^{g}	1.52^{h}	1.25^{h}	1.63^{w}	0.088					
	T mean	1.31^{a}	1.05^{b}	0.74^{c}		0.044					
Propionic acid	0	0.06^{g}	ND^{h}	ND^{h}	0.02^{w}	0.005	<0.01	<0.01	<0.01	<0.01	0.08
	7	ND^{h}	ND^{h}	ND^{h}	ND^{x}	0.005					
	21	ND^{h}	ND^{h}	ND^{h}	ND^{x}	0.005					
	60	ND^{h}	ND^{h}	ND^{h}	ND^{x}	0.005					
	T mean	0.02^{a}	ND^{b}	ND^{b}		0.002					
Total acid	0	0.33^{m}	0.16^{m}	0.09^{m}	0.19^{z}	0.180	<0.01	<0.01	<0.01	<0.01	0.09
	7	5.03^{ij}	3.83^{k}	2.20^{l}	3.68^{y}	0.180					
	21	6.87^{h}	4.98^{ij}	3.67^{k}	5.17^{x}	0.180					
	60	8.10^{g}	5.51^{i}	4.24^{k}	5.95^{w}	0.180					
	T mean	5.08^{a}	3.62^{b}	2.55^{c}		0.090					
Lactic:Acetic ratio	0	0.15	0.68	0.15	0.32^{y}	0.158	<0.01	<0.01	0.05	<0.01	0.43
	7	2.75	2.19	1.98	2.31^{x}	0.158					
	21	2.93	2.68	2.45	2.69^{w}	0.158					
	60	2.86	2.62	2.43	2.64^{wx}	0.158					
	T mean	2.17^{a}	2.04^{a}	1.75^{b}		0.079					
Lactic, % of total acid	0	10.77	30.69	37.14	26.20^{x}	7.083	0.78	<0.01	0.28	0.54	0.74
	7	73.37	68.62	66.20	68.40^{w}	7.083					
	21	74.56	72.79	71.00	72.78^{w}	7.083					

Item	Day of ensiling	CRM:AH Blend 0:100	25:75	50:50	Day mean	SEM	*P*-values[1] Trt	d	Trt × d	Linear	Quadratic
	60	73.85	72.36	70.53	72.25^{w}	7.083					
	T mean	58.14	61.12	61.22		3.541					
Ethanol	0	0.07	0.07	0.03	0.06^{z}	0.043	0.05	<0.01	0.99	<0.05	0.39
	7	0.32	0.33	0.23	0.29^{y}	0.043					
	21	0.39	0.36	0.31	0.35^{w}	0.043					
	60	0.38	0.34	0.29	0.33^{x}	0.043					
	T mean	0.29	0.27	0.22		0.021					
Methanol	0	0.36^{gi}	0.04^{i}	ND^{i}	0.14^{x}	0.077	<0.05	<0.01	<0.01	<0.01	0.52
	7	ND^{i}	0.06^{i}	0.07^{i}	0.05^{x}	0.077					
	21	0.12^{i}	0.11^{i}	0.11^{i}	0.11^{x}	0.077					
	60	0.51^{gh}	0.62^{g}	0.20^{hi}	0.44^{w}	0.077					
	T mean	0.25^{a}	0.21^{ab}	0.10^{b}		0.038					
Propanediol	0	ND	0.04	ND	0.02	0.009	0.38	0.4	0.44	1.00	0.16
	7	ND	ND	ND	ND	—					
	21	ND	ND	ND	ND	—					
	60	ND	ND	ND	ND	—					
	T mean	ND	0.02	ND		0.004					
Ammonia NPC	0	0.56^{jl}	0.39^{lm}	0.28^{m}	0.41^{z}	0.040	<0.01	<0.01	<0.01	<0.01	0.10
	7	0.77^{i}	0.55^{jl}	0.35^{mn}	0.56^{y}	0.040					
	21	1.14^{h}	0.73^{ij}	0.52^{kln}	0.79^{x}	0.040					
	60	1.52^{g}	1.07^{h}	0.67^{jk}	1.09^{w}	0.040					
	T mean	1.00^{a}	0.68^{b}	0.45^{c}		0.020					
Ammonia, % of CP	0	2.41^{ik}	1.28^{kl}	0.94^{l}	1.54^{y}	0.274	<0.01	<0.01	<0.01	<0.01	0.05
	7	3.33^{hi}	1.97^{jkl}	1.05^{kl}	2.12^{y}	0.274					
	21	4.42^{h}	2.93^{ij}	1.55^{kl}	2.96^{x}	0.274					
	60	6.00^{g}	3.33^{hi}	2.03^{il}	3.79^{w}	0.274					
	T mean	4.04^{a}	2.38^{b}	1.39^{c}		0.137					

$^{a-b}$Means in the same row (T mean) with unlike letters differ ($P < 0.05$).

$^{g-m}$For variables with significant treatment × day interactions, means across all treatments and time points with unlike letters differ ($P < 0.05$).

$^{x-z}$Means in the same column (Day mean) with unlike letters differ ($P < 0.05$).

[1]Trt = treatment; d = day.

[2]% of DM.

Table 7.5. Fermentation profile over days of ensiling of treatment blends (0:100, 25:75, and 50:50) of carinata meal with corn silage (CRM:CS)

	Day of	CRM:CS Blend					*P*-values[1]				
Item	ensiling	0:100	25:75	50:50	Day mean	SEM	Trt	d	Trt × d	Linear	Quadratic
pH	0	4.75	5.07	5.32	5.05^{w}	0.070	<0.01	<0.01	0.9	<0.01	0.67
	7	3.82	4.15	4.37	4.12^{x}	0.070					
	21	3.65	3.85	4.15	3.88^{y}	0.070					
	60	3.60	3.85	4.02	3.82^{y}	0.070					
	T mean	3.96^{c}	4.23^{b}	4.47^{a}		0.035					
Lactic acid	0	0.08^{k}	0.01^{k}	0.01^{k}	0.04^{z}	0.099	<0.01	<0.01	<0.01	<0.01	0.38
	7	2.35^{j}	2.52^{j}	2.14^{j}	2.34^{y}	0.099					
	21	3.90^{h}	3.38^{i}	3.11^{i}	3.46^{x}	0.099					
	60	4.24^{g}	3.47^{hi}	3.35^{hi}	3.69^{w}	0.099					
	T mean	2.64^{a}	2.35^{b}	2.15^{c}		0.500					
Acetic acid	0	0.06	0.07	0.10	0.08^{z}	0.060	0.22	<0.01	0.61	0.22	0.21
	7	0.94	1.04	0.94	0.97^{y}	0.060					
	21	1.20	1.21	1.29	1.23^{x}	0.060					
	60	1.34	1.52	1.43	1.43^{w}	0.060					
	T mean	0.89	0.96	0.94		0.030					
Total acid	0	0.07^{k}	0.08^{k}	0.06^{k}	0.07^{z}	0.136	<0.01	<0.01	<0.05	<0.01	0.92
	7	3.32^{j}	3.56^{j}	3.08^{j}	3.32^{y}	0.136					
	21	5.11^{gh}	4.50^{hi}	4.40^{i}	4.67^{x}	0.136					
	60	5.58^{g}	5.10^{gh}	4.79^{hi}	5.16^{w}	0.136					
	T mean	3.52^{a}	3.31^{ab}	3.08^{b}		0.068					
Lactic:Acetic ratio	0	0.12^{i}	0.06^{i}	ND^{i}	0.06^{y}	0.114	<0.01	<0.01	<0.01	<0.01	0.21
	7	2.50^{h}	2.46^{h}	2.31^{h}	2.49^{x}	0.114					
	21	3.25^{g}	2.82^{gh}	2.41^{h}	2.82^{w}	0.114					
	60	3.15^{g}	2.37^{h}	2.37^{h}	2.63^{wx}	0.114					
	T mean	2.26^{a}	1.93^{b}	1.77^{b}		0.057					
Lactic, % of total acid	0	8.33	5.00	50.00	21.11^{x}	8.834	0.26	<0.01	0.05	0.26	0.23
	7	71.55	70.96	69.65	70.72^{w}	8.834					
	21	76.39	73.61	70.65	73.55^{w}	8.834					

Item	Day of ensiling	CRM:CS Blend 0:100	25:75	50:50	Day mean	SEM	*P*-values[1] Trt	d	Trt × d	Linear	Quadratic
	60	75.88	70.25	70.19	72.11^{w}	8.834					
	T mean	58.04	54.96	65.12		4.417					
Ethanol	0	0.01^{k}	0.02^{k}	0.01^{k}	0.02^{y}	0.037	<0.01	<0.01	<0.01	<0.05	<0.05
	7	0.16^{k}	0.21^{hj}	0.21^{hj}	0.19^{x}	0.037					
	21	0.34^{gh}	0.20^{h}	0.27^{hi}	0.27^{wx}	0.037					
	60	0.51^{g}	0.20^{h}	0.25^{hi}	0.32^{w}	0.037					
	T mean	0.26^{a}	0.16^{b}	0.19^{b}		0.018					
Ammonia	0	ND	0.04	0.07	0.04	1.160	0.45	0.42	0.45	0.28	0.53
NPC	7	4.11	0.22	0.20	1.51	1.160					
	21	0.19	0.27	0.25	0.24	1.160					
	60	0.28	0.42	0.43	0.38	1.160					
	T mean	1.15	0.24	0.24		0.580					
Ammonia, %	0	0.16^{m}	0.29^{m}	0.29^{m}	0.25^{z}	0.128	<0.01	<0.01	<0.01	<0.01	<0.01
of CP	7	2.47^{ij}	1.37^{kl}	0.76^{lm}	1.53^{y}	0.128					
	21	3.22^{h}	1.81^{k}	1.08^{l}	2.04^{x}	0.128					
	60	4.46^{g}	2.79^{hi}	1.86^{jk}	3.04^{w}	0.128					
	T mean	2.58^{a}	1.56^{b}	1.00^{c}		0.064					

[a-b]Means in the same row (T mean) with unlike letters differ ($P < 0.05$).

[g-m]For variables with significant treatment × day interactions, means across all treatments and time points with unlike letters differ ($P < 0.05$).

[x-z]Means in the same column (Day mean) with unlike letters differ ($P < 0.05$).

[1]Trt = treatment; d = day.

[2]% of DM.

Table 7.6. Dry matter degradation variables of day 60 ensiled blends (0:100, 25:75, and 50:50) of carinata meal with corn silage forage (CRM:CS) or with alfalfa haylage (CRM:AH), carinata meal (CRM), and soybean meal (SBM)

	Treatment[1]									
	CRM:CS			CM:AH						*P*-
Item	50:50	25:75	0:100	50:50	25:75	0:100	CRM	SBM	SEM	value
DM disappearance, %										
A[2]	42.68^{a}	40.18^{ab}	42.38^{a}	32.82^{c}	30.69^{c}	29.24^{c}	41.55^{a}	33.99^{bc}	1.357	<0.01
B[3]	33.92^{cd}	28.00^{d}	26.19^{d}	50.86^{abc}	56.56^{ab}	49.97^{abc}	45.99^{bc}	66.03^{a}	3.445	<0.01
C[4]	23.40^{ab}	31.82^{a}	31.43^{a}	16.32^{abc}	12.75^{bc}	20.79^{ab}	12.46^{bc}	0.00^{c}	3.635	<0.01
K_d[5], % h	9.51^{ab}	13.96^{a}	6.98^{ab}	9.95^{ab}	4.22^{b}	4.79^{b}	7.83^{ab}	5.81^{b}	2.206	<0.01
RDDM[6], % of DM	61.10^{bc}	58.11^{cd}	54.78^{d}	58.99^{c}	50.70^{e}	48.17^{e}	65.19^{a}	63.07^{a}	1.334	<0.01

[1]CRM:CS = carinata meal and corn silage; CRM:AH = carinata meal and alfalfa haylage; 50:50, 25:75 or 0:100 = ratio of blend of carinata meal to forage

[2]Soluble DM.

[3]Potentially degradable DM

[4]Undegradable DM.

[5]Rate of DM degradation.

[6]Ruminally degradable DM (RDDM).

[abcde] Values with unlike subscripts differ by $P < 0.05$.

Table 7.7. Crude protein degradation variables day 60 ensiled blends (0:100, 25:75, and 50:50) of carinata meal with corn silage (CRM:CS) or with alfalfa haylage (CRM:AH), carinata meal (CRM), and soybean meal (SBM)

	Treatment[1]									
	CRM:CS			CM:AH						*P*-
Item	50:50	25:75	0:100	50:50	25:75	0:100	CRM	SBM	SEM	value
CP disappearance, %										
A[2]	73.59^{b}	79.54^{ab}	80.74^{a}	65.50^{c}	79.84^{ab}	77.90^{ab}	43.83^{d}	22.86^{e}	1.320	<0.01
B[3]	19.08^{cd}	11.89^{de}	4.93^{e}	25.41^{c}	18.67^{cd}	17.70^{d}	47.59^{b}	77.14^{a}	1.459	<0.01
C[4]	7.33^{bc}	8.87^{ab}	14.33^{a}	9.09^{ab}	1.49^{cd}	4.40^{bcd}	8.58^{ab}	0.00^{d}	1.194	<0.01
K_d[5], % h	16.25	23.14	49.03	25.47	4.29	4.01	10.27	4.92	11.59	0.17
RDP[6], % of CP	86.45^{ab}	87.97^{a}	84.24^{ab}	85.23^{ab}	86.45^{ab}	83.07^{b}	71.56^{c}	53.76^{d}	1.041	<0.01
RUP[7], % of CP	13.55^{cd}	12.03^{d}	15.76^{cd}	14.77^{cd}	13.55^{cd}	16.93^{c}	28.44^{b}	46.24^{a}	1.041	<0.01
IDP[8], % of RUP	71.26^{bc}	70.61^{bc}	75.77^{b}	63.84^{cd}	70.84^{bc}	67.95^{bcd}	61.24^{d}	94.19^{a}	2.019	<0.01
IADP[9], % of CP	9.65^{c}	8.49^{c}	11.93^{c}	9.40^{c}	9.61^{c}	11.51^{c}	17.40^{b}	43.56^{a}	0.874	<0.01
TDP[10], % of CP	96.10^{ab}	96.47^{ab}	96.17^{ab}	94.64^{b}	96.06^{ab}	94.58^{b}	88.96^{c}	97.31^{a}	0.479	<0.01

[1]CRM:CS = carinata meal and corn silage; CRM:AH = carinata meal and alfalfa haylage; 50:50, 25:75 or 0:100 = ratio of blend of carinata meal to forage

[2]Soluble CP.

[3]Potentially degradable CP.

[4]Undegradable CP.

[5]Rate of CP degradation.

[6]Ruminally degradable protein (RDP).

[7]Ruminally undegradable protein (RUP).

[8]Estimated intestinal digestible protein (IDP) after 16 h incubation in Dacron bag and pepsin-pancreatin digestion

[9]Intestinally absorbable digestible protein (IADP; % CP) = Rumen undegradable protein (RUP, % of CP) × intestinal CP digestion (% of RUP).

[abcde]Values with unlike subscripts differ by $P < 0.05$.

Table 7.8. Fermentation profile over days of ensiling of corn silage (CS) and the treatment blends (25:75) corn silage:carinata meal cold-pressed (CS:CPR) or solvent-extracted (CS:SLV)

		Treatment					*P* - value		
Item[1]		CS	CRM-CPR	CRM-SLV	day	SEM	Trt	d	Trt × d
DM, %	Trt Mean	37.38^{c}	43.69^{b}	45.43^{a}		0.36	< 0.01	0.07	0.40
	0	36.69	42.98	45.52	41.73	0.72			
	7	38.68	43.61	45.84	42.71	0.72			
	21	37.51	45.43	45.42	42.79	0.72			
	60	36.64	42.75	44.95	41.45	0.72			
	Linear				< 0.01				
	Quadratic				< 0.01				
pH	Trt Mean	4.25^{b}	4.42^{a}	4.40^{a}		0.02	< 0.01	< 0.05	< 0.05
	0	5.47^{h}	5.57^{gh}	5.73^{g}	5.59^{w}	0.04			
	7	3.90^{j}	4.17^{i}	4.07^{ij}	4.04^{x}	0.04			
	21	3.83^{j}	4.00^{ij}	3.90^{j}	3.91^{x}	0.04			
	60	3.80^{j}	3.97^{j}	3.90^{j}	3.89^{y}	0.04			
	Linear				< 0.01				
	Quadratic				< 0.01				
Lactic acid	Trt Mean	3.66^{a}	3.22^{b}	3.47^{a}		0.11	< 0.01	< 0.05	0.36
	0	0.01	0.01	0.01	0.01^{y}	0.22			
	7	4.03	3.33	3.86	3.74^{x}	0.22			
	21	5.14	4.75	5.25	5.05^{w}	0.22			
	60	5.48	4.77	4.74	5.00^{w}	0.22			
	Linear				0.22				
	Quadratic				< 0.05				
Acetic acid	Trt Mean	0.63^{c}	0.83^{a}	0.76^{b}		0.02	< 0.01	< 0.01	< 0.05
	0	0.01^{l}	0.01^{l}	0.01^{l}	0.01^{y}	0.04			
	7	0.60^{k}	0.84^{j}	0.76^{jk}	0.73^{x}	0.04			
	21	0.93^{ij}	1.28^{gh}	1.09^{hi}	1.10^{w}	0.04			
	60	1.00^{h}	1.31^{g}	1.18^{gh}	1.16^{w}	0.04			
	Linear				< 0.01				
	Quadratic				< 0.01				
Ethanol	Trt Mean	0.43^{a}	0.18^{c}	0.28^{b}		0.01	< 0.01	< 0.01	< 0.01
	0	0.01^{l}	0.01^{l}	0.01^{l}	0.01^{y}	0.02			
	7	0.49^{h}	0.20^{k}	0.34^{ij}	0.34^{x}	0.02			
	21	0.61^{g}	0.26^{jk}	0.43^{hi}	0.43^{w}	0.02			
	60	0.62^{g}	0.26^{jk}	0.36^{i}	0.41^{w}	0.02			
	Linear				< 0.01				
	Quadratic				< 0.01				
CP	Trt Mean	7.34^{b}	16.25^{a}	16.44^{a}		0.21	< 0.01	0.14	< 0.05
	0	7.12^{i}	14.98^{h}	16.67^{gh}	12.92	0.41			
	7	7.23^{i}	16.68^{gh}	16.77^{gh}	13.56	0.41			
	21	7.54^{i}	17.43^{g}	16.03^{gh}	13.67	0.41			
	60	7.49^{i}	15.92^{gh}	16.30^{gh}	13.24	0.41			
	Linear				< 0.01				
	Quadratic				< 0.01				
Ammonia NCP	Trt Mean	0.19^{c}	0.41^{a}	0.28^{c}		0.01	< 0.01	< 0.01	< 0.01
	0	0.03^{m}	0.14^{kl}	0.07^{lm}	0.08^{z}	0.01			
	7	0.16^{k}	0.34^{i}	0.20^{jk}	0.24^{y}	0.01			

	21	0.25 [j]	0.54 [gh]	0.35 [i]	0.38 [x]	0.01			
	60	0.33 [i]	0.61 [g]	0.51 [h]	0.48 [w]	0.01			
	Linear				< 0.01				
	Quadratic				< 0.01				
Ammonia N%CP	T Mean	0.26 [a]	0.25 [a]	1.73 [b]		0.05	< 0.01	< 0.01	< 0.01
	0	0.41 [l]	0.98 [k]	0.40 [l]	0.60 [z]	0.10			
	7	2.33 [j]	2.07 [j]	1.21 [k]	1.87 [y]	0.10			
	21	3.29 [hi]	3.12 [i]	2.17 [j]	2.86 [x]	0.10			
	60	4.38 [g]	3.82 [h]	3.12 [i]	3.77 [w]	0.10			
	Linear				< 0.01				
	Quadratic				< 0.01				
Total acids	Trt Mean	4.37	4.07	4.22		0.12	0.27	< 0.01	0.52
	0	0.01	0.01	ND	0.00 [y]	0.25			
	7	4.92	4.17	4.62	4.57 [x]	0.25			
	21	6.07	6.03	6.34	6.15 [w]	0.25			
	60	6.48	6.09	5.92	6.16 [w]	0.25			
	Linear				0.41				
	Quadratic				0.17				
Lactic:acetic	Trt Mean	4.45 [a]	2.83 [c]	3.48 [b]		0.04	< 0.01	< 0.01	< 0.01
	0	ND	ND	ND	ND	0.08			
	7	6.78 [g]	3.96 [k]	5.06 [ij]	5.27 [w]	0.08			
	21	5.56 [h]	3.71 [k]	4.81 [j]	4.69 [x]	0.08			
	60	5.97 [hi]	3.64 [k]	4.03 [k]	4.38 [y]	0.08			
	Linear				< 0.01				
	Quadratic				< 0.01				
Lactic % total	Trt Mean	72.47	67.59	61.60		6.80	0.54	< 0.01	0.79
	0	33.33	33.33	0.01	22.22 [x]	13.61			
	7	87.81	79.81	83.50	83.48 [w]	13.61			
	21	84.87	78.74	82.77	82.13 [w]	13.61			
	60	84.54	787.47	80.13	81.05 [w]	13.61			
	Linear				0.27				
	Quadratic				0.95				
NDF	Trt Mean	31.16 [a]	25.88 [b]	30.11 [a]		0.59	< 0.01	< 0.01	0.34
	0	33.97	27.25	29.90	30.37 [w]	1.18			
	7	28.28	25.71	29.10	27.70 [x]	1.18			
	21	29.74	23.51	29.27	27.51 [x]	1.18			
	60	32.64	27.07	32.17	30.63 [w]	1.18			
	Linear				0.22				
	Quadratic				< 0.01				
ADF	Trt Mean	15.81 [a]	14.13 [b]	14.84 [a]		0.38	< 0.05	< 0.05	0.20
	0	17.68	14.76	14.42	15.62 [w]	0.75			
	7	13.97	13.73	14.63	14.11 [x]	0.75			
	21	15.44	12.88	14.90	14.41 [x]	0.75			
	60	16.17	15.14	15.40	15.57 [w]	0.75			
	Linear				0.08				
	Quadratic				< 0.01				
OM	Trt Mean	96.18 [a]	94.92 [c]	95.07 [b]		0.07	< 0.01	0.22	0.42
	0	96.00	94.91	95.17	95.36	0.13			
	7	96.45	94.88	95.18	95.50	0.13			
	21	96.25	95.00	95.03	95.42	0.13			
	60	96.02	94.90	94.91	95.28	0.13			

	Linear				< 0.01				
	Quadratic				< 0.01				
Ash	Trt Mean	3.82 [b]	5.07 [a]	4.93 [b]		0.65	< 0.01	0.22	0.39
	0	4.00	5.60	4.83	4.63	0.13			
	7	3.56	5.12	4.82	4.50	0.13			
	21	3.75	5.00	4.97	4.57	0.13			
	60	3.98	5.10	5.09	4.72	0.13			
	Linear				< 0.01				
	Quadratic				< 0.01				
EE	Trt Mean	2.55 [b]	7.59 [a]	2.48 [b]		0.09	< 0.01	< 0.01	< 0.05
	0	2.10 [j]	6.67 [i]	2.22 [j]	3.67 [x]	0.18			
	7	2.64 [j]	7.40 [hi]	2.44 [j]	4.16 [w]	0.18			
	21	2.76 [j]	8.37 [g]	2.49 [j]	4.54 [w]	0.18			
	60	2.68 [j]	7.91 [gh]	2.75 [j]	4.45 [w]	0.18			
	Linear				0.59				
	Quadratic				< 0.01				
Sinigrin, mg/g	Trt Mean	< 0.01	0.14 [a]	1.72 [b]		0.04	< 0.01	< 0.01	< 0.01
	0	ND	0.20 [i]	4.81 [g]	2.50 [w]	0.07			
	7	ND	0.14 [i]	1.75 [h]	0.94 [x]	0.07			
	21	ND	0.16 [i]	0.24 [i]	0.20 [y]	0.07			
	60	ND	0.05 [i]	0.09 [i]	0.07 [y]	0.07			

[a-b]Means in the same row (T mean) with unlike letters differ ($P < 0.05$).
[g-m]For variables with significant treatment × day interactions, means across all treatments and time points with unlike letters differ ($P < 0.05$).
[x-z]Means in the same column (Day mean) with unlike letters differ ($P < 0.05$).
[1]Trt = treatment; d = day.
[2]% of DM.

Figure 7.1. Sinigrin concentrations in carinata meal and alfalfa haylage (CRM:AH) blends (0:100; 25:75, and 50:50) over days of ensiling (0, 7, 21, and 60).

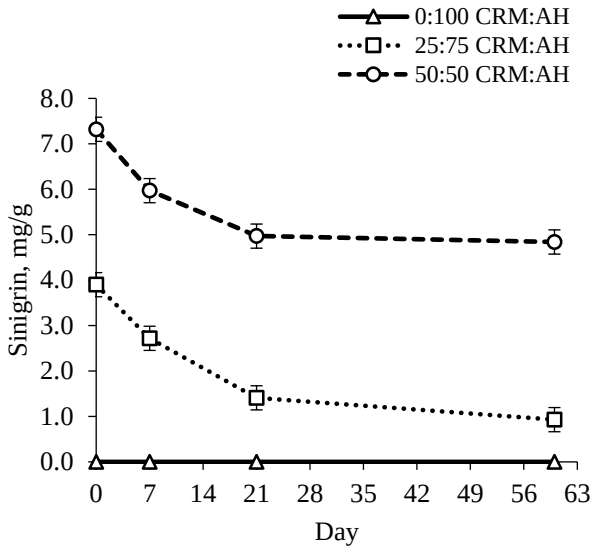


Figure 7.2. Sinigrin concentrations in carinata meal and corn silage (CRM:CS) blends (0:100; 25:75, and 50:50) over days of ensiling (0, 7, 21, and 60).

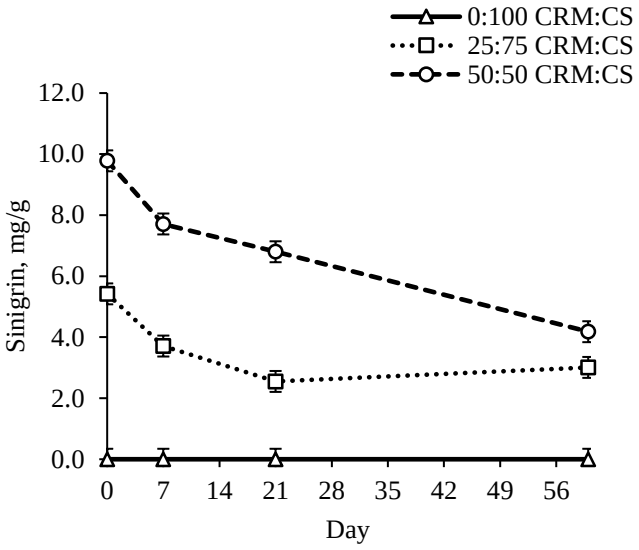
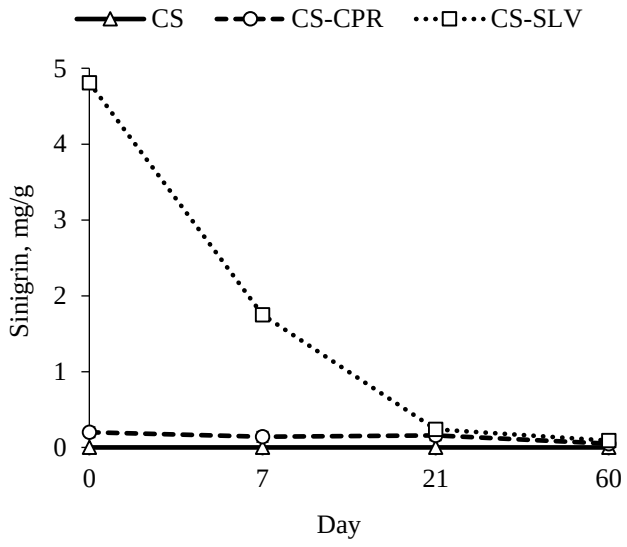


Figure 7.3. Sinigrin concentrations in corn silage (CS) and the treatment blends (25:75) corn silage:carinata meal cold-pressed (CS:CPR) or solvent-extracted (CS:SLV).



OVERALL SUMMARY AND CONCLUSIONS

This research fulfilled our initial overall objective to evaluate carinata meal as a feedstuff for dairy heifers and lay the foundations for future research in lactating dairy cows. In Chapter 2 we identified the short-term taste preference of carinata meal compared with other oilseeds and distillers dried grains with solubles (DDGS). In Chapters 3 and 4 we determined how feeding cold-pressed carinata meal affects the growth, nutrient utilization, metabolic profile and onset of puberty compared with DDGS. In Chapters 5, and 6 we determined how feeding solvent-extracted carinata meal affects the growth, nutrient utilization, metabolic profile and onset of puberty compared with canola meal and soybean products. Finally, in Chapter 7 we evaluated ensiling cold-pressed or solvent extracted carinata meal with corn silage or alfalfa haylage and its effects on glucosinolate content, silage fermentation, and nutrient quality of silage.

It was hypothesized that as CRM has high crude protein content and quality, its inclusion in the diet will maintain or enhance the growth performance of dairy heifers and age at puberty without negatively affecting the health and thyroid hormone concentrations. Secondly, as the content and type of glucosinolates vary depending on the oilseed meal, the taste preference could be different, affecting the dry matter intake of dairy heifers. And third, we hypothesized that the fermentation process during ensiling of carinata meal with forages would decrease the glucosinolates content without affecting the fermentation characteristics of the silage.

The literature review conducted, did not show other short-term studies of Holstein heifers preference for oilseeds meals with glucosinolates. The content of glucosinolates was the greatest in carinata meal, but it was preferred similar to canola meal, which had the least content of glucosinolates. Carinata meal had greater preference compared to camelina meal, and less preference compared to DDGS or linseed meal for dairy heifers. Apparently, the profile of glucosinolates is the factor that may affect preference, at least in the short-term. Findings of this study are important because dairy producers need to be aware that taste preference may cause heifers the need for an adjustment period to different oilseed meals or that cattle may consume them better if they are mixed with more palatable feeds.

Research described in Chapters 3 and 4 is one of the first studies, which could be found, on feeding cold-pressed carinata meal to growing dairy heifers. In this study, we confirmed that despite containing some glucosinolates, heifers can adapt to the taste of cold-pressed carinata meal and dry matter intake was not affected after two weeks of feeding. Body frame growth and average daily gain (ADG) were maintained at recommended rates (Zanton and Heinrichs, 2006) throughout the study. Metabolic profile, thyroid function, and onset of puberty were comparable to the heifers fed the DDGS diet. Additionally, our results were consistent with other research with dairy heifers of similar age that were fed other feedstuffs that are co-products of the biofuels industry (Anderson et al., 2009; Anderson et al., 2015a; Anderson et al., 2015b, Lawrence et al., 2016; Manthey and Anderson, 2017; Manthey et al., 2017; Manthey et al., 2018).

From results of Chapters 5 and 6 we found that despite containing some glucosinolates, solvent-extracted carinata meal did not have the short-term effect on reduction of dry matter intake that was observed with cold-pressed carinata meal. Body frame growth and average daily gain (ADG) were 0.2 kg greater than recommended rates (Zanton and Heinrichs, 2006), which was in all treatments including the heifers fed canola meal or soybean products diets. Metabolic profile, thyroid function, and onset of puberty were comparable to the heifers fed canola meal or the soybean products diets. The proportion of cycling heifers fed the CRM or CON diets was greater by 270 kg of BW compared with heifers fed the CAN diet. Additionally, these results are consistent with other research with dairy heifers of similar age by our research group.

Ensiling cold-pressed or solvent extracted carinata meal effectively reduced the glucosinolates content, increased protein content and quality of alfalfa haylage and corn silage without affecting the fermentation characteristics of the silage. The fermentation profiles of the ensiled blends were similar to those recommended by other researchers (McDonald et al., 1991; Kung et al., 2018).

Overall, this body of research on feeding carinata meal has demonstrated that it is a viable protein and energy source for dairy heifers that can maintain growth performance when included at 10% of diet DM. Carinata meal shows potential as a by-product of the biofuels industry that can be used as a new feedstuff for growing dairy heifers and replace canola meal and part of the DDGS and soybean products of heifers diets. When feeding cold-pressed carinata meal, producers need to be aware that heifers may initially

need a week or two adaptation period to adjust to the cold-pressed carinata flavor. In these initial studies, a limit-feeding strategy was utilized to control overall intakes. Based on these initial positive results, more research is now warranted using other feeding strategies such as in diets fed ad libitum as TMR and in dairy cattle at different stages of life, such as during lactation. Overall, this research shows that carinata meal could be a valuable new feedstuff for use in dairy cattle rations and it proves that it can be fed to dairy heifers and maintain growth, nutrient utilization, and metabolic status compared to commonly used feedstuffs.

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