EVALUATION OF BRASSICA CARINATA MEAL ON ANIMAL PERFORMANCE AND METABOLISM IN BEEF CATTLE

By

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To my children

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AA	Amino acid
AAD	Amino acid digestibility
AAFC	Agriculture and Agri-Food Canada
ADF	Acid detergent fiber
ADFI	Acid detergent fiber intake
ADG	Average daily gain
A:P	Acetate to propionate ratio
APP	Acute phase protein
APR	Acute phase response
BCAA	Branched-chain amino acid
BCM	*Brassica carinata* meal
BCVFA	Branched-chain volatile fatty acid
BMR	Basal metabolic rate
BUN	Blood urea nitrogen
BW	Body weight
CNS	Central nervous system
CP	Crude protein
CPI	Crude protein intake
Cp	Ceruloplasmin
CRP	C-reactive protein
CSM	Cottonseed meal
CTL	Control
Cu	Copper
D	Potentially degradable fraction

DDGS	Dry distillers grains plus solubles
DIT	Diiodotyrosine
DM	Dry matter
DMI	Dry matter intake
DoD	Department of defense
DPD	N, N dimethyl-p-phenylenediamine sulfate
EFSA	European Food Safety Authority
EIA	U.S. Energy Information Administration
EPA	Environmental Protection Agency
Exp	Experiment
FA	Fatty acid
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
FEF	Feed Efficiency Facility
GE	Gross energy
G:F	Gain to feed ratio
GH	Growth hormone
GHG	Greenhouse gas emissions
Hb	Hemoglobin
Hp	Haptoglobin
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric acid
IAAA	Intestinally absorbable amino acids
IADP	Intestinally absorbable dietary protein
IDP	Intestinally digestible protein

IGFs	Insulin-like growth factors
IL-	Interleukin
ILUC	Indirect land use change
iNDF	Indigestible neutral detergent fiber
IPS	Inter Press Service
IVOMD	In vitro organic matter digestibility
IVTDMD	In vitro true dry matter digestibility
K^+	Potassium
K_d	Rate of degradation (% h^{-1})
K_p	Rate of passage (% h^{-1})
L	Lag time
LCA	Life cycle assessment
MBW	Metabolic body weight ($BW^{0.75}$)
MCP	Microbial crude protein
MIT	Monoiodotyrosine
MP	Metabolizable protein
N	Nitrogen
Na^+	Sodium
NAABB	National Alliance for Advanced Biofuels and Bio-products
NADH	Nicotinamide adenine dinucleotide
NDF	Neutral detergent fiber
NDFI	Neutral detergent fiber intake
NFREC	North Florida Research and Education Center
NH_3-N	Ruminal ammonia nitrogen
NIMSS	National Information Management and Support System

NRC	National Research Council Canada
OM	Organic matter
OMI	Organic matter intake
PAMPs	Pathogen-associated molecular patterns
REAP	Rural Energy for America Program
RDP	Rumen degradable protein
rpm	Revolutions per minute
r-T_3	Reverse-triiodothyronine
RUP	Rumen undegradable protein
S	Sulfur
SAA	Serum amyloid-A
SF	Soluble fraction
SBM	Soybean meal
SD	Standard deviation
SEM	Standard error of means
T_3	Triiodothyronine
T_4	Thyroxine
TDN	Total digestible nutrients
TDP	Total dietary protein
TTDP	Total tract digestibility of protein
Tg	Thyroglobulin
TNF-α	Tumor necrosis factor-α
Undeg	Undegradable fraction
US	United States
USDA	United States Department of Agriculture

VFA	Volatile fatty acid
VLCFA	Very long-chain fatty acid

Abstract of Thesis Presented to the Graduate School
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Brassica carinata, a novel oilseed crop, yields high-quality biofuel, resulting in a high-protein byproduct. This meal has not been tested as a cattle protein supplement, therefore our objective was to evaluate the effects of supplementation of *B. carinata* meal.

Eight Angus crossbred steers were utilized in a duplicated 4 × 4 Latin square design, over 4 periods of 28 d each, to assess ruminal fermentation parameters, nutrient digestibility, and blood profile. Within period, steers were assigned to one of four treatments: 1.39 kg d^{-1} of *Brassica carinata* meal pellets (BCM), 1.62 kg d^{-1} of cottonseed meal (CSM), 2.15 kg d^{-1} of dry distiller's grains plus solubles (DDGS), or 1.17 kg d^{-1} of soybean meal (SBM). No effect of treatment ($P > 0.05$) was observed for ruminal pH, NH_3-N, total VFA, plasma glucose, DMI, or apparent total tract digestibility of nutrients. Steers receiving SBM had the greatest ($P < 0.01$) concentration of BUN.

A ruminal in situ degradability study was performed utilizing steers from the previous experiment. Supplement remaining after 16 h of incubation was subjected to serial solutions simulating post-ruminal digestion. Rate of degradation of DM and CP was greatest ($P < 0.01$) in SBM; total tract digestibility was greater in SBM. Nearly equivalent amounts of RDP and RUP

were observed in CSM and DDGS, which differed ($P < 0.01$) from SBM and BCM. Compared with DDGS, SBM had a greater IDP ($P < 0.01$), with CSM having the greatest IADP ($P < 0.01$).

Animal performance, attainment of puberty, and blood profile was evaluated in 64 Angus crossbred heifers. Stratified and blocked by initial BW, heifers were randomly allocated into 18 pens over 2 consecutive years. Pens were randomly assigned, within block, to one of two treatments: 0 (CTL) or 0.3% of BW d^{-1} (as fed) of BCM pellets. Bermudagrass hay was provided ad libitum. Compared with CTL, BCM increased ($P < 0.01$) ADG; treatment did not affect interval to attainment of puberty ($P = 0.68$). Concentrations of ceruloplasmin were greater ($P < 0.01$) in CTL heifers, with an effect of day ($P < 0.01$) observed for T_3, T_4, and ceruloplasmin.

CHAPTER 1
INTRODUCTION

Greenhouse gas emissions in the US are principally anthropogenic, resulting from burning fossil fuels for heat, electricity, and transportation (EPA, 2015). Replacing petroleum-based products with renewable resources offers probable solutions in reducing emissions (EIA, 2017), and technological advances have enabled an increasingly efficient conversion of renewable resources into fuels and chemicals (IPS, 2014).

Alternatives have been employed to decrease dependency on fossil fuels. The current ethanol industry was revitalized in the 1970s in response to increasing fuels costs (EIA, 2017), and began utilizing corn as a feedstock for the production of this biofuel. The ethanol industry is insufficient in meeting demands, partially due to EPA (2011) regulation allowing only 10 to 15% ethanol mixtures in gasoline, decreased fuel efficiency compared with gasoline alone (Knoll et al., 2009), and competition in land allocated for increased crop production with land utilized to produce food for humans and livestock (FAO, 2008). Algal oil has been successfully converted to biofuel, however acquisition of materials, pricing, and efficiency of conversion have presented challenges (NAABB, 2014). Similarly, lignocellulose has been successfully converted to biofuel and used to generate electricity in local industries where biomass is abundant. Despite success, the conversion of lignocellulose to biofuel for use on a large scale is expensive, less efficient, and environmentally taxing (Carroll and Somerville, 2009). Recently incurring greater interest are non-food oilseeds, which may provide a sustainable alternative without competing directly with human food, or livestock production systems.

Non-food oilseeds undergo efficient treatments for oil extraction, accounting for 10% of the total costs of the final fuel (NIMSS, 2016). Due to a favorable fatty acid profile, *Brassica carinata* has been successfully used as a 100% drop-in biofuel (NRC, 2013). After oil extraction,

a residual meal with approximately 40% CP results. Additional benefits include cold and drought tolerance, heat and disease resistance, and potential use as a pesticide and rotational crop (AAFC, 2015), which offer great promise in the Southeast US. *Brassica carinata* has not been extensively tested as a protein supplement for beef cattle, therefore, research is necessary to evaluate the effects of supplementation on metabolism, feed intake, nutrient digestibility, ruminal fermentation, and performance in beef cattle.

CHAPTER 2
LITERATURE REVIEW

Brassica carinata

Concern over stability and negative effects of fossil-fuels has increased awareness and motivated an effort to replace petroleum-derived sources with renewable and sustainable resources (EIA, 2017). *Brassica carinata* is a non-food oilseed crop, belonging to the mustard family, *Brassicaceae*, and originates from the highlands of Ethiopia. Ethiopian mustard, or more commonly carinata, results from interspecific hybridization between *B. nigra* L. and *B. oleracea* L. (Prakash and Hinata, 1980). As an amphidiploid (BBCC, 2n = 34), carinata possesses a complete diploid set of chromosomes from each parent, thereby acquiring valuable traits inherent to each parent. Carinata has many potential benefits including: a favorable very long-chain fatty acid (VLCFA) profile for conversion to biofuel, a low indirect land use change (ILUC) rating, a favorable Life Cycle Assessment (LCA), drought and cold tolerance, heat and disease resistance, usage as a rotational crop, and a high concentration of crude protein within the meal residue after oil extraction (AAFC, 2015).

Favorable Long-chain Fatty Acid Profile

The VLCFA profile of carinata consists of erucic and nervonic acid, favorable alternatives for not only biofuel production, but also bio-plastics, lacquers, and paints (Carlsson, 2009; Impallomeni et al., 2010; Newson et al., 2013). Genetically modifying the fatty acid (FA) composition of native *B. carinata* seed oil, approximately 40% of seed DM (Warwick et al., 2006), significantly increased concentration of erucic acid or proportions of nervonic acid resulting in more than 40% of erucic or nervonic acid in second generation lines (Marillia et al., 2013). Erucic acid (22:1; C_{22}) is a precursor to nervonic acid (24:1; C_{24}), both resultant from elongation of oleic acid (18:1; Taylor et al., 2010). The increase in C_{22} and C_{24} resulted in a

decrease of C_{18} FA proportions (Marillia et al., 2013), which is uncommon for an oilseed, considering soybean and canola meal typically have FA profiles of C_{20} or less. This alteration of the FA profile is beneficial as an increase in VLCFA improves performance properties such as cold-temperature flow characteristics, oxidative stability, and NO_x emissions in biodiesel (Durrett et al., 2008); decreases GHG emissions by more than 80%, reduces fuel consumption, and decreases black carbon, or incomplete combustion of biofuels, up to 50% in jet biofuel (ARA, 2017).

Potential Effects of Utilizing Renewable Resources

Land use change, or diversion of land from its existing uses (Searchinger et al., 2008), is a concern when considering potential growth of renewable resource industries utilizing feedstock for biofuels. Potential GHG mitigation through utilization of feedstock is promising, however unintended effects of ILUC are not taken into account when calculating emissions, destruction of forests and grasslands, or competition between human and livestock for feed (Milazzo et al., 2013).

A Life Cycle Assessment (LCA) is an intensive study used to evaluate environmental impacts, energetic balance, and economic performance of a biomass crop regarding cultivation, collection, transportation, and conversion of biomass to energy in the form of fuel or electricity (Gasol et al., 2007; Butnar et al., 2010). A LCA was conducted for carinata in southern Europe (Gasol et al., 2007), Spain (Butnar et al., 2010), and Italy (Cardone et al., 2003) as a lignocellulosic biomass crop for energy use and biofuel. Butnar et al. (2010) analyzed the impacts of carinata and a native crop on six categories: global warming, acidification, human toxicity, ozone layer depletion, abiotic depletion, and photochemical oxidation. Biomass crops used for generating power were found to be more environmentally harmful than current electricity-producing systems, however Butnar et al. (2010) observed that negative impacts

decreased with an increase in biomass productivity. The use of carinata was deemed as a viable source for production of energy in southern Europe, and Gasol et al. (2009) concluded that varying management practices to improve crop production and performance may further increase energy and environmental benefits. Fertilizers used for carinata presented the greatest impact, however utilizing an alternative, such as livestock waste, was suggested as a potential improvement (Gasol et al., 2009). Agronomic performance and energetic balance resulted in a favorable analysis in Italy concerning use of carinata as a biofuel (Cardone et al., 2003). Carinata required less fertilizer, weed control, and tillage compared with *Brassica napus*, and due to the tolerance of carinata to harsher environments, outperformed *B. napus* in production in coastal regions. Further, biofuel from carinata exhibited positive characteristics similar to commercial biodiesel, with the potential of decreasing costs associated with production of biofuel (Cardone et al., 2003).

Utilizing renewable resources for national energy security, climate change mitigation, and sustainability are motivations behind majority of studies evaluating feedstocks as potential biofuels (Seepaul et al., 2016). Carinata eliminates competition for land allocated for food crop production through its ability to be utilized in unfavorable environments as it is drought and cold tolerant, heat and disease resistant (AAFC, 2015), and potentially able to be used in rotation with other crops, or for use in fallow land, when food production crops would not normally be present (Marois et al., 2015). Additionally, use of carinata in the southeastern U.S. promotes the agenda of Rural Energy for America Program (REAP) established by United States Department of Agriculture (USDA, 2013), through domestically grown renewable and sustainable resources. Residual meal waste may also be beneficial in the southeastern U.S., due to high concentration of CP, as a potential protein supplement for livestock.

Previous Research on Utilization of Carinata as a Supplement for Livestock

Although carinata is not a new crop, the residual meal after oil extraction has not been extensively investigated as a protein supplement for livestock. Tadelle et al. (2003) evaluated the performance of Hubbard broiler chicks fed carinata as a rapeseed cake at various inclusion rates (0, 7, 14, 21, 28, and 35%) of the basal diet. Additionally, no differences were observed in intake of feed or water, feed to gain ratio, or in dressing percentage. Interestingly, percentage of mortality was increased for inclusion of 28% compared with 7%, but was similar for all other treatments; however, an increase in size of thyroid follicles was observed for broiler chicks receiving 35% inclusion. Wheater (1987) observed a negative correlation regarding thyroid activity, i.e., an increase in thyroid follicle size results in a decrease in thyroid hormone secretion. Tadelle et al. (2003) concluded that the effects observed were related to the concentration of rapeseed cake inclusion; however, comparing effects of the control with dietary inclusion amounts of 7 through 21% indicated no differences were observed for these treatments. Further, the economical aspect of feeding broiler chicks seemed to be the focus and it was concluded that the inclusion of 28% required the least quantity of feed per unit gain and promoted greatest dressing percentage. Considering mortality was highest for 28% inclusion, with no significance observed in dressing percentage, intake, or gain, the conclusion is questionable.

Inclusion of 10% carinata meal as a protein supplement in a diet of barley silage and grain, compared with canola meal at the same rate, was evaluated for yearling steers, in which no differences in live weight, ADG, DMI, or subcutaneous rib or rump fat were observed (McKinnon et al., 2012). A tendency was observed for decreased gain:feed ($P = 0.09$) in carinata compared with canola (0.09 vs 0.10, respectively), however it was concluded that inclusion of

10% carinata meal of diet DM resulted in similar effects on animal performance, compared with canola meal, at the same rate of inclusion.

Ruminal degradation, intestinal, and total tract digestion characteristics, and metabolizable protein supply of carinata meal was evaluated, in comparison with canola meal, using dry Holstein cows. An in situ study was performed to estimate the nutritive value of carinata meal and ruminal degradation kinetics, observing an increase in the rate of degradation of OM and CP for carinata meal when compared with canola meal (Xin and Yu, 2014). Proportions of degradable and undegradable fractions were similar for OM and CP, with soluble fractions similar for OM, but increased for CP in carinata, compared with canola. Undegradable OM tended to be decreased for carinata, compared with canola, however effective degradability of OM, CP, and NDF tended to be increased for carinata, compared with canola. Carinata and canola meals differed only in intestinally digestible rumen bypass OM, with canola being greater; therefore carinata meal performed similarly in intestinal digestibility of nutrients, except for rumen bypass OM compared with canola meal, with an increased rate of ruminal degradation for carinata compared with canola meal.

Protein Supplementation in Ruminants

Fractionation of Dietary Protein

Dietary CP results from the assumption that the average N content of proteins is 16%, and is partitioned in two fractions based on derivation of N: true protein (TP) or non-protein nitrogen (NPN). However, when calculating dietary CP content, differences between TP and NPN are not delineated. Upon consumption, dietary CP is further separated by ruminal activity, resulting in potentially degradable and undegraded fractions (Orskov and McDonald, 1979).

Specifically in ruminants, CP can be divided into two fractions: ruminally degradable protein (RDP) or ruminally undegradable protein (RUP). The RDP fraction includes NPN and

TP and is often referred to as fraction A is composed of water soluble proteins and NPN, or nitrogenous containing entities, such as nucleic acids, urea, NH_3, and nitrates, which are rapidly degradable (Dryden, 2008). A potentially degradable fraction B is comprised of TP, i.e., proteins, peptides, and amino acids, a portion of which may escape ruminal degradation. Ruminally undegradable CP is often referred to as fraction C. Thus, fraction A and degraded protein in fraction B compose RDP, whereas the undegraded portion of fraction B, and all of fraction C constitute RUP.

Microbial degradation of fraction B includes hydrolysis of proteins to oligopeptides by proteolytic enzymes, with subsequent degradation to smaller peptides and AA for cellular uptake to occur (NRC, 2016). Short peptides and AA are preferentially utilized by ruminal microorganisms, particularly fibrolytic bacteria (NRC, 2016), however NH_3 resulting from soluble NPN or deamination of AA, can be assimilated into microbial protein synthesis (Hungate, 1966). Excessive NH_3 is absorbed across the ruminal epithelium into the portal vein system, and subsequently converted to urea in the liver, to avoid toxicity. Urea, which is relatively non-toxic, is either excreted in urine, returned to the rumen via saliva, or diffused through the ruminal epithelium wall where it will be converted into NH_3 for utilization by ruminal microorganisms, or further recycled. Thus, efficiency of nitrogen recycling is dependent upon dietary intake of nitrogen, i.e., when nitrogen intake is decreased, efficiency of nitrogen recycling increases (NRC, 2016).

Ruminally undegraded protein escapes degradation by microorganisms, with subsequent digestion and absorption occurring within the small intestine, and further utilized by the animal for tissue growth or lactation. Importantly, protozoa are capable of deamination, however their involvement with regards to protein metabolism include engulfing and degradation of insoluble

particulate proteins, bacterial, and fungal cells. Thus, contribution of ciliate protozoa, autolysis and activity of bacteriophages results in nearly 50% of ruminal microbial crude protein (MCP; NRC, 2016). The RDP, which is degraded and ultimately transformed into MCP, in combination with RUP and endogenous protein, comprises metabolizable protein (MP), which more succinctly defines the availability of true protein for use by the ruminant (Dryden, 2008).

Ruminal microbes are able to utilize NPN sources for growth, however MCP yield is poor (Hume, 1970); therefore, provision of TP is essential for effective ruminal degradation and fermentation of feedstuffs. Further, maximizing ruminal MCP synthesis yields high quality protein, specifically amino acids, available to the small intestine, however additional RUP is necessary for optimizing yield in high-producing animals (Stern et al., 2006). It has been suggested that approximately 80 to 90% of dietary protein is inefficiently utilized and subsequently excreted as waste (NRC, 2016). Thus, despite the importance of protein, it is secondary to energy requirements, as efficiency of MCP synthesis is a function of energy utilized by ruminal microbes for maintenance and growth (NRC, 2016). Therefore, feeding cattle involves careful synchronization of energy and protein provided, complementing the needs of the animal, environment in which they live, and resources available. It is therefore important to understand dietary requirements of cattle, specifically protein, which vary with stage of production, animal size, and expected performance, as well as to recognize the needs of meeting the requirements of both the ruminant host and its microorganisms.

Cattle Production in the Southeast U.S.

Cattle production in the Southeast U.S. is typically comprised of cow-calf operations (McBride and Matthews, 2011), with management of a cow-calf herd based upon breeding cattle to suit conditions within a certain region. Often this is accomplished through crossbred cattle (Greiner, 2009), thereby increasing hybrid vigor through utilization of the F-1 hybrid, and

employing breeds which are most economical and complementary to management and available resources. In Florida alone, there are approximately 1,700,000 cattle, and nearly half of that total represent beef cows and replacement heifers (USDA, 2017).

Geographical location defines available resources, i.e., beef cattle production in southern Florida differs from practices in northern Florida. Due to heat stress, production in southern Florida typically results in calving season beginning in late fall (Vendramini and Arthington, 2008) with earlier weaning of calves. Cattle production in the Florida panhandle differs slightly with heifers calving, in the majority of the operations in which a breeding season is defined, anywhere from October to mid-December, and mature cows from November to January. Cow-calf production systems throughout the state, and Southeast U.S. in general, may deviate slightly from these two examples, however, these will be used for discussion of resources available at each stage of production.

Warm-season perennial grasses are the basis for beef-production in the southeastern U.S., with bahiagrass (*Paspalum notatum*) being the most common forage resources throughout the majority of Florida. In an attempt to improve the quality of the forage base over that of bahiagrass, bermudagrasses (*Cynodon dactylon*) are often planted, with Tifton 85 being preferred to Coastal bermudagrass due to increased digestibility and consequently, animal performance (Vendramini et al., 2008). During warmer months, Tifton 85 may be an ideal option for grazing cattle, however long periods of regrowth decrease the nutritive value of Tifton 85. Regarding forages to be used in the cool season, annual ryegrass is commonly utilized in central and north Florida, due to productivity and increased nutritive value, with the addition of small grains mixed in to provide sufficient forage until ryegrass is most productive (Dubeux et al., 2016). As previously mentioned, calving season occurs earlier in southern Florida and is often

accompanied with earlier weaning, which is suggested as an effective method of preparing first-calf heifers for rebreeding (Arthington and Kalmbacher, 2003). In northern Florida, however, calves are not weaned until approximately 6 to 10 months of age. Though climates differ, resulting in diverse growth patterns of forages and available resources, both regions face a common challenge: feeding beef cattle at various stages of production, size, and levels of nutrient requirements, with calf production, lactating cows, and developing heifers relying on supplementation and grazing (Banta et al., 2016). In order to determine the protein supplementation needed for these animals, it is necessary to review their dietary requirements.

Protein requirements of beef cattle in cow-calf operations of the southeastern U.S.

The publication Nutrient Requirements of Beef Cattle (NRC, 2016), provides in-depth, general information resulting from an abundance of research for provision of nutrients to beef cattle. With consideration to the prevalent production system in the Southeast U.S., energy and protein requirements are discussed for early-weaned calves, growing steer calves, lactating cows, and heifers. A summary of nutrient requirements for growing and lactating cattle is presented in Table 2-1 (NRC, 2016).

Vendramini et al. (2006) reported a decrease in DMI in early-weaned calves grazing wheat, and inefficient N utilization when consuming high-protein, annual cool-season pastures. Performance was improved, however, with addition of concentrate supplemented in synchronization with crude protein. Early-weaned calves should therefore be provided with a diet containing at least 15% CP and concentrate when grazing pastures to facilitate improved ruminal degradation and N utilization, and subsequent feed intake.

Growing steer calves, depending upon expected performance, should be supplemented with protein coinciding with recommended TDN values. Calves expected to weigh 550 kg at finishing vary in requirements depending on length of time for gain, i.e., calves with an expected

ADG of 1.17 kg d^{-1} require less DMI, but increased TDN, CP, and MP compared with calves with an ADG of 1.04 kg d^{-1} (Table 2-1; NRC, 2016). Yearling stocker calves are typically grown on high-roughage rations (Field, 2016), however the quality grade of beef is improved with marbling, which increases linearly with growth, genetic predisposition, and time on feed (Kern et al., 2014). Therefore, to attain expected growth and marbling, supplementation of protein may be necessary, depending on the basal diet provided.

Lactating cows in southern Florida present a challenge in terms of nutrition, as intake requirements increase approaching the peak milk yield (~ 60 DIM) and subsequent rebreeding, which corresponds with the cool season and limited pasture growth. During this critical period warm-season pastures are typically poor in quantity and quality, and therefore insufficient in meeting nutritional demands (Bohnert et al., 2011), without additional supplementation. Cool-season annuals in northern and southern Florida are typically sufficient to meet nutrient requirements, due to increased concentration of protein. However, weather variability (mainly their dependence on precipitation) may lead to failure during the establishment of cool season annual forages, having to incur additional supplementation. Protein supplementation for lactating cows should include sources higher in RUP to optimize milk production (Stern et al., 2006). Conversely, dry cows and mid-gestation, older cows in adequate to good condition can be sustained on lower quality feed (Field, 2016).

The success of a cow-calf operation is dependent upon management of cattle, specifically replacement heifers, as they are an important resource and their requirements differ from calves and mature cows. Nutrition of yearling heifers is similar to that of yearling steers, however diets are modified slightly to prevent excessive adipose tissue accretion, as this may affect development of the mammary gland and subsequent lactation (Field, 2016). Heifers are expected

to attain a certain weaning weight, become pregnant in the first estrous cycle, carry a calf to full term without complications, remain disease-free, and achieve a successful lactation while maintaining or increasing BW, despite not having reached maturity (Bellows et al., 2002).

Nutrition and attainment of puberty in beef heifers

The onset of puberty, primarily a function of age and weight, is the first ovulation that is accompanied by visual signs of estrus and normal luteal function (Perry and Cushman, 2013). Attainment of puberty in heifers is expected at approximately 15 months of age, depending on breed (Day and Nogueira, 2013). Funston and Deutscher (2004) suggest breeding at a target weight of 50 to 57 % of mature BW, as body conditions are primed for initiation of the estrous cycle. In order to meet that goal, heifers should gain approximately 0.22 to 0.68 kg d^{-1}, depending on weaning weight. In a cow-calf operation, the length of breeding season is reduced to optimize production of a uniform calf crop. Heifers are expected to calve at 24 months, and therefore require quality feed and increased intake to ensure reproductive performance, as the first calving tends to set precedence for subsequent parturitions and calving events (Patterson et al., 1992). Further, heifers calving earlier in the season tend to produce heavier calves and have more time to prepare for rebreeding (Arthington and Kalmbacher, 2003). Additionally, an increase in ADG has been observed when calving occurs 2 to 3 months prior to availability of green forage production, as opposed to calves born earlier or later (Field, 2016). Calving 2 to 3 months prior to green forage production correlates with a peak in milk yield of dams, thereby supplying calves with adequate nutrition, and displacing production pressure from the cow.

Glucosinolates

Carinata has been classified as a botanical impurity, with further classification in the Annex to Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 as an undesirable substance in animal feed due to glucosinolates present (EFSA, 2008).

Glucosinolates are a common occurrence in oilseed crops, especially those of *Brassicaceae*, and are found throughout the plant, with higher concentrations often found in seeds. Glucosinolates are a general term for any alkyl aldoxime-*O*-sulfate ester with a β-D-thioglucopyranoside group, which vary in structure and configuration of their side-chain (EFSA, 2008).

Evolution and Degradation

Glucosinolates, possibly evolved to prevent herbivory and are inactive until, upon mastication of plant leaves, myrosinase is released, unfolding the toxicity of the compound (van Doorn et al., 1998; Collett et al., 2014). Additionally, bacterial myrosinases are present in the gut of humans and animals (Fahey et al., 2001). Glucosinolates are hydrophilic and rather stable, therefore remaining in the seed after solvent-extraction, until myrosinase acts to convert glucosinolates into various products through hydrolysis (Tripathi and Mishra, 2007). More than 140 glucosinolates have been identified, however this review will focus on those relevant to carinata, primarily sinigrin, representing more than 95% of total glucosinolate content, and in lower concentration, progoitrin (Bellostas et al., 2007; Marillia et al., 2013).

Glucosinolates in Carinata: Sinigrin and Progoitrin

The hydrolysis of sinigrin by myrosinase produces an unstable thiohydroximate-*O*-sulfate and glucose, in which the glucose is subsequently removed resulting in spontaneous decomposition of thiohydroximate-*O*-sulfate to allyl isothiocyanate and free sulfate (Yuan et al., 2016) and 3-butenenitrile, also known as allyl cyanide (Duncan and Milne, 1992). The content of sinigrin in carinata seed meal was evaluated by Yuan et al. (2016) and found to contain 46.72 ± 0.92 μg mg^{-1} compared with a crude oil concentration of 0.075 ± 0.05 μg mg^{-1}, through a reversed-phase HPLC method. Progoitrin is hydrolyzed by myrosinase to (*S*)-goitrin, with activity of myrosinase being pH dependent, thus increasing concentration of (*S*)-goitrin under biologically relevant pH conditions (6.5 to 8; Xie et al., 2011), and goitrin is a sulfur-containing

oxazolidine (Ishikawa et al., 2014). Additionally, progoitrin can be hydrolyzed to nitriles (Forss and Barry, 1983; Collett et al., 2014).

Sinigrin and progoitrin breakdown products: Sinigrin and progoitrin are related to the characteristically bitter taste found in mustard plants (van Doorn et al., 1998), with palatability being an issue when crops containing high concentrations (~ 90 to 140 μmol g^{-1}; Lardy and Kerley, 1994) of glucosinolates are consumed by humans and animals. However, the effects on health of the breakdown products of sinigrin and progoitrin are of greater concern. Thiocyanate (or the isothiocyanate ion) and goitrin alter thyroid metabolism, including potential enlargement of the thyroid gland (Spiegel et al., 1993), through selectively binding iodine, which can be remedied with iodine supplementation; however, goitrin inhibits the synthesis of thyroid hormones, which cannot be corrected with supplementation (Zukalova and Vasak, 2002). Isothiocyanate and nitriles have been confirmed to be involved in growth retardation (Schone et al., 1997), inhibition of copper (Cu) and selenium absorption and metabolism, and fertility impairment (Taljaard, 1993). Further, damage to endothelium and epithelium, cell membranes, liver damage, transient impairment of locomotion, and disorientation (Schmid and Schmid, 1992) have been observed as effects of isothiocyanate and nitriles. Decreased intake and low amino acid absorption (Barry, 2013), in addition to irritation and edema of gastro-intestinal mucosa (Mason and Lucas, 1983), have also been reported. Isothiocyanate has been implicated in the tainting of animal products consumed by humans, referring to unpleasant taste or odor as the consumption by the animal increases (Fenwick et al., 1983). Furthermore, thiocyanate is absorbed into the blood and secreted into the milk, which may affect lactation performance in cows and growth of calves (Tayo et al., 2012).

Thyroid Metabolism

The thyroid gland consist of follicular cells, arranged into fluid-filled spheres, forming a follicle, which encloses an inner lumen filled with colloid. Thyroglobulin (Tg), a large, complex glycoprotein produced by the endoplasmic reticulum/Golgi complex of the thyroid follicular cells incorporates the amino acid tyrosine, and is exported via exocytosis into the colloid (Sherwood et al., 2013). The thyroid, stimulated by thyroid stimulating hormone from the adenohypophysis (Chiamolera and Wondisford, 2009), captures iodide from blood, trapping it within the thyroid, and transfers it into the colloid via a Na^+/K^+ pump located at the basolateral membrane (Dukes et al., 1993). Before reaching the colloid, iodide is oxidized by thyroperoxidase, thus indicating its active state (Sherwood et al., 2013). Once active iodide is within the colloid, it is coupled to a tyrosine yielding monoiodotyrosine, or two iodide coupling to a tyrosine yield diiodotyrosine. The coupling of iodide to tyrosine is attached to Tg through peptide bonding, and remains stored until cleaved off and secreted (Sherwood et al., 2013).

Thyroid Hormones

Iodination of tyrosine resulting in monoiodotyrosine (MIT) and diiodotyrosine (DIT) is the precursor to formation of thyroid hormones. Triiodothyronine (T_3) results from the coupling of one MIT and DIT, with thyroxine (T_4) resulting from coupling of two DITs, and these products being biologically active and stored until needed (Boelaert and Franklyn, 2005). Once stimulated for secretion, a portion of thyroglobulin-hormone complex is internalized by follicular cells and phagocytosis results in a membrane-bound droplet of colloid. This droplet will coalesce with lysosomes, whose enzymes will split off biologically active T_3 and T_4, as well as any inactive iodotyrosines for recycling (Sherwood et al., 2013). Released T_3 and T_4 will enter blood where they will quickly bind with plasma proteins, except for a small concentration which will remain in the unbound form, and are biologically effective. Most of the secreted hormone is T_4,

though it is subsequently converted to T_3 in peripheral tissues by a deiodinase enzyme, as T_3 is the major biologically active form (van der Spek et al., 2017) at the cellular level due to its affinity of binding to receptors, thereby giving peripheral cells the ability to activate their own hormone stimulation (Zhang and Lazar, 2000). Selecting for inactive hormone, reverse-triiodothyronine (r-T_3), allows for conservation of energy during limited food availability.

Effects of thyroid hormones: Thyroid hormones have no specific target, rather they affect every tissue in the body through crossing the plasma membrane and binding to nuclear receptors bound to thyroid-response element of DNA (Gerebens et al., 2015). Binding alters transcription of specific mRNAs, thus synthesizing specific new proteins for cellular response (Yen et al., 2006). Thyroid hormone synthesis is considered slow compared to other hormones, thus elevated concentrations of T_3 and T_4 are not detectable for several hours, with maximal response taking days to detect (Sherwood et al., 2013).

Thyroid hormones increase basal metabolic rate (BMR) by regulating mitochondrial function and certain mitochondrial proteins (Wrutniak-Cabello et al., 2001), and decrease BMR through regulating the rate of oxygen consumption and energy expenditure under resting conditions. Effects on metabolism are complex, as T_3 and T_4 can influence synthesis and degradation of carbohydrate, fat, protein, yet varying concentrations of hormones may have opposite effects (Sherwood et al., 2013). Fluctuations in thyroid hormones may vary by day and season, depending on animal species (Yoshimura, 2013).

Thyroid hormones increase target cell responsiveness to catecholamines, thus a sympathomimetic effect is observed (Silva, 2009). In the cardiovascular system, thyroid hormones increase cardiac output through increased responsiveness to circulating catecholamines (Jabbar et al., 2016). Essential for normal growth and development (Mullur et al.,

2014), thyroid hormones act permissively in concert with other hormones in stimulating growth process. Required for growth hormone (GH) secretion, T_3 and T_4 also promote effects of GH and IGFs onsynthesis of new structural proteins and on skeletal growth (Nilsson et al., 2005), with stunted growth observed in thyroid-deficient animals.

Thyroid hormones play a crucial role in development of the nervous system, especially the central nervous system (CNS) (Zoeller and Rovet, 2005), with thyroid hormones being essential for normal CNS activity in adult animals (Beydoun et al., 2015), as well as in conduction velocity of peripheral nerves which varies directly with availability of thyroid hormones (Zhang et al., 2015). Thyroid hormones also act on the development of steroidogenesis, testicular development, and spermatogenesis, as indicated by the presence of thyroid hormone receptors (Wagner et al., 2008).

Disorders of the Thyroid

Disorders of the thyroid gland are mainly grouped in two categories which reflect excess of thyroid hormone secretion, hyperthyroidism, or the deficient secretion of thyroid hormones, hypothyroidism.

Hyperthyroidism results in an elevated BMR resulting in increased heat production, and subsequent excessive perspiration or panting and poor tolerance of heat. An increased BMR can also result in increased appetite and intake, yet decreased weight gain, leading to loss of skeletal muscle protein and weakness, and negative effects on the cardiovascular system (Klein and Ojamaa, 2001). Hyperthyroidism affects the cardiovascular system, resulting in abnormalities from direct effects of thyroid hormones, as well as their interactions with catecholamines, potentially leading to heart failure (Klein and Danzi, 2007). Effects on the nervous system are observed in hyperthyroidism, manifested by excessive alertness, potentially resulting in irritability and anxiety.

Hypothyroidism, or low thyroid activity, is also characterized by BMR activity although markedly decreased (Reinehr, 2010) compared with hyperthyroidism. Hypothyroidism results in poor tolerance to cold, loss of hair or fur, fatigue, tendency for excessive weight gain, slow and weak pulse, slow reflexes, and decreased mental awareness and memory (Roberts and Ladenson, 2004). Hypothyroidism may be a result of thyroid failure, a deficiency of thyroid releasing or secreting hormone, an inadequate supply of dietary iodine, or certain chemicals or compounds which may affect uptake or trapping of iodide (Zoeller, 2010).

Immune Response

Under immunological stress, the first line of defense is the innate, antigen non-specific response, which can be elicited immediately or within several hours (Gruys et al., 2005). Innate immunity includes chemical and physical barriers preventing entry of pathogenic substances, such as skin, tears, urine, and stomach acid, in addition to beneficial microorganisms which will compete with foreign invaders for resources. Should pathogens penetrate these barriers, cellular defenses are activated with a release of phagocytic cells, natural killer cells, and cells that release inflammatory mediators (Sherwood et al., 2013). Phagocytic cells are activated at the site of infection and able to recognize pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors. Binding of PAMPs to these receptors initiates a killing mechanism in macrophages and neutrophils (Carroll and Forsberg, 2007). Natural killer cells do not attack pathogens, but attack and kill cells which have been contaminated by pathogens through chemically perforating the cell membrane leading to an influx of fluids and rupturing of the cell. Natural killer cells also release cytokines, or interleukins (IL), thereby initiating further immunological response (Carroll and Forsberg, 2007).

Acute Phase Response

An acute phase response (APR) is a component of the innate immunity defense system, in which the response is activated due to stressors from infection, inflammation, disease, trauma, stress, and bacterial components, and is detected for several days after the stimulus (Petersen et al., 2004). Indicators of an APR include fever, decreased food and water intake, catabolism of muscle proteins, decreased sexual and social behavior, alterations in plasma iron (Fe), zinc, Cu, calcium, and vitamin A, increases in circulating leukocytes, and increased sensitivity to pain (Gruys et al., 2005, 2006; Carroll and Forsberg, 2007; Moriel and Arthington, 2013).

Induced by pro-inflammatory cytokines (IL-1, IL-6, and TNF-α), secretions are predominantly from monocytes which have been activated as a response to bacterial toxins or local tissue damage, and subsequently released into the blood stream (Sherwood et al., 2013). Cytokines communicate between sites of infection or inflammation and hepatocytes within the liver synthesizing acute phase proteins. Pro-inflammatory cytokines act in tandem through multiple, overlapping pathways exhibiting effects on both cells surrounding the affected area, as well as systemic effects through transportation in the blood stream. A transient increase occurs in serum concentrations of pro-inflammatory cytokines a few hours after initial stimulus, however concentration decreases within several hours (Carroll and Forsberg, 2007).

Acute Phase Proteins

Proteinase inhibitors, enzymes, coagulation proteins, and metal-binding and transport proteins (APP) are all produced in hepatocytes of the liver at a relatively steady rate under normal conditions, however IL-1, IL-6, and TNF-α mediate hepatocyte synthesis and secretion of APP during an APR (Carroll and Forsberg, 2007). In response to stimuli, protein production is either increased (positive acute phase protein) or decreased (negative acute phase protein), with hepatic mRNA upregulation of APP produced in response to stressors negatively correlated with

normal production proteins (Gruys et al., 2005), in addition to production of APP being species-specific (Petersen et al., 2004). Due to the increase in concentrations of positive APPs during an APR, APPs such as haptoglobin (Hp), serum amyloid-A (SAA), C-reactive protein (CRP), and ceruloplasmin (Cp) have been used as potential indicators of bovine acute and chronic inflammation, however this review will only focus on Hp and Cp. It is important to note that while APPs are useful in assessing an APR, a single APP is not sufficient to evaluate "healthy" vs. "non-healthy" conditions, and should therefore be combined with other indicators to obtain a broader perspective (Gruys et al., 2006).

Haptoglobin: Haptoglobin is a positive APP, indicating an increase in synthesis and secretion is observed during an APR (Lomborg et al., 2008), therefore, Hp has been used as an indicator of health in cattle (Gruys et al., 2006), as diagnostic biomarkers and prognostic aids in veterinary medicine, and evaluation of inflammatory response (Nazifi et al., 2009). Haptoglobin is an α2-globulin synthesized by the liver during APR, with IL-6 inducing synthesis of Hp within hepatocytes (Yoshioka et al., 2002).

Haptoglobin strongly binds hemoglobin (Hb; Gruys et al., 2005) acting as a scavenger of free Hb in blood and assisting in an anti-oxidant role of Fe stabilization, thereby reducing oxidative effects on albumin, lipids, and kidneys (Ceciliani et al., 2012). Additionally, Hp has anti-inflammatory capabilities and binds to CD11b/CD18 integrines on cell membranes of leukocytes (Gruys et al., 2005), in addition to binding of Hp-Hb complex to CD163 of monocytes, thus increasing upregulation of anti-inflammatory mediators (Ceciliani et al., 2012).

In cattle, circulating concentrations of Hp are negligible during non-pathogenic conditions, however an increase of more than a 100-fold may be observed during an APR (Cooke and Arthington, 2013). Eckersall and Bell (2010) observed Hp serum concentrations in

cattle without display of pathological conditions to be < 20 mg L^{-1} with potential to increase 1000-fold within 2 d of infection, however Tourlomoussis et al. (2004) observed mean concentrations of Hp in plasma of beef cattle without pathological conditions to be 0.11 ± 0.08 mg mL^{-1}, and those displaying pathological conditions had increased concentrations of Hp in plasma, 0.27 ± 0.40 mg mL^{-1}.

In addition to Hp being used effectively in the diagnosis and prognosis of mastitis (Akerstedt et al., 2008), respiratory disease (Yoshioka et al., 2002), and endometritis (Eckersall and Bell, 2010), elevated concentrations of Hp have also been reported in cows at parturition (Trevisi et al., 2012), during transport stress (Lomborg et al., 2008), and exposure to stressful management practices (Cooke and Arthington, 2013). Haptoglobin is therefore an indicator of stress, in addition to APR, through potential activation of the hypothalamus-pituitary-adrenal axis resulting in increased production of glucocorticoids and subsequent induction of hepatic APP synthesis (Lomborg et al., 2008).

Ceruloplasmin: Ceruloplasmin is a positive APP, indicating an increase in synthesis and secretion is observed during an APR (Nazifi et al., 2009), although less commonly used as a diagnostic marker of APR compared with Hp. A metalloenzyme with oxidase activity, Cp is associated with Fe and Cu metabolism (Blakely and Hamilton, 1985), and similar to Hp, Cp scavenges for free Hb in blood, thereby reducing Fe availability for bacterial growth (Weinberg, 1984). Additionally, Cp has been used to evaluate Cu deficiency in cattle as Cp transports approximately 90 to 95% of serum Cu, an essential nutrient (Carroll and Forsberg, 2007).

Arthington et al. (1996) found that inducing Cu deficiency with molybdenum supplementation, resulted in decreased concentrations of Cp, compared with heifers without Cu deficiency. Dietary sulfur and molybdenum are known to induce Cu-deficiency in cattle, through

decreasing absorption of Cu (Carroll and Forsberg, 2007). When steers were provided increasing levels of metabolizable protein (MP) following vaccination (Moriel and Arthington, 2013), decreased concentrations of ceruloplasmin were observed for the greatest level of supplementation (115% MP), compared with 85 and 100% MP, from d 10 to the end of the study (d 29). This decrease in concentration of ceruloplasmin, approximately 10 to 16 mg dL^{-1} in 115% MP, compared with approximately 16 to 24 mg dL^{-1} in 85 and 100% MP treatments, appears to be related to the increased content of molybdenum and sulfur, however this is speculative.

Trevisi et al. (2012) observed an increase in concentrations of IL-6 which subsequently led to an increase in concentrations of ceruloplasmin in response to dystocic calving, placental retention, ketosis, fever, diarrhea, mastitis, delayed uterine involution, swelling of joints and lameness both pre/post-calving. This differed from concentrations of Hp, which were slightly elevated before calving and markedly increased post-calving, indicating that Cp may be a more effective tool in evaluating some responses compared with Hp. Further, increase in positive APP, Cp, was at the expense of negative APPs, albumin, lipoproteins and bilirubin, indicating the use of Cp to asses liver function during an APR (Trevisi et al., 2012). Concentrations of Cp in dairy cows exhibiting low liver function ranged from 3 to 3.5 μmol L^{-1}, compared with dairy cows exhibiting high liver function, 2.5 to 3 μmol L^{-1}, with an increase in concentrations occurring around calving.

Table 2-1. Summary of nutrient requirements of beef cattle[1].

Description	SBW (kg)	ADG (kg d^{-1})	DMI (kg d^{-1})	TDN (% DM)	CP (% DM)	RDP (% CP)	MP (g d^{-1})
Growing and finishing cattle (550 kg at finishing)	250	1.04	5.93	70	14.2	48.1	607
		1.17	5.72	75	15.7	46.3	652
		1.25	5.42	80	17.2	45.0	680
		Peak Milk (kg d^{-1})					
Lactating cow, 90 d post-calving	550	8	12.2	60	10.0	55.5	376
			12.8	65	11.7	50.8	
			13.5	70	13.5	47.5	

[1]Nutrient Requirements of Beef Cattle, 2016.

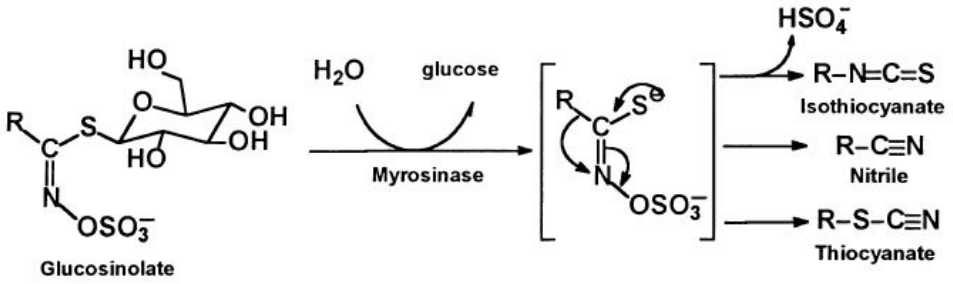

Figure 2-1. Breakdown products of a general glucosinolate structure under the action of myrosinase.

CHAPTER 3
EVALUATION OF BRASSICA CARINATA MEAL ON RUMINAL METABOLISM AND NUTRIENT DIGESTIBILITY OF BEEF CATTLE

Introduction

Brassica carinata is a non-food oilseed crop with a favorable very long chain fatty acid composition for conversion to biofuel (Marillia et al., 2013). Oil extracted from the seed has been utilized as a 100% drop-in jet biofuel, promoting the use of *B. carinata* as a renewable and potentially sustainable resource (AAFC, 2015). In the southeastern U.S., *B. carinata* would be an ideal candidate for use in crop rotation and cover crop due to its heat and drought tolerance, and cold and disease resistance (AAFC, 2015; Seepaul et al., 2016). A high-protein meal (~40% CP) is obtained as a byproduct of oil extraction; however, this meal has not been extensively tested as a potential protein supplement for cattle. As the southeastern U.S. is typically comprised of cow-calf operations, cattle often graze pastures of limited nutritive value which are not adequate to support high levels of production, especially during critical periods, necessitating supplementation of protein (Hersom et al., 2011; McBride and Matthews, 2011). Common protein supplements in this region result from byproducts of various industries and in conjunction with the poor quality hay available in winter, provide an opportunity to meet the nutritional requirements of growing cattle (Schulmeister et al., 2015). *Brassica carinata* meal has been evaluated as a high quality source of crude protein for ruminants utilizing an in situ procedure (Xin and Yu, 2014), however research in feeding *B. carinata* to cattle is limited. Thus, the objective of this study was to evaluate the effects of supplementation with *B. carinata* meal in comparison with common protein supplements on ruminal fermentation parameters, metabolism, and blood profile in Angus crossbred steers consuming bahiagrass hay.

Materials and Methods

All procedures involving animals were approved by the Animal Care and Use Committee of the Institute of Food and Agricultural Sciences at the University of Florida, study # 201308011.

Experimental design and sample collection

The experiment was conducted at the University of Florida, Feed Efficiency Facility (FEF) in Marianna, FL, beginning in October, 2014. Eight ruminally-cannulated Angus crossbred steers (473 ± 119 kg of initial BW) were used in a duplicated 4 × 4 Latin square design conducted over four consecutive 28-d periods. Steers were randomly allocated to 8 pens, and within each period steers were randomly assigned to one of four treatments: 1.39 kg d^{-1} *B. carinata* meal pellets (BCM), 1.62 kg d^{-1} cottonseed meal (CSM), 2.15 kg d^{-1} dry distillers grain plus solubles (DDGS), or 1.17 kg d^{-1} soybean meal (SBM), supplemented daily. Treatments were calculated to be isonitrogenous based on total N provided by supplementation of 1.39 kg d^{-1} of BCM. On d 0, steers were shrunk weighed (after 16 h of feed and water withdrawal) and housed individually in pens at the FEF with ad libitum access to water and bahiagrass hay (*Paspalum notatum*). Each pen at the FEF was equipped with 2 GrowSafe feed bunks (GrowSafe System Ltd., Airdrie, Alberta, Canada) to record individual hay intake by weight change measured to the nearest gram. Steers were acclimated to the facility, hay, and supplements from d 0 to 14, and d 14 through 18 consisted of a digestibility period in which hay and fecal samples were collected twice daily for four d each. Day 19 involved a 24 h collection of ruminal fluid, blood, and ruminal pH, every 3 h. Day 28 was the final and initial day of a period in which BW was measured, and the adaptation period began for the next supplement.

Ruminal fluid and blood samples were collected before feeding (0 h) and every 3 h post-feeding for 24 h. Ruminal fluid was strained from a representative sample of digesta through 4 layers of cheesecloth and pH was immediately measured using a manual pH meter (Corning

Pinnacle M530, Corning Inc., Corning, NY). A 10-mL sample was taken and 0.1 mL of a 20% (vol/vol) H_2SO_4 solution was added to stop fermentation. Ruminal fluid samples were stored at -20°C for further analysis. Blood samples were collected from jugular venipuncture in 10-mL evacuated tubes containing sodium heparin, placed on ice following collection, and centrifuged for 15 min at 4,000 × *g* at 4 °C. After centrifugation, plasma was transferred into polypropylene vials (12 mm × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20 ˚C for further analysis.

Beginning on d 14 and d 15, feed and fecal samples were collected, respectively, for 4 consecutive days to determine apparent total tract digestibility of DM, OM, CP, NDF, and ADF. Feed samples were collected twice daily immediately after delivery of protein supplement and stored at -20°C. Fecal samples were collected twice daily at 0800 h and 1600 h from the ground, inside the pen, immediately after the animal defecated. After collection, fecal samples were stored at -20°C. At the end of each period, hay and fecal samples were thawed and dried at 55°C for 48 h in a forced-air oven, ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm screen, and pooled within steer for further determination of nutrient content and digestibility marker concentration. Indigestible NDF (iNDF) was used as an internal indigestible marker (Cole et al., 2011; Krizsan and Huhtanen, 2013).

Laboratory analyses

Supplement subsamples were weighed (0.5 g) in duplicate, dried in a forced-air oven at 100°C overnight to calculate DM, and subsequently ashed in a muffle furnace at 650°C for 6 h to calculate OM. To determine NDF concentration, samples were weighed (0.5 g) in duplicate in F57 filter bags and analyzed in an Ankom 200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY) using sodium sulfite and heat-stable α-amylase. Samples were subsequently

analyzed for ADF concentration as described by van Soest et al. (1991). Concentrations of CP in feed and feces was determined by rapid combustion using a macro elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) following official method 992.15 (AOAC, 1995). Protein supplements and bahiagrass hay were analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY.).

Concentrations of VFA in ruminal fluid samples was determined in a water-based solution using ethyl acetate extraction (Ruiz-Moreno et al., 2015). Samples were centrifuged for 10 min at 10,000 × *g*. Ruminal fluid supernatant was mixed with a meta-phosphoric acid:crotonic acid (internal standard) solution at a 5:1 ratio and samples were frozen overnight, thawed and centrifuged for 10 min at 10,000 × *g*. Supernatant was transferred into glass tubes and mixed with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant. After shaking tubes vigorously, the ethyl acetate fraction (top layer) was transferred to vials. Samples were analyzed by gas chromatography (Agilent 7820A GC, Agilent Technologies, Palo Alto, CA) using a flame ionization detector and a capillary column (CP-WAX 58 FFAP 25 m 0.53 mm, Varian CP7767, Varian Analytical Instruments, Walnut Creek, CA). Column temperature was maintained at 110°C, and injector and detector temperatures were 200 and 220 ˚C, respectively.

Concentration of NH_3-N was measured after centrifuging ruminal fluid samples at 10,000 × *g* for 15 min at 4°C (Avanti J-E, Beckman Coulter Inc., Palo Alto, CA) following the phenol-hypochlorite technique described by Broderick and Kang (1980) with the following modification: absorbance was read at 620 nm in flat-bottom 96-well plates using a plate reader (DU-500, Beckman Coulter Inc.). Plasma was analyzed for concentration of BUN using a quantitative colorimetric kit (B7551-120; Pointe Scientific Inc., Canton, MI). Plasma was

analyzed for glucose using a quantitative colorimetric kit (G7521-1L; Pointe Scientific Inc., Canton, MI).

Concentrations of iNDF in hay and feces were determined as described by Cole et al. (2011) with modifications proposed by Krizsan and Huhtanen (2013). Briefly, samples were weighed (0.5 g) into Ankom F57 filter bags, and then incubated at 39°C using a 4:1 ratio of McDougall's buffer:ruminal fluid in a Daisy^{II} incubator (Ankom Technology Corp.) for 288 h to ensure complete digestion of potentially digestible NDF fraction. After incubation, samples were rinsed and analyzed for NDF concentration as previously described.

Calculations and statistical analysis

Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were calculated as follows:

$$100 - 100 \times \left[\left(\frac{marker\ concentration\ in\ feed}{marker\ concentration\ in\ feces}\right) \times \left(\frac{nutrient\ concentration\ in\ feces}{nutrient\ concentration\ in\ feed}\right)\right]$$

Data were analyzed as a duplicated 4 × 4 Latin square design using the MIXED Procedure of SAS (SAS Institute Inc., Cary, NC). The model for intake and digestibility included fixed effects of treatment, square, and period, and the random effects of steer within square. Animal within period was the subject. Autoregressive was the best covariance structure based upon the smallest Akaike Information Criterion (AIC) values.

Data repeated over time (ruminal pH, NH_3-N, VFA, and BUN) were analyzed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc.). The model included the fixed effects of treatment, time, the treatment x time interactions, square, and period; random effects included effects of steer within square, and steer within treatment, with animal as the experimental unit (n = 8). Animal within period was the subject and the covariance structure used for all the parameters was unstructured, with the exception for total VFA, which was

analyzed using compound symmetry. Unstructured and compound symmetry were the best covariance structures based upon the smallest AIC values.

Differences between treatment means were identified by Tukey's least squares means comparison and significance was declared at $P \leq 0.05$ and tendencies considered when $0.05 < P \leq 0.10$.

Results and Discussion

The chemical and nutrient composition of hay and protein supplements fed to steers is available in Table 3-1. Neither intake nor digestibility of nutrients was affected by protein supplementation, which was averaged over four periods (Table 3-2). Dry matter intake (P = 0.49) for all treatments averaged between 6.2 and 7.2 kg d-^{1}, with OMI (P = 0.475) ranging from 5.8 to 6.7 kg d^{-1}. Intake of CP (P = 0.47) was similar for all treatments ranging from 0.33 to 0.37 kg d^{-1}, confirming isonitrogenous supplementation of protein. Digestibility of DM (P = 0.99) and OM (P = 0.98) was similar across treatments with averaged values of approximately 51 and 53% for DM and OM, respectively.

Bahiagrass (*Paspalum notatum*) is a common perennial grass grown in Florida and is often utilized for grazing beef cattle, or production of hay (Chambliss and Sollenberger, 1991); however, bahiagrass hay is of poor quality and often requires additional supplementation (Moore et al., 1991). The nutritive value of the bahiagrass hay fed to steers in this study was poor as illustrated by the digestible organic matter (DOM) or TDN:CP ratio, however supplementation of protein would be expected to increase intake, thus potentially explaining the lack of differences observed in intake (Moore et al., 1995). An effect of treatment (P = 0.65; Table 3-3) was not observed for ruminal pH, with averaged values ranging from 6.61 to 6.67, indicating a favorable ruminal environment for cellulolytic microorganisms activity (Russell and Wilson, 1996). An effect of time post-feeding of supplementation (P < 0.001; Figure 3-1) was observed

for pH, however there was no treatment × time interaction ($P = 0.37$). Steers were provided ad libitum access to bahiagrass hay and water, and as a result the initial pH was greater at the 0 h, however upon consumption of the protein supplements, a decrease in pH was observed between 3 h and 9 h, stabilizing through 18 h and then increasing through 24 h. We speculate that the protein supplements decreased the pH partially due to other constituents of the supplements, such as non-fiber carbohydrates (NFC), and the tendency of the steers to consume the protein supplements immediately upon arrival. The stabilization of pH may be related to an achieved balance after supplements were consumed, followed by an increase in pH due to consumption of bahiagrass hay alone, coupled with rumination and subsequent buffering effects. Concentrations of ruminal NH_3-N ($P = 0.37$) were not affected by treatments, however an effect of time ($P < 0.001$; Figure 3-1) was observed, yet no treatment × time interaction ($P = 0.60$) detected. Concentration of ruminal NH_3-N peaked at 3 h and steadily declined through 18 h, stabilizing between 21 and 24 h. Conversely to pH, the concentration of ruminal NH_3-N increased after consumption of protein supplements, which was expected and indicative of microbial degradation of provisional protein. Interestingly, Figure 3-1 illustrates the balance between pH and protein supplementation, i.e., upon consumption of protein supplements, pH decreases and concentration of NH_3-N increases, yet as fermentation ensues a stability is reached until, presumably, protein has been either completely degraded or removed from the rumen. Concentrations of ruminal NH_3-N, ranging from 2.27 to 3.18 m*M*, are lower than the value of 3.57 m*M* often quoted in reference to Satter and Slyter (1974) to maximize microbial protein synthesis. However, Satter and Slyter (1974) suggest that the "precise limiting concentration is perhaps closer to 20 mg NH_3-N/L, [1.43 m*M*] but use of the higher value gives a slight margin of excess". Furthermore, dietary requirements vary with age, stage of production, and size of cattle

(NRC, 2016); however, despite apparently low concentrations of ruminal NH_3-N, the values observed in this study are within the suggested values to maximize microbial crude protein synthesis.

An effect of treatment ($P < 0.001$) was observed for concentrations of BUN, with steers supplemented with SBM having the greatest and DDGS having the least concentrations (10.86 and 6.85 mg dL^{-1}, respectively). Concentrations of ruminal NH_3-N and BUN are highly correlated and indicative of the energy to protein ratio in healthy cattle (Hammond, 1992). Supplementation of protein in steers grazing bahiagrass and limpograss pastures resulted in concentrations of BUN between 9 and 12 mg dL^{-1}, thus indicating a transition range in which responding to protein supplementation, ADG was greater in steers with values below, and lesser in values above that range (Hammond, 1997). An increase in concentrations of BUN in steers supplemented with SBM in the current study, may be a result of poor synchronization of energy and protein when feeding a low quality forage and a protein source readily degradable in the rumen. However, BCM and DDGS values are within the range of 7 to 8 mg dL^{-1} suggested by Preston et al. (1978) for finishing steers, and are therefore potentially more favorable in terms of ADG and decreased N loss. A tendency for a time effect ($P = 0.085$) and treatment × time interaction ($P = 0.085$) was observed.

Concentrations of plasma glucose ($P = 0.37$) were not different between treatments, however an effect of time ($P < 0.001$; Figure 3-2) was observed, with no treatment × time interaction ($P = 0.99$) detected. Plasma glucose concentrations are tightly regulated, however an increase may result following a high-carbohydrate meal or endogenous synthesis of glucose in the liver (Dukes et al., 1993). Moreover, glucose is not readily absorbed, thus as NH_3-N decreases, an increase in glucose and subsequently insulin is observed (van Soest, 1994).

Therefore, the increase in plasma glucose at 21 h may be related to the time effects observed in NH_3-N, in which gluconeogenesis within the liver is induced by precursors resulting from ruminal fermentation and digestion of the diet and subsequently detected in plasma (Reynolds, 2005). Treatment × time interactions ($P < 0.001$) were observed for molar proportions of acetate (Table 3-4; Figure 3-3), propionate (Figure 3-4), and butyrate (Figure 3-5). Molar proportions of acetate were similar for all treatments ($P > 0.10$), however a decrease at 3 h post-feeding was observed in steers consuming DDGS, followed by a gradual increase until 12 h in which no differences were detected between DDGS, SBM, and BCM. Molar proportions of acetate increased in steers supplemented with CSM at 6 h differing from all treatments except for 18 and 24 h. Consequently, an increase in molar proportions of propionate and butyrate at 3 h post-feeding was observed for DDGS supplementation, compared with the remaining protein supplements. Molar proportions of propionate gradually declined in DDGS until 12 h, in which no further differences were detected compared with SBM and BCM. Molar proportions of propionate fluctuated throughout the 24 h observation with regards to CSM; nonetheless, molar proportions of propionate were decreased for CSM compared with other supplements. Despite the effect of supplementation over time, the increase in molar proportions of propionate did not affect the concentration of plasma glucose (i.e., there was not an effect of treatment).

As previously stated, endogenous secretions of glucose from the liver result from ruminal activity, with gluconeogenic precursors including propionate, amino acids, glycerol, and lactate (Dukes et al., 1993); therefore, the increase in plasma glucose may be related to one of the other precursors, however this was not evaluated. Molar proportions of butyrate were similar to propionate with an increase observed in DDGS at 3 h and a gradual decline through 15 h in which no further differences were detected in treatments. Molar proportions of branched-chain

volatile fatty acid (BCVFA) were greater ($P < 0.001$) in SBM compared with CSM and DDGS, and gradually decreased post-feeding ($P = 0.004$; Figure 3-6) for all treatments until 12 h, however a treatment × time interaction ($P = 0.30$) was not observed. Production of BCVFA results from fermentation of branched-chain amino acid (BCAA), which are either used for AA resynthesis, or as growth factors for other microbial species (Allison, 1978); however, BCVFA production is mediated through the availability of glucose, depending upon microbial species. Thus, an increase in concentration of BCVFA in steers supplemented with SBM and BCM may indicate a greater availability of BCAA within the rumen.

No treatment ($P = 0.39$), time ($P = 0.55$), or treatment × time interaction ($P = 0.15$) was observed for molar proportions of caproate. A treatment × time interaction ($P < 0.001$; Figure 3-7) was observed for molar proportions of valerate, with a peak at 3 h, and no further differences detected. Concentrations of total VFA was not affected by treatment ($P = 0.93$), nor was a treatment × time interaction ($P = 0.30$) observed; however, concentrations of total VFA differed from 9 h compared with 18 and 24 h ($P < 0.001$; Figure 3-8). Absorption rates of individual VFAs vary with concentrations of VFAs or changes in ruminal pH (Dijkstra, 1993), therefore the differences observed in concentration of total VFA may be related to the fluctuations observed in ruminal pH and subsequent absorption of VFA. Despite differences observed in time post-feeding, concentration of total VFA did not differ between treatments indicating similar fermentation, and as the fermentation rate of feed is positively associated with microbial efficiency (van Soest, 1994), BCM performed similarly to commonly provided protein supplements. A treatment × time interaction ($P < 0.001$; Figure 3-9) was observed for A:P, which further reflects the relationship of DDGS and CSM with regards to production of acetate and propionate.

Conclusion

Brassica carinata is not a new crop, however the residual meal remaining after oil extraction has not been extensively tested as a protein supplement for cattle. The effects of supplementing *B. carinata* meal on ruminal fermentation parameters and blood profile in Angus crossbred steers were similar when compared with commonly used protein supplements, thus indicating its viability as a protein supplement for beef cattle.

Table 3-1. Analyzed[1] chemical and nutrient composition (DM basis) of hay and protein supplements fed to ruminally-cannulated Angus crossbred steers.

		Treatment[2]			
Item	Bahiagrass hay[3]	BCM	CSM	DDGS	SBM
DM, %	94.0	89.8	88.9	86.3	90.7
CP, %	7.2	43.3	49.2	32.8	52.9
NFC[4], %	--[5]	21.7	13.2	20.2	28.7
NDF, %	71.4	23.5	28.6	30.7	10.2
ADF, %	41.8	12.8	18.7	14.3	8.4
TDN, %	56	80	67	83	79
S, %	0.35	1.75	--	--	--

[1]Dairy One Forage Testing Laboratory, Ithaca, NY.
[2]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.
[3]Bahiagrass hay (*Paspalum notatum*).
[4]NFC = non-fiber carbohydrates.
[5]-- Indicates this item was not analyzed.

Table 3-2. Effects of protein supplementation on nutrient intake and apparent total tract digestibility[1] of ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum with iNDF utilized as an internal marker.

	Treatment[2]					*P*-value[3]
Item	BCM	CSM	DDGS	SBM	SEM[4]	TRT
Intake, kg/d						
DM	6.83	6.87	6.23	7.19	0.771	0.49
OM	6.37	6.42	5.79	6.73	0.727	0.48
CP	0.37	0.37	0.33	0.37	0.053	0.47
NDF	5.08	5.06	4.53	5.27	0.555	0.48
ADF	2.54	2.51	2.28	2.63	0.246	0.46
Digestibility, %						
DM	51.52	51.11	50.90	51.37	1.968	0.99
OM	53.59	53.22	52.74	53.42	1.939	0.98
CP	67.10	69.03	63.64	65.53	4.934	0.39
NDF	50.03	48.69	49.57	47.45	2.464	0.65
ADF	51.24	50.92	52.67	49.24	3.403	0.45

[1]Hay and fecal samples were collected twice daily for 4 d; intake of bahiagrass hay was measured using the GrowSafe System Ltd., Airdrie, Alberta, Canada.
[2]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.
[3]Observed significance levels for treatment (TRT).
[4]Pooled standard error of treatment means, n = 8 steers/treatment.

Table 3-3. Effects of protein supplementation on ruminal fermentation parameters and blood profile of ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

	Treatment[1]					*P*-value[2]		
Item	BCM	CSM	DDGS	SBM	SEM[3]	TRT	TIME	TRT × TIME
Ruminal pH[4]	6.63	6.63	6.61	6.67	0.044	0.65	< 0.001	0.38
NH_3-N[4], m*M*	2.27	2.75	2.37	3.18	0.458	0.38	< 0.001	0.60
Glucose[4], m*M*	3.67	3.63	3.63	3.72	0.045	0.37	< 0.001	0.99
BUN[4], mg/dL	8.87[b]	9.21[b]	6.85[c]	10.86[a]	0.532	< 0.001	0.085	0.085

[a,b,c]Within a row, means with different superscripts differ, $P < 0.05$.
[1]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.
[2]Observed significance levels for treatment (TRT) and time post-feeding (TIME) effects, and for their interaction (TRT × TIME).
[3]Pooled standard error of treatment means, $n = 8$ steers/treatment.
[4]Ruminal fluid and blood samples were collected every 3 h for 24 h; NH_3-N = ruminal ammonia nitrogen, glucose = plasma glucose, BUN = plasma urea nitrogen.

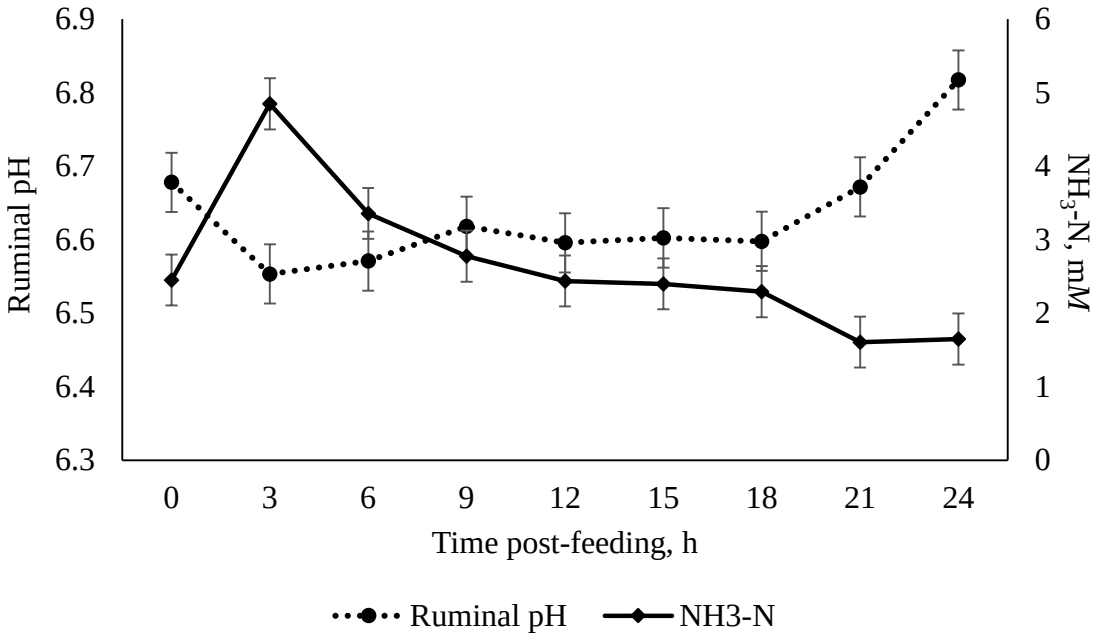

Figure 3-1. Effects of protein supplementation post-feeding in ruminal pH and concentrations of ruminal ammonia nitrogen ($P < 0.0001$) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

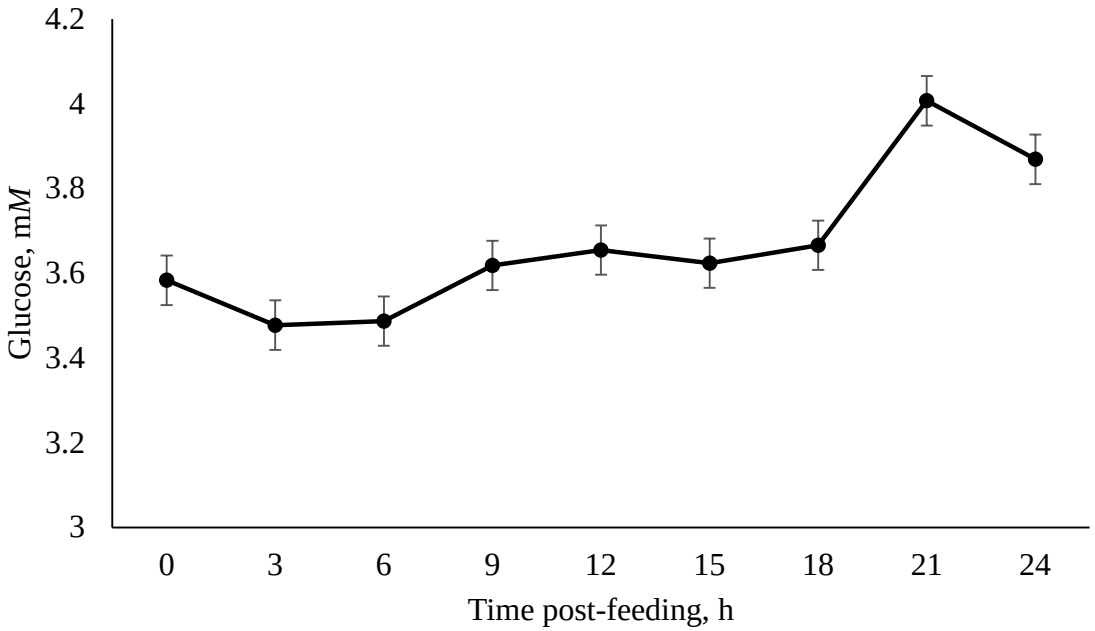

Figure 3-2. Effects of protein supplementation post-feeding in concentrations of plasma glucose ($P < 0.0001$) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

Table 3-4. Effects of protein supplementation on proportions of VFA (mol 100 mol^{-1}), total VFA concentrations (m*M*), and acetate to propionate ratio (A: P) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

	Treatment[1]					*P*-value[2]		
Item	BCM	CSM	DDGS	SBM	SEM[3]	TRT	TIME	TRT × TIME
Acetate	74.18	75.40	72.39	74.25	0.316	< 0.001	< 0.001	< 0.001
Propionate	15.75	15.03	16.23	15.37	0.205	< 0.001	< 0.001	< 0.001
Butyrate	7.95	7.57	9.47	8.01	0.144	< 0.001	< 0.001	< 0.001
BCVFA[4]	1.34[ab]	1.20[b]	1.14[b]	1.57[a]	0.063	< 0.001	0.004	0.30
Valerate	0.63	0.59	0.60	0.62	0.016	0.20	< 0.001	< 0.001
Caproate	0.13	0.18	0.15	0.16	0.016	0.40	0.55	0.15
Total VFA	98.31	99.90	98.72	94.89	5.854	0.93	0.03	0.025
A:P	4.71	5.03	4.49	4.84	0.084	< 0.001	< 0.001	< 0.001

[a,b] Within a row, means with different superscripts differ, $P < 0.05$.
[1]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.
[2]Observed significance levels for treatment (TRT) and time effects, and for their interaction (TRT × TIME).
[3]Pooled standard error of treatment means, $n = 8$ steers/treatment.
[4]BCVFA = Branched chain volatile fatty acids: isobutyrate + isovalerate + 2-methylbutyrate.

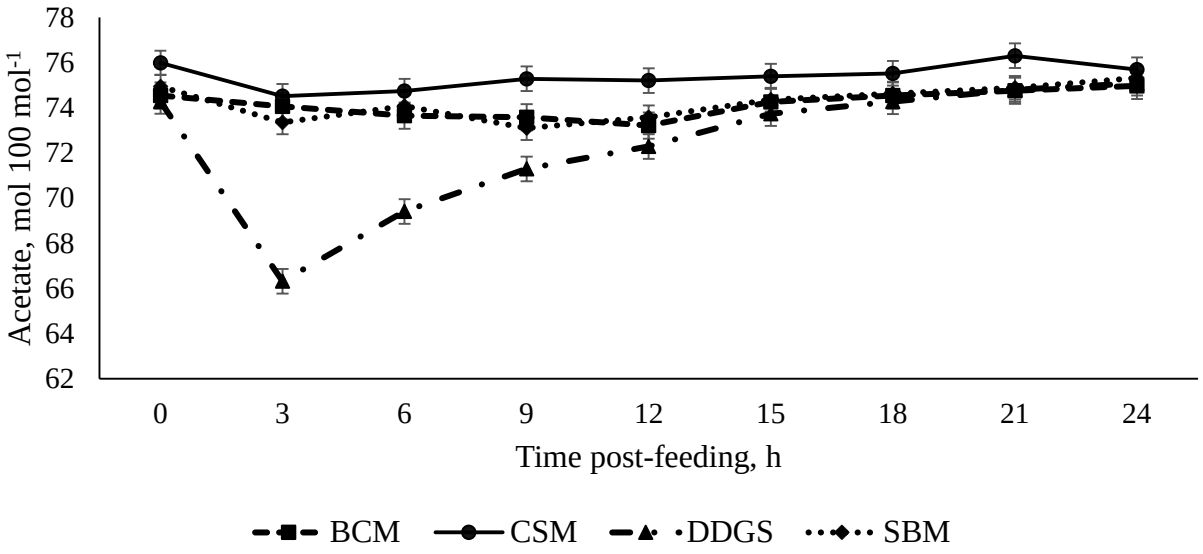

Figure 3-3. Effects of protein supplementation post-feeding on molar proportions of acetate (mol 100 mol^{-1}) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.0001$). BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

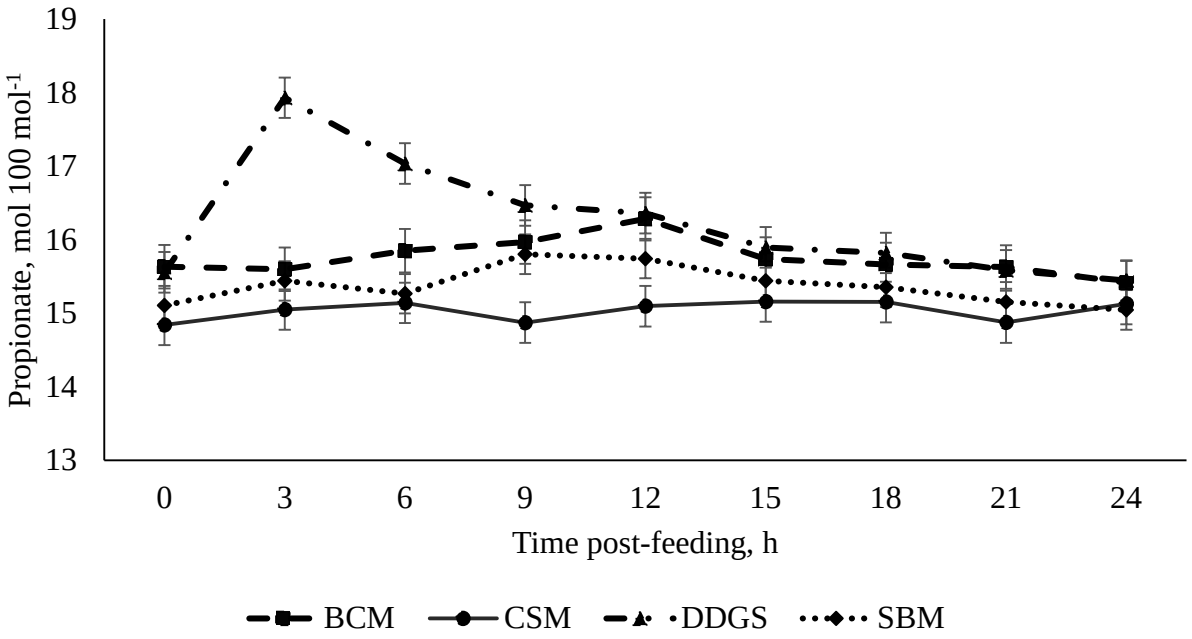

Figure 3-4. Effects of protein supplementation post-feeding on molar proportions of propionate (mol 100 mol^{-1}) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.0001$). BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

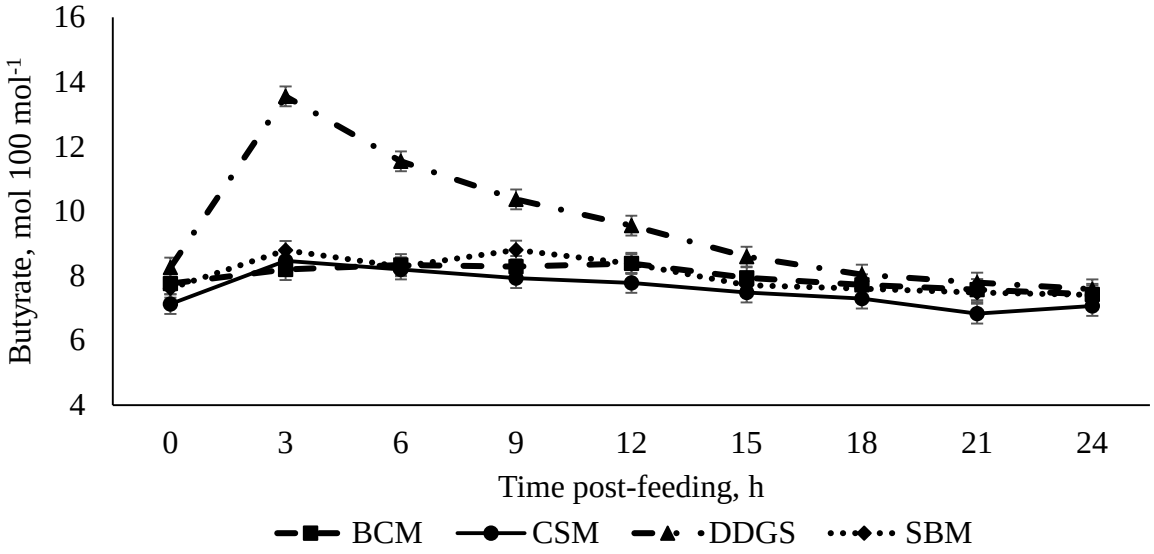

Figure 3-5. Effects of protein supplementation post-feeding on molar proportions of butyrate (mol 100 mol^{-1}) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.0001$). BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

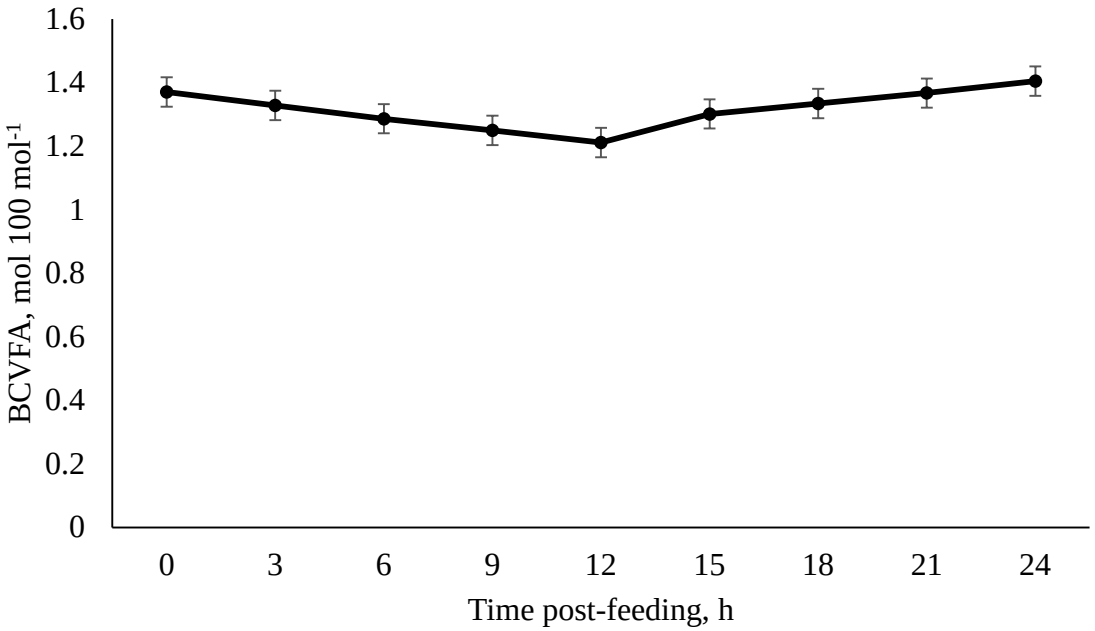

Figure 3-6. Effects of protein supplementation post-feeding on molar proportions of BCVFA (P = 0.004) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

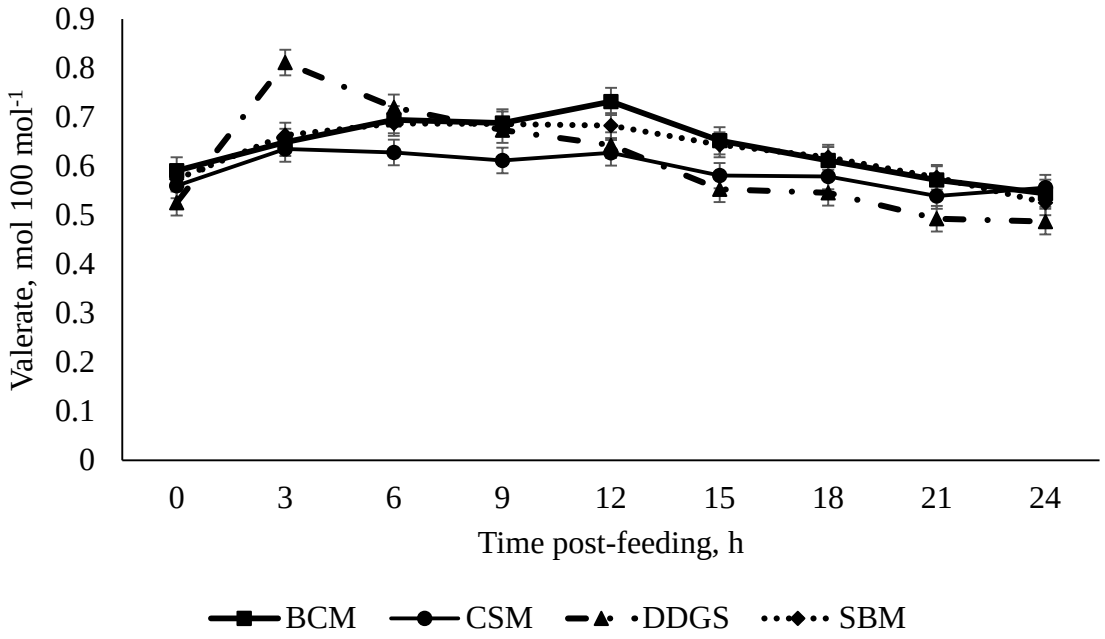

Figure 3-7. Effects of protein supplementation post-feeding on molar proportions of valerate (mol 100 mol^{-1}) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.0001$). BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

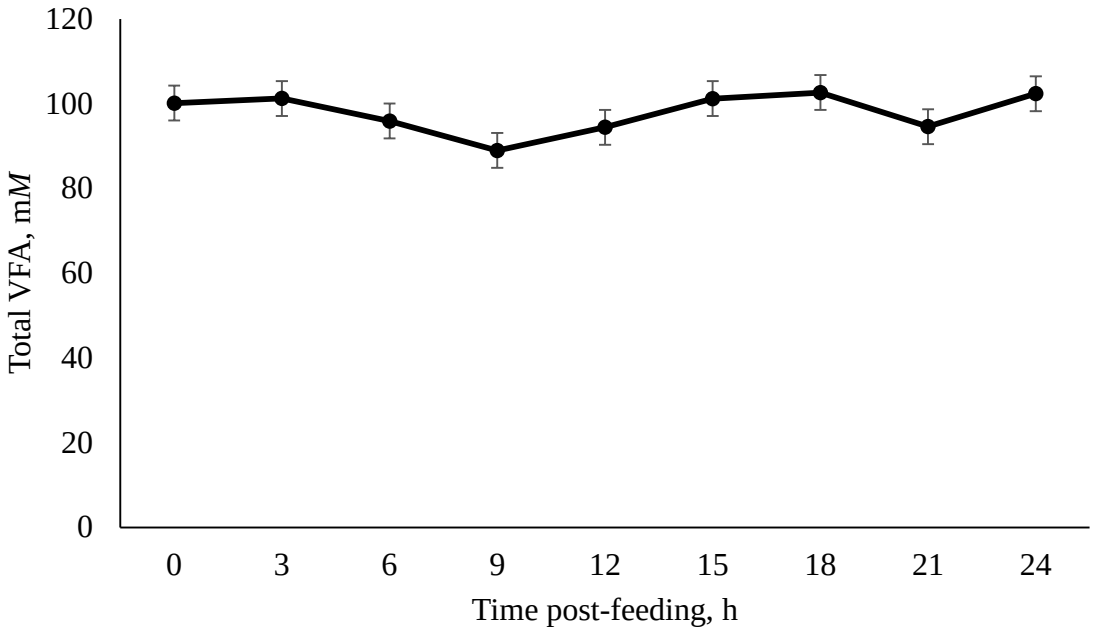

Figure 3-8. Effects of protein supplementation post-feeding on concentrations of total VFA (*P* = 0.025) in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

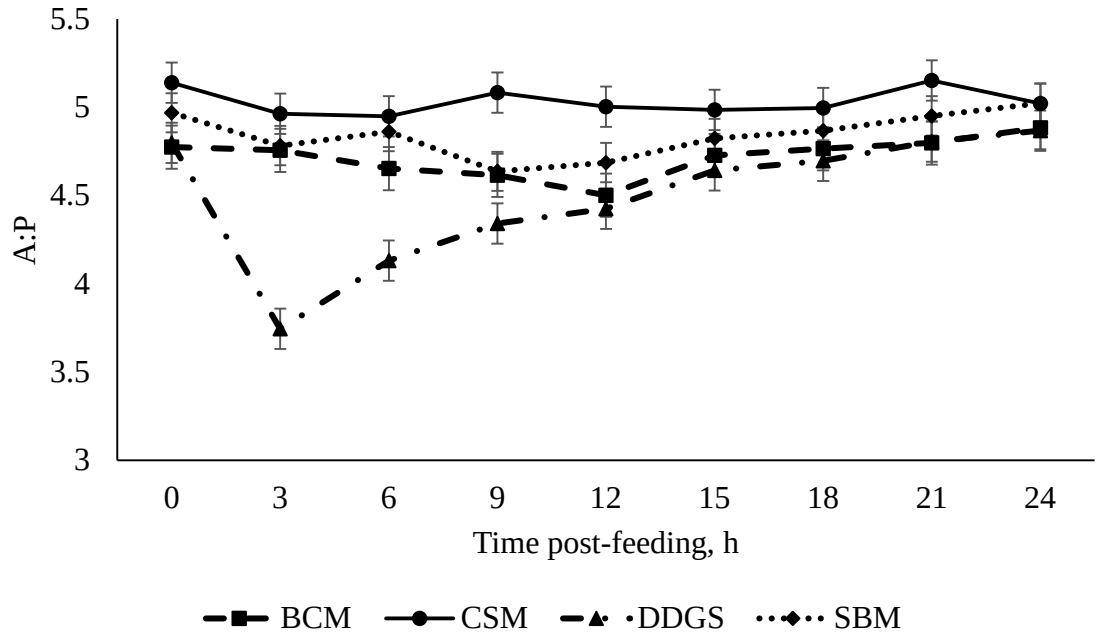

Figure 3-9. Effects of protein supplementation post-feeding on acetate to propionate ratio in ruminally-cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.0001$). BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

CHAPTER 4
CHARACTERIZATION OF THE DIETARY PROTEIN IN BRASSICA CARINATA

Introduction

Brassica carinata is a non-food oilseed crop with a favorable very long chain fatty acid composition for conversion to biofuel (Marillia et al., 2013). Oil extracted from the seed has been utilized as a 100% drop-in jet biofuel, promoting the use of *B. carinata* as a renewable and potentially sustainable resource (AAFC, 2015). In the southeastern U.S., *B. carinata* would be an ideal candidate for use in crop rotation and cover crop due to its heat and drought tolerance, and cold and disease resistance (AAFC, 2015; Seepaul et al., 2016). A high-protein meal (~40% CP) is obtained as a byproduct of oil extraction; however, this meal has not been extensively tested as a potential protein supplement for cattle. As the southeastern U.S. is typically comprised of cow-calf operations (McBride and Matthews, 2011), cattle of various ages, stages of production and size often graze medium to poor quality pastures with limited protein content, thus high quality protein supplementation is necessary. *Brassica carinata* meal has been evaluated as a high quality source of crude protein for dry Holstein cows (Xin and Yu, 2014), when compared with canola meal. However, to our knowledge, *B. carinata* has not been evaluated compared with commonly used protein supplements in the southeastern U.S., or in beef cattle. Thus, the objective of this experiment was to characterize ruminal protein fractionation, and subsequent post-ruminal degradation of protein in *B. carinata* compared with common protein supplements, and to determine the amino acid profile of *B. carinata* upon ruminal and post-ruminal degradation.

Materials and Methods

All procedures involving animals were approved by the Animal Care and Use Committee of the Institute of Food and Agricultural Sciences at the University of Florida, study # 201308011.

Experimental design and sample collection

The experiment was conducted at the University of Florida, Feed Efficiency Facility (FEF) in Marianna, FL, beginning in October, 2014. Eight ruminally cannulated Angus crossbred steers (473 ± 119 kg of initial BW) were used in a duplicated 4 × 4 Latin square design conducted over four consecutive 28-d periods. Steers were randomly allocated to 8 pens, and within each period steers were randomly assigned to one of four treatments: 1.39 kg d^{-1} *B. carinata* meal pellets (BCM), 1.62 kg d^{-1} cottonseed meal (CSM), 2.15 kg d^{-1} dry distillers grains plus solubles (DDGS), or 1.17 kg d^{-1} soybean meal (SBM), supplemented daily. Treatments were calculated to be isonitrogenous based on total N provided by supplementation of 1.39 kg d^{-1} of BCM. On d 0, steers were shrunk weighed and housed individually in pens at the FEF with ad libitum access to water and bahiagrass hay (*Paspalum notatum*). Each pen at the FEF was equipped with two GrowSafe feed bunks (GrowSafe System Ltd., Airdrie, Alberta, Canada) to record hay intake by weight change measured to the nearest gram. Steers were acclimated to the facility, hay, and supplements from d 0 to d 14, and a ruminal in situ degradability procedure was conducted from d 21 to d 25, in which bags were placed in the rumen of supplement-specific adapted steers for 0, 3, 6, 9, 12, 16, 24, 48, 72, and 96 h. Following ruminal incubation, the undegraded supplement after 16 h incubation was subjected to serial solutions simulating post-ruminal digestion (Calsamiglia and Stern, 1995; Gargallo et al. 2006), with subsequent analysis of concentration of CP and determination of the BCM AA profile.

Ruminal in situ DM disappearance of treatments was determined using duplicate bags within steer. Supplement samples were taken at the beginning of each period, dried for 48 h at

55°C, and weighed (5 g) into 10 × 20 cm Ankom in situ bags (R1020, Ankom Technology Corp., Macedon, NY), with 50 μm pore size and ratio of surface area to supplement equal to 12.5 mg/cm^2. In situ bags were heat-sealed, placed in mesh laundry bags fitted with a zipper, and suspended in the ventral sac of the rumen from a nylon rope and carabiner attached to a U-bolt on the stopper of the cannula after soaking in warm (39°C) water for 15 min. Bags were placed in the rumen altogether and incubated for 0, 3, 6, 9, 12, 16, 24, 48, 72, and 96 h, except for the 0 h bag, which was soaked in 39°C water for 15 min to determine the 'A' fraction of protein. Bags were removed at predetermined times, as previously mentioned, rinsed with cold running water to remove adherent particles and bacteria, and then rinsed with tap water 3 times and distilled water 5 times. Bags were dried for 48 h at 55°C and weighed. Residues from the in situ incubation were composited by incubation time within steer and composite samples were analyzed for determination of DM, OM, CP, NDF, and ADF. The 16 h bag was removed and analyzed separately to determine intestinally absorbable CP by the three-step procedure (Calsamiglia and Stern, 1995; Gargallo et al. 2006).

Laboratory analyses

Samples were weighed (0.5 g) in duplicate, dried in a forced-air oven at 100ºC overnight to calculate DM, and subsequently ashed in a muffle furnace at 650ºC for 6 h to determine OM. To determine NDF concentration, samples were weighed (0.5 g) in duplicate in F57 filter bags and analyzed in an Ankom 200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY) using sodium sulfate and heat-stable α-amylase. Samples were subsequently analyzed for concentrations of ADF as described by van Soest et al. (1991). Concentrations of CP in feed and feces was determined by rapid combustion using a macro elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) following official method 992.15 (AOAC, 1995).

Brassica carinata meal pellets and bahiagrass hay were analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY.).

Determination of intestinally absorbable CP was analyzed according to the three step procedure (Calsamiglia and Stern, 1995; Gargallo et al. 2006), with modifications. Briefly, the 16 h bag was removed from the rumen, washed until runoff was clear, and dried in a forced-air oven at 55ºC for 48 h. Contents of ruminal in situ residue bags were composited and analyzed for DM, OM, CP, NDF, ADF, and AA profile (University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO). Contents were then weighed into 5 × 10 cm nylon bags (Ankom R510, pore size 50 µm; Ankom, Fairport, NY; Boucher et al., 2009) in duplicate, heat sealed and suspended in a Daisy^{II} incubator (Ankom, Fairport, NY) in a 2 L solution of pre-warmed 0.1 *N* HCl solution (pH 1.8) containing 1 g L^{-1} of pepsin (P-3000, Sigma, St. Louis, MO) at 39ºC for 1 h, under constant rotation. Nylon bags were removed from the incubator, rinsed with tap water until runoff was clear, and then further incubated in a 2 L pre-warmed pancreatin solution (0.5 *M* KH_2PO_4 buffer, pH 7.7, containing 50 ppm of thymol and 3 g L^{-1} of pancreatin; Sigma P-7545) for 24 h at 39ºC, under constant rotation. After incubation, bags were removed from solution, washed with tap water until runoff was clear, and dried in a forced-air oven at 55ºC for 48 h. Contents from duplicate bags were composited, analyzed for DM and CP content (CP content was determined by rapid combustion using a macro elemental N analyzer; Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ), and sent for AA profile analysis (University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO).

Calculations and statistical analysis

Residues from in situ incubations were fitted to a first order kinetic model according to Ørskov and McDonald (1979) using the nonlinear procedure of SAS (SAS Inst. Inc., Cary, NC). The model used was:

$$R_{(t)} = \text{Undeg} + \text{D} \times \text{e}^{-K_d \times (\text{t}-T_0)}$$

Where $R_{(t)}$ = residue at each given incubation time (%); t = time incubated in the rumen (h); Undeg = undegradable fraction (%); D = potentially degradable fraction (%); e = 2.71828; K_d= degradation rate of D (% h^{-1}); and T_0 = lag time (h).

Effective rumen degradability (E) of DM, OM, NDF, and ADF was calculated according to the model:

$$E_x = SF + [D \times \left(\frac{K_d}{K_d + K_p}\right)]$$

Where, x = nutrient evaluated; SF = Soluble fraction, which is the proportion of material that washed out from the bags without rumen incubation (0 h); and K_p = fractional rate of passage, in this study assumed to be 5 % h^{-1} (Foster et al., 2011).

Effective rumen CP degradability representing RDP was determined by the equation (Mjoun et al., 2010):

$$RDP = A + [B \times \left(\frac{K_d}{K_d + K_p}\right)]$$

Where, A = rapidly degradable CP that disappeared at 0 h after the rinsing procedure; B = potentially degradable CP; K_d and K_p are degradation constants described previously. Estimated RUP of feeds was calculated as 100 - % RDP. Intestinally absorbable digestible protein (IADP) was determined as RUP × intestinally digestible protein (IDP). Total tract digestibility of CP was calculated as the sum of RDP and IADP. Contribution of RUP to intestinally absorbable AA (g/kg of CP) was calculated for each AA as (100 - % rumen degradability at 16 h) × % intestinal disappearance in situ × AA concentration in feed /10 (Mjoun et al., 2010).

Pepsin-pancreatin digestion (PPD) of protein was calculated using the model of Gargallo et al. (2006):

$$PPD = [\frac{\left(IS\ (N) - P{:}P\ (N)\right)}{S\ (N)}]$$

Where: 'IS (N)' = N content of the rumen-exposed residue; 'P:P (N)' = N content of the pepsin-pancreatin residue; and 'S (N)' = N content of the sample.

In situ digestibility data were analyzed as a duplicated 4 × 4 Latin square using the MIXED procedure of SAS (SAS Inst. Inc.). The model included the fixed effects of treatment, square, period within square, and animal within square.

Three step procedure data were analyzed using PROC MIXED of SAS (SAS Inst. Inc.). The model for protein characterization included fixed effects of treatment, and random effects of square, period, and steer within square. Differences between treatment means were identified by Tukey's least squares means comparison, significance was declared at $P \leq 0.05$ and tendencies considered when $0.05 < P \leq 0.10$.

Results and Discussion

The chemical and nutrient composition of the hay and protein supplements provided to steers is available in Table 4-1. Concentrations of nutrients for CSM, SBM, and DDGS were comparable with published values, with exception to a slightly less DM in DDGS (NRC, 2016). Fractionation of ruminal protein differed ($P < 0.01$; Table 4-2) between treatments, with CSM and DDGS having nearly equivalent amounts of RDP (approximately 51% of CP), which differed from SBM and BCM having approximately 72% RDP as a percentage of CP. The RDP for CSM was lesser than published values reported in the NRC (2016), while the RDP for DDGS was greater than values reported in the NRC (2016). Protein fractionation for SBM was similar to published values for RDP and RUP (NRC, 2016). Compared to DDGS, SBM had the greatest IDP ($P < 0.01$), with CSM having the greatest IADP ($P < 0.01$), and similar for BCM and SBM.

Total tract digestibility (TTDP; $P < 0.01$) of CP was greatest for SBM compared with CSM and DDGS.

Metabolizable protein (MP) is defined as the true protein digested in the intestine, supplied by microbial protein and RUP (NRC, 2016). Though MP is the common nomenclature, TTDP has also been utilized in various studies; nonetheless, the concept is the same. The NRC (1996, 2001) assumed an 80 % digestibility of RUP as a result of insufficient information regarding digestibility; however, to accurately predict MP valid estimates are necessary (NRC, 2016). Consequently, intestinal digestibility values for RUP (IDP) or MP are not available in the newest edition of the NRC (2016). Erasmus et al. (1994) observed an approximately 98% intestinal digestibility of RUP when SBM was fed to lactating dairy cows. This value is similar to the IDP of 94.53% observed in the current study for SBM, but further illustrates the variability in digestibility of substrates.

Retention time of ruminal protein will affect estimates of RDP and RUP, i.e., a shorter retention time will result in an estimation of greater values for RUP and subsequent overestimates of MP (NRC, 2016). Estimates of RDP and RUP observed in the current study resulted from ruminal incubation for 16 h, considered to be the mean residence time of CP in the rumen (Calsamiglia and Stern, 1995). Ruminal in situ degradation kinetics are presented in Table 4-3. The ruminal degradation rates of DM, OM, and CP were greatest ($P < 0.01$) for SBM. The potentially degradable fraction of DM was greater ($P < 0.01$) for SBM and CSM compared with DDGS, despite a greater ($P < 0.01$) soluble fraction of DM for DDGS compared with CSM. A delay ($P < 0.01$) in lag time of DM was observed in CSM and SBM, compared with BCM. More time was required ($P < 0.01$) by CSM and SBM to degrade OM compared with DDGS, despite a greater ($P = 0.02$) undegradable fraction of OM in DDGS compared with SBM, and a tendency

($P = 0.06$) for the potentially degradable fraction of OM to be increased in SBM. While BCM was similar to other treatments in both lag time and the potentially degradable fraction of OM, BCM tended ($P = 0.07$) to have a greater soluble fraction. Crude protein in BCM and SBM required less lag time ($P < 0.01$) than CSM, and BCM had the greatest ($P < 0.01$) soluble fraction; however, the potentially degradable fraction of CSM was greater ($P < 0.01$) compared with BCM and DDGS. The undegradable fraction of DM ($P = 0.20$) and CP ($P = 0.24$) were not different between treatments. Soybean meal is a more rapidly fermentable substrate in the rumen, as indicated by the increased degradation rate, despite greater lag times in DM and OM. As a protein supplement, SBM is often recommended as a source of RDP, with CSM and DDGS utilized as a source of RUP (Lee et al., 2016; NRC, 2016), supporting the data observed in the current study. Similar in proportions of RDP and RUP, BCM has a decreased rate of degradation compared to SBM, but a greater soluble fraction contributing to an increase in ruminally degradable protein.

Determining the protein fractionation of supplements is important in formulating rations for cattle, however the availability of amino acids (AA) post-ruminally is of greater interest as these will be available as a portion of the MP (Merchen and Titgemeyer, 1992). The AA composition of BCM in the original feed sample, 16 h rumen sample, and post-rumen residue is presented in Table 4-4. The total tract digestibility of individual AA, ruminally and post-ruminally, is presented in Table 4-5, with the contribution of RUP to intestinally absorbable AA (IAAA). Previous research on the fractionation and characterization of protein in *B. carinata* (Xin and Yu, 2014) resulted in values of RUP and TTDP (123 and 358 g/kg DM, respectively). Upon initial evaluation, these values may seem lesser than the current values, but the original concentration of CP in the diet used by Xin and Yu (2014) was not presented, dry Holstein

ruminally-cannulated cows were used, and a total mixed ration (forage:concentrate = 78:22) was fed. The differences in experimental designs may be the main reason for the discrepancies in the values presented by Xin and Yu (2014) and those observed in this study.

Mjoun et al. (2010) compared fractionation of protein and subsequent AA profiles in distiller's grains products in common soybean meal products, utilizing the in situ technique and the modified three-step procedure described by Gargallo et al. (2006). The RDP and RUP values for SBM (67.7 and 32.3 % of CP) and DDGS (47.7 and 52.3 % of CP) in lactating Holstein cows reported by Mjoun et al. (2010) were similar to the estimates obtained in the current study. Furthermore, the protein digestibility parameters (IDP, % of RUP; IADP and TTDP, % of CP) of SBM and DDGS were similar, confirming the values observed in the current study (Mjoun et al., 2010).

The total tract digestibility of essential AA of *B. carinata* meal pellets and contribution of RUP to IAAA is presented in Table 4-6. As rumen microbes are able to synthesize all of the essential AAs (D'Mello, 2003), ruminants have no theoretical requirement for dietary pre-formed protein or AA (Bach et al., 2005). Production of MCP alone, resulting from RDP, may be insufficient in supplying adequate amounts of AA for optimal production (Kung Jr. and Rode, 1996), especially during periods of rapid growth in cattle and high rates of production (Klopfenstein et al., 1978). Thus, limiting AAs, such as methionine and lysine, are of more concern and should therefore be supplied as RUP in order to meet the dietary requirements of ruminants. Depending upon the diet fed, the post-ruminal AA supply will be altered (i.e., in corn-based diets, lysine may be the limiting AA) differing from methionine as the limiting AA with barley-fed diets (Fenderson and Bergen, 1975; Burris et al., 1976; Merchen and Titgemeyer, 1992). Therefore, defining the total tract composition, digestibility, and availability of AA in *B.*

carinata is important in order to synchronize the supplementation of energy and protein when using a variety of feedstuffs.

Conclusion

Brassica carinata is not a new crop, however the residual meal remaining after oil extraction has not been extensively tested as a protein supplement for cattle. Furthermore, *Brassica carinata* meal has not been previously evaluated with regards to fractionation of protein, AA composition, or digestibility and subsequent absorption of AA, which have been described in this study. The evaluation of *Brassica carinata* meal as protein supplemented for cattle consuming a forage-based diet, resulted in a protein fraction comprised of 71.8% RDP, and a total tract digestibility of dietary protein of 97%, thus indicating its viability as a high-value protein supplement for beef cattle.

Table 4-1. Analyzed[1] chemical and nutrient composition (DM basis) of hay and protein supplements fed to ruminally-cannulated Angus crossbred steers.

		Treatment[2]			
Item	Bahiagrass hay[3]	BCM	CSM	DDGS	SBM
DM, %	94.0	89.8	88.9	86.3	90.7
CP, %	7.2	43.3	49.2	32.8	52.9
NFC[4], %	--[5]	21.7	13.2	20.2	28.7
NDF, %	71.4	23.5	28.6	30.7	10.2
ADF, %	41.8	12.8	18.7	14.3	8.4
TDN, %	56	80	67	83	79
S, %	0.35	1.75	--	--	--

[1]Dairy One Forage Testing Laboratory, Ithaca, NY.
[2]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.
[3]Bahiagrass hay (*Paspalum notatum*).
[4]NFC = non-fiber carbohydrates.
[5]-- Indicates this item was not analyzed.

Table 4-2. Characterization of protein supplements fed to ruminally-cannulated Angus crossbred steers[1] fed bahiagrass hay ad libitum.

	Treatment[2]					*P*-value[3]
Item[4]	BCM	CSM	DDGS	SBM	SEM[5]	TRT
RDP, % CP	71.79[a]	47.80[b]	55.05[b]	72.30[a]	3.298	< 0.001
RUP, % CP	28.20[b]	52.19[a]	44.94[a]	27.69[b]	3.298	< 0.001
IDP, % RUP	89.94[ab]	89.91[ab]	85.38[b]	94.53[a]	2.194	0.007
IADP, % CP	25.24[c]	46.99[a]	36.46[b]	26.37[c]	2.847	< 0.001
TTDP, % CP	97.06[ab]	94.80[bc]	93.74[bc]	98.66[a]	0.958	< 0.001

[a,b,c]Within a row, means with different superscripts differ, $P < 0.05$.

[1]Steers from Exp. 1.

[2]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}).

[3]Observed significance levels for treatment (TRT).

[4]K_d = rate of degradation of fraction D; IDP = estimated intestinal protein digestibility (Gargallo et al., 2006); RDP = A + B [K_d /(K_d + K_p)], where K_p is the rate of passage from the rumen, estimated to be 5% h^{-1}; RUP = 100 - % RDP; IADP = intestinally absorbable dietary protein (IDP × RUP); TTDP = total tract digestibility of dietary protein (TTDP = RDP + IADP); also MP.

[5]Pooled standard error of treatment means, n = 8 steers/treatment.

Table 4-3. In situ digestion kinetics on DM, OM, and CP of protein supplements fed to ruminally-cannulated Angus crossbred steers[1] fed bahiagrass hay ad libitum.

	Treatment[2]					*P*-value[3]
Item[4]	BCM	CSM	DDGS	SBM	SEM[5]	TRT
DM						
K_d, % h^{-1}	6.61[b]	2.85[c]	5.16[bc]	10.87[a]	0.929	< 0.001
T0, h	0.53[b]	2.87[a]	1.20[ab]	2.52[a]	0.530	0.0094
SF, %	42.97[b]	32.25[c]	48.81[a]	40.70[b]	1.580	< 0.001
D, %	54.74[ab]	59.78[a]	45.83[b]	59.11[a]	3.223	0.0086
Undeg, %	2.38	7.98	5.36	0.12	3.063	0.2049
OM						
K_d, % h^{-1}	6.71[b]	2.54[c]	5.31[bc]	11.27[a]	0.888	< 0.001
T0, h	0.99[ab]	2.64[a]	0.78[b]	2.68[a]	0.613	0.0096
SF, %	7.49	5.28	5.96	2.33	1.608	0.0674
D, %	92.48	94.68	93.95	97.65	1.604	0.0636
Undeg, %	0.03[ab]	0.04[ab]	0.09[a]	0.01[b]	0.017	0.0287
CP						
K_d, % h^{-1}	7.59[b]	3.86[c]	4.68[bc]	11.50[a]	0.877	< 0.001
T0, h	0.87[b]	8.89[a]	3.44[ab]	2.80[b]	1.779	0.0066
SF, %	22.10[a]	0.24[d]	15.66[b]	7.78[c]	1.929	< 0.001
D, %	76.80[bc]	98.70[a]	74.67[c]	88.60[ab]	3.771	< 0.001
Undeg, %	0.69	1.06	9.67	3.94	3.876	0.2409

[a,b,c,d]Within a row, means with different superscripts differ, $P < 0.05$.

[1]Steers from Exp. 1.

[2]BCM: *Brassica carinata* meal pellets (1.39 kg d^{-1}); CSM: cottonseed meal (1.62 kg d^{-1}); DDGS: dry distillers grain plus solubles (2.15 kg d^{-1}); SBM: soybean meal (1.17 kg d^{-1}); composited over 4 periods.

[3]Observed significance levels for treatment (TRT).

[4]K_d = rate of degradation of fraction D, T0 = Lag time, SF = soluble fraction, D = potentially degradable fraction, and Undeg = undegradable fraction.

[5]Pooled standard error of treatment means, $n = 8$ steers/treatment.

Table 4-4. Amino acid composition of *Brassica carinata* meal pellets in original meal, ruminally incubated residue, and post-ruminal residue.

AA	AA composition[1] (w/w %) BCM[2]	In situ 16 h residue[3]	Post-rumen residue[4]
Taurine	0.10	0.15	0.13
Hydroxyproline	0.24	0.93	0.53
Aspartic Acid	2.39	0.78	2.95
Threonine	1.43	0.58	1.83
Serine	1.28	0.51	1.59
Glutamic Acid	6.68	1.08	6.36
Proline	2.24	0.83	2.18
Glycine	1.80	0.57	1.98
Alanine	1.55	0.45	1.92
Cysteine	0.97	0.35	0.85
Valine	1.83	0.72	2.42
Methionine	0.70	0.15	0.80
Isoleucine	1.52	0.60	1.94
Leucine	2.58	0.72	3.10
Tyrosine	0.91	0.38	1.27
Phenylalanine	1.49	0.49	1.90
Hydroxylysine	0.05	0.03	0.03
Ornithine	0.02	0.01	0.04
Lysine	1.61	0.57	1.88
Histidine	0.98	0.19	0.95
Arginine	2.51	0.44	2.44

[1]AA profiles analyzed by University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO.
[2]Original *Brassica carinata* meal pellets as supplied by Agrisoma Biosciences, Inc., Gatineau, Quebec.
[3]Rumen disappearance (%) at 16 h of incubation using in situ technique.
[4]Post-rumen disappearance (%) using modified three step procedure.

Table 4-5. Total tract digestibility of amino acids from *Brassica carinata* meal pellets and contribution of RUP to intestinally absorbable amino acids.

Total AA composition	AA digestibility[1]		
	In situ 16 h residue[2] (%)	Post-rumen residue[3] (%)	Contribution of RUP to IAAA[4] (g/kg CP)
Taurine	78.80	68.87	0.39
Hydroxyproline	67.99	50.75	1.07
Aspartic Acid	79.92	92.69	11.29
Threonine	79.26	91.22	6.89
Serine	79.99	91.21	5.95
Glutamic Acid	84.55	95.33	24.82
Proline	84.29	89.49	8.04
Glycine	82.12	92.19	7.54
Alanine	79.85	93.64	7.40
Cysteine	86.09	88.77	3.06
Valine	78.60	91.87	9.14
Methionine	81.56	94.84	3.09
Isoleucine	79.28	91.50	7.33
Leucine	80.52	93.73	11.91
Tyrosine	77.18	91.75	4.85
Phenylalanine	79.36	93.01	7.25
Hydroxylysine	91.80	60.04	0.07
Ornithine	69.54	89.35	0.14
Lysine	80.92	91.55	7.16
Histidine	84.28	94.64	3.68
Arginine	84.19	95.15	9.53
Total			140.61

[1]Amino acid digestibility was calculated as [((initial sample DM × original BCM AA profile) - (16 h residue × sample DM remaining))/(initial sample DM × original BCM AA profile)].
[2]Rumen disappearance (%) at 16 h of incubation using in situ technique.
[3]Post-rumen disappearance (%) using modified three step procedure.
[4]IAAA = Intestinally absorbable amino acids; Contribution of RUP to IAAA is defined as (100 - % rumen degradability at 16 h) × (% intestinal disappearance in situ) × AA concentrations in feed/10.

Table 4-6. Total tract digestibility of essential amino acids of *Brassica carinata* meal pellets and contribution of RUP to intestinally absorbable essential amino acids.

Essential AA composition	AA digestibility[1]		
	In situ 16 h residue[2] (%)	Post-rumen residue[3] (%)	Contribution of RUP to IAAA[4] (g/kg CP)
Arginine	84.19	95.15	9.53
Histidine	84.28	94.64	3.68
Isoleucine	79.28	91.50	7.33
Leucine	80.52	93.73	11.91
Lysine	80.92	91.55	7.16
Methionine	81.56	94.84	3.09
Phenylalanine	79.36	93.01	7.25
Threonine	79.26	91.22	6.89
Valine	78.60	91.87	9.14
Total			65.98

[1]Amino acid digestibility was calculated as [((initial sample DM × original BCM AA profile) - (16 h residue × sample DM remaining))/(initial sample DM × original BCM AA profile)].
[2]Rumen disappearance (%) at 16 h of incubation using in situ technique.
[3]Post-rumen disappearance (%) using modified three step procedure.
[4]IAAA = Intestinally absorbable digestibility of amino acids; Contribution of RUP to IAAA is defined as (100 - % rumen degradability at 16 h) × (% intestinal disappearance in situ) × AA concentrations in feed/10.

CHAPTER 5
EVALUATION OF BRASSICA CARINATA MEAL AS A PROTEIN SUPPLEMENT FOR GROWING BEEF HEIFERS

Introduction

Brassica carinata is a non-food oilseed crop with a favorable very long chain fatty acid composition for conversion to biofuel (Marillia et al., 2013). Oil extracted from the seed has been utilized as a 100% drop-in jet biofuel, promoting the use of *B. carinata* as a renewable and potentially sustainable resource (AAFC, 2015). In the southeastern U.S., *B. carinata* would be an ideal candidate for use in crop rotation and as a cover crop due to its heat and drought tolerance, and cold and disease resistance (AAFC, 2015; Seepaul et al., 2016). A high-protein meal (~40% CP) is obtained as a byproduct of oil extraction; however, this meal has not been extensively tested as a potential protein supplement for cattle. Analysis of the meal yields low concentrations of sinigrin and progoitrin, byproducts of ruminal degradation of glucosinolates (EFSA, 2008), which have been implicated in decreased intake, interference of thyroid hormone metabolism, and impaired fertility and reproductive performance in cattle. Cattle in the southeastern U.S. often graze pastures of limited nutritive value which are not adequate to support high levels of production, especially during critical periods, necessitating supplementation of protein (Hersom et al., 2011). Common protein supplements in this region result from byproducts of various industries and in conjunction with the poor quality hay available in winter, provide an opportunity to meet the nutritional requirements of growing heifers (Schulmeister et al., 2015). *Brassica carinata* meal has been evaluated as a high quality source of crude protein for ruminants utilizing an in situ procedure (Xin and Yu, 2014), however research in feeding *B. carinata* to cattle is limited. Thus, the objective of this study was to determine the effects of supplementation with *B. carinata* meal on performance, attainment of puberty, and blood profile in growing Angus crossbred heifers consuming bermudagrass hay.

Materials and Methods

All procedures involving animals were approved by the Animal Care and Use Committee of the Institute of Food and Agricultural Sciences at the University of Florida, study # 201308011.

Experimental design and sample collection

The experiment was conducted at the North Florida Research and Education Center in Marianna, FL. Sixty-four Angus crossbred heifers (240 ± 39 kg initial BW) were used in a generalized randomized block design. Heifers were stratified and blocked (2 blocks) by initial BW and randomly allocated to 18 pens over 2 consecutive years (10 pens in yr 1 and 8 pens in yr 2). Within block, pens were randomly assigned to one of two treatments: 0% BCM pellets (CTL) or 0.3% of BW d^{-1} (as fed) of BCM pellets (BCM). Heifers were provided ad libitum access to bermudagrass hay (*Cynodon dactylon*) and water, and BCM pellets were supplemented daily in the pen. Initial BW was considered as the average of d -1 and 0 BW. Blood samples were collected on d 0, before feeding, for baseline analysis of initial concentrations of thyroid hormones, progesterone, and acute phase proteins in plasma. Body weight and blood samples were then collected every 7 d for the 70 d period, before the daily BCM supplementation.

Blood was collected from jugular venipuncture every 7 d in the morning, before BCM supplementation, in 10-mL evacuated tubes containing sodium heparin, placed on ice following collection, and subsequently centrifuged for 15 min at 4,000 × *g* at 4°C. After centrifugation, plasma was transferred into polypropylene vials (12 mm × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C for further analysis of concentrations of progesterone, triiodothyronine (T_3), thyroxine (T_4), haptoglobin (Hp), and ceruloplasmin (Cp).

Laboratory analyses

Hay samples were collected every 7 d, composited by pen within period and analyzed for DM, OM, CP, NDF, and ADF. Samples were weighed (0.5 g) into tared beakers, placed in an

oven at 100ºC overnight to calculate DM, and subsequently placed in a muffle furnace at 650ºC for 6 h to calculate OM. To determine concentrations of NDF, samples were weighed (0.5 g) into F57 filter bags and analyzed in an Ankom 200 Fiber Analyzer (Ankom Technology) using sodium sulfate and heat-stable α-amylase. Samples were subsequently analyzed for concentrations of ADF. Concentrations of N in feed and feces was determined by rapid combustion using a macro-elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) following official method 992.15 (AOAC, 1995), with CP calculated as concentrations of N multiplied by 6.25. *Brassica carinata* meal pellets were analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY.).

Concentrations of progesterone were determined by an immunoassay (Immulite 1000, Siemens Health, Inc., Malvern, PA) according to manufacturer's instructions. Females were considered to have attained puberty after the first increase in concentrations of progesterone in plasma samples exceeding 1.0 ng mL^{-1}. Briefly, 200 uL of plasma was placed in a sample cup, loaded onto a conveyor belt with a kit-specific (kit # LKPW1) test unit following, with samples and reagents then pipetted into the sample cup. After incubating for 30 min in a temperature controlled carousel, the unbound portion of sample and reagent was washed away, chemiluminescent substrate added and the signal read by a photomultiplier tube, in which the signal generated was proportional to the bound enzyme, which was then converted to concentration. Concentrations of T_3 and T_4 were analyzed similarly, using a solid-phase, competitive chemiluminescent enzyme immunoassay (kit # LKT31 and LKT41, for T_3 and T_4, respectively).

Plasma concentrations of Hp were determined using a biochemical assay measuring haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity

(Makimura and Suzuki, 1982). Results were obtained as arbitrary units resulting from the absorption reading at 450 nm. Quality control standards were analyzed by quantitative determination of bovine Hp in plasma (bovine haptoglobin ELISA test kit; Life Diagnostics, Inc., West Chester, PA). The ELISA standard curve was used to convert the arbitrary units obtained from the biochemical procedures into mg mL^{-1} (Cooke and Arthington, 2013), with the lowest detectable value of 0.03 mg mL^{-1}. Inter- and intra-assay CV of Hp assays using the biochemical procedure were 3.65 and 3.02%, respectively.

Plasma Cp oxidase activity was measured using the colorimetric procedures described by Demetriou et al. (1974) and expressed as mg dL^{-1} as described by King (1965). Inter- and intra-assay CV for Cp assays were 2.34 and 2.46%, respectively.

Statistical analysis

Data were analyzed as a generalized randomized block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included fixed effects of treatment, week, treatment × week interactions, block, and block × treatment interactions, with the random effect of year. Repeated measures, with pen within year as subject, were used to analyze T_3, T_4, Cp, and Hp concentrations over time. A survival analysis was conducted using the LIFETEST procedure of SAS, to determine time to attainment of puberty. Differences between treatment means were identified by Tukey's least squares means comparison and significance was declared at $P \leq 0.05$ and tendencies considered when $0.05 < P \leq 0.10$.

Results and Discussion

The nutritional composition of bermudagrass hay and BCM pellets fed to heifers is presented in Table 5-1. Bermudagrass hay used in this study had a CP concentration of 13.3%, and depending on expected growth and performance of yearling heifers, it should be sufficient to meet their nutritional demands. However, the concentration of TDN in bermudagrass hay

consumed in the current study was 55%, and could be considered limiting for achieving sufficient weight gains in developing heifers. Heifers supplemented with BCM at 0.3% of their BW for 70 d, had increased ADG ($P < 0.001$) when compared to CTL (0.14 vs. 0.42 kg d^{-1}; Table 5-2). An increase in performance is expected when supplementing protein in hay-based diets, due to an increase in the ruminal supply of substrate for microbial growth and fermentative activity, thus increasing MCP flow to the small intestine.

Differences in initial BW (Table 5-2) were not observed for treatment or treatment × block interactions ($P > 0.10$); however, an effect of block was observed ($P < 0.001$) for both initial and final BW. This was expected because of the initial stratification and blocking of heifers based on BW. A tendency for a treatment effect was observed on final BW ($P = 0.088$). Heifers in the second year of study weighed less at the initiation of the trial than those in the first year, which we speculate may account for the tendency of treatments to affect final BW, however this may be confounded by effect of block.

Brassica carinata belongs to the mustard family, *Brassicaceae*, which contain high concentrations of glucosinolates. Lardy and Kerley (1994) suggested 90 to 140 μmol g^{-1} as high concentrations of glucosinolates in growing crossbred beef steers, however the meal in the current study had 28 μmol g^{-1}; nevertheless, it was imperative to evaluate their potential effects on growth performance, as upon digestion, bacterial myrosinases will degrade the stable, intact compound (Duncan and Milne, 1992). Sinigrin and progoitrin are glucosinolates relevant to carinata, and upon ruminal degradation, unstable compounds are produced, resulting in formation of isothiocyanate and thiocyanate (EFSA, 2008). Thiocyanate and isothiocyanate are problematic with regards to fertility/reproductive impairment, thyroid metabolism, growth retardation, and inhibition of copper (EFSA, 2008). In the current study, the interval to

attainment of puberty ($P = 0.67$), was not affected by supplementation of BCM, compared with CTL heifers. An effect of block ($P < 0.001$) was observed, indicating that light BW block heifers attained puberty earlier than those in the heavy BW. While this was unexpected, similarly to the effects of treatment on final BW, we speculate that this may be due to observed differences in weight of heifers between the first and second year of study.

No effect of treatment or block ($P > 0.05$) on concentrations of T_3 or T_4 was observed, however, an effect of day ($P < 0.001$) demonstrated an increase in T_3 and T_4 (Figure 5-3), on d 7 which may be attributed to environmental factors such as cooler temperatures (Guyton, 1986; Figure 5-4). Lardy and Kerley (1994) observed a significant decrease in concentrations of T_4 with increasing inclusion concentrations of glucosinolates. This was not observed in the current study, however there was a tendency ($P = 0.087$) for heavy heifers to have an increase in plasma T_4 and a subsequent tendency ($P = 0.077$) for heavy CTL heifers to have an increased concentrations of plasma T_4 compared with light CTL heifers. Concentration of plasma T_3 observed for light lactating and non-lactating cows (approx. 488 kg BW) was similar to heifers in the current study (128.9 to 109.2 ng mL^{-1} vs. 122.7 to 128.7 ng mL^{-1}, respectively), however, Previous research indicates that as BW increases, plasma T_4 and T_3 decrease, which was observed in a study between light and heavy cows (488 kg and 573 kg BW, respectively; Walker et al., 2015). A positive correlation between growth rate of calves and concentrations of plasma T_3, has previously been reported, which may explain similar concentrations of plasma T_3 between lighter cows and heifers in the present study, however thyroid hormones fluctuate depending on age, size, environment, and a host of other factors (Tripathi et al., 2001).

Thiocyanate has the potential to bind iodine, preventing trapping and uptake of iodine by the thyroid gland (Barrett et al., 1997), however it is possible to alleviate the resulting deficiency

by supplementing additional iodine. Plasma and serum T_4 have been used as indicators for iodine status assessment in cattle (Hemingway et al., 2001; Takahashi et al., 2001), and it has been suggested that long-term iodine deficiency can be diagnosed with concentrations of T_4 below 1.56 μg dL^{-1} (Whittaker, 1999). Furthermore, thiocyanate has the potential to interfere with thyroid hormone synthesis (Guyton, 1986), in which case, additional supplementation of iodine is not effective. Concentrations of thyroid hormones are variable within blood and fluctuate with age, sex, and weight. Paulikova et al. (2011) assessed serum concentrations of T_4 and T_3 in apparently healthy cattle at various ages, with concentrations of T_4 in calves and heifers significantly different ($P < 0.05$; 8.10 ± 2.78; 9.15 ± 3.67, μg dL^{-1}; respectively) and concentrations of T_3 similar ($P > 0.05$; 1.91 ± 0.65; 3.92 ± 0.71, ng mL^{-1}; respectively). Circulating thyroid hormones are positively correlated with energy balance, thus during negative energy balance, dairy cows responded with decreasing concentrations of T_4 and T_3 (McGuire et al., 1991), which has been implicated in fatty liver syndrome (Kapp et al., 1978), hormonal imbalance, and potential reproduction disorders (Paulikova et al., 2011).

Concentration of plasma Cp was decreased (Table 5-3; $P < 0.001$; 9.78 vs 11.47 mg dL^{-1}, respectively) in BCM supplemented heifers compared with CTL heifers. An effect of day was observed in concentration of plasma Cp ($P < 0.001$; Figure 5-5), in which concentrations decreased from d 14 through 35, peaked at d 49, and stabilized for the duration of the study. Moriel and Arthington (2013) observed a peak of concentrations of plasma positive APPs between d 8 and 14, which coincided with vaccinations, yet concentrations returned to baseline values between d 21 and 29. A similar pattern was observed in the latter part of the current study, however, concentrations were significantly decreased between d 14 and 35, despite treatments. Glucosinolates are sulfur-containing moieties, and high concentrations of S may inhibit copper

absorption (Yu and Benyen, 1996), subsequently affecting immune function, copper transport, and iron metabolism. Ceruloplasmin has been utilized as an indicator of nutritional Cu status in cattle as plasma Cu and Cp are highly correlated (Blakley and Hamilton, 1985). Additionally, Cp and Hp have been used as indicators of an acute phase response, which is elicited during periods of stress, and in response to inflammation or disease, due to cytokine stimulation of hepatocytes to increase production of positive APPs (Carroll and Forsberg, 2007). Previous research indicates plasma concentration of Cp decreases during periods of Cu deficiency (Mulhern and Koller, 1988) as Cp is a major transporter of plasma Cu (Cousins, 1985). During an immune challenge or in response to stress, protein deposition may be negatively affected as nutrients are partitioned to support immune function, thereby ensuring survival (Elasser et al., 2008). Therefore, heifers under an acute phase response would be expected to decrease intake and consequently weight gain, however, ADG was increased in heifers supplemented with BCM compared with CTL heifers. Qiu et al. (2007) observed elevated concentrations of plasma Cp for heifer calves compared with steer calves after exposure to stressors ($P < 0.05$; 20.1 vs 18.9 mg dL^{-1}, respectively), but concentrations were similar at weaning (11.08 mg dL^{-1}). Dietary concentrations of sulfur have been implicated in decreasing absorption of Cu leading to a Cu deficiency (Arthington et al., 1996), and subsequently a decrease in plasma concentration of Cp. Therefore, differences in plasma Cp may have resulted from dietary sulfur content, as BCM contains approximately 1.7%, whereas CTL heifers were not receiving additional sulfur. Assessment of Cu status was not within the scope of this study, yet it may be of benefit to examine potential Cu deficiency resulting from BCM supplementation in future studies.

Concentration of plasma Hp was not affected by supplementation of BCM ($P = 0.28$; 0.08 and 0.04 mg mL^{-1} for BCM and CTL, respectively). These results are similar to

concentrations observed in “healthy” dairy cows (0.08 mg mL^{-1}), compared with cows infected with *Theilera annulata*, in which case plasma Hp ranged from 0.13 to 1.01 mg mL^{-1}, indicating a significant increase in Hp synthesis in response to infection (Nazifi et al., 2009).

Conclusion

Supplementation of BCM for 70 d in growing heifers consuming bermudagrass hay ad libitum, increased ADG by 0.28 kg d^{-1} when compared with CTL, without altering the interval to attainment of puberty, or thyroid hormone metabolism. Supplementation of BCM led to variable results on plasma concentrations of APPs: 1) concentration of plasma Hp was not affected; 2) and concentration of plasma Cp was decreased in BCM heifers compared with CTL heifers. Additional research is necessary to understand the effects of supplementing BCM on concentrations of plasma Cp, and to determine whether the decrease in plasma Cp was elicited by an acute phase response.

Table 5-1. Analyzed[1] chemical and nutrient composition (DM basis) of diet fed to growing Angus crossbred heifers.

	Treatment[2]	
Item	BCM	Bermudagrass hay
DM, %	89.1 ± 1.06	92.7 ± 1.84
Glucosinolates[3], µmol g^{-1}	28.7	--[4]
CP, %	43.6 ± 0.35	13.3 ± 2.12
NFC[5], %	21.7 ± 0.10	6.0 ± 6.58
NDF, %	23.6 ± 0.14	71.2 ± 8.13
ADF, %	13.2 ± 0.57	38.0 ± 8.91
EE[6], %	2.5 ± 0.10	--
S, %	1.7 ± 0.02	--
TDN, %	76 ± 5.66	55 ± 2.83

[1]Dairy One Forage Testing Laboratory, Ithaca, NY.
[2]BCM: *Brassica carinata* meal pellets; Bermudagrass hay (*Cynodon dactylon*) fed ad libitum; values averaged over 2 years.
[3]Analyzed by Agrisoma Biosciences, Inc., Gatlineau, Quebec.
[4]-- Indicates this item was not analyzed.
[5]NFC = non-fiber carbohydrates.
[6]EE = ether extract.

Table 5-2. Effects of protein supplementation on average daily gain, initial and final BW, and attainment of puberty in Angus crossbred heifers fed bermudagrass hay ad libitum.

	Treatment[1]			*P*-value[2]		
Item	BCM	CTL	SEM[3]	TRT	BLK	TRT × BLK
ADG, kg	0.42[a]	0.14[b]	0.101	< 0.001	0.86	0.47
Initial BW, kg	243.8	243.2	34.14	0.96	< 0.001	0.80
Final BW, kg	272.8	253.3	40.88	0.088	< 0.001	0.97
Puberty[4], d	430.6	427.5	5.84	0.68	< 0.001	0.50

[a,b] Within a row, means with different superscripts differ, $P < 0.05$.

[1]BCM: *Brassica carinata* meal pellets; CTL: bermudagrass hay (*Cynodon dactylon*) fed ad libitum; values averaged over 2 years.

[2]Observed significance levels for treatment (TRT) and block (BLK) effects, and for their interaction (TRT × BLK).

[3]Pooled standard error of treatment means, $n = 9$ pens/treatment.

[4]Puberty is defined as concentrations of plasma progesterone ≥ 1 ng mL^{-1} over two consecutive 7 d measurements.

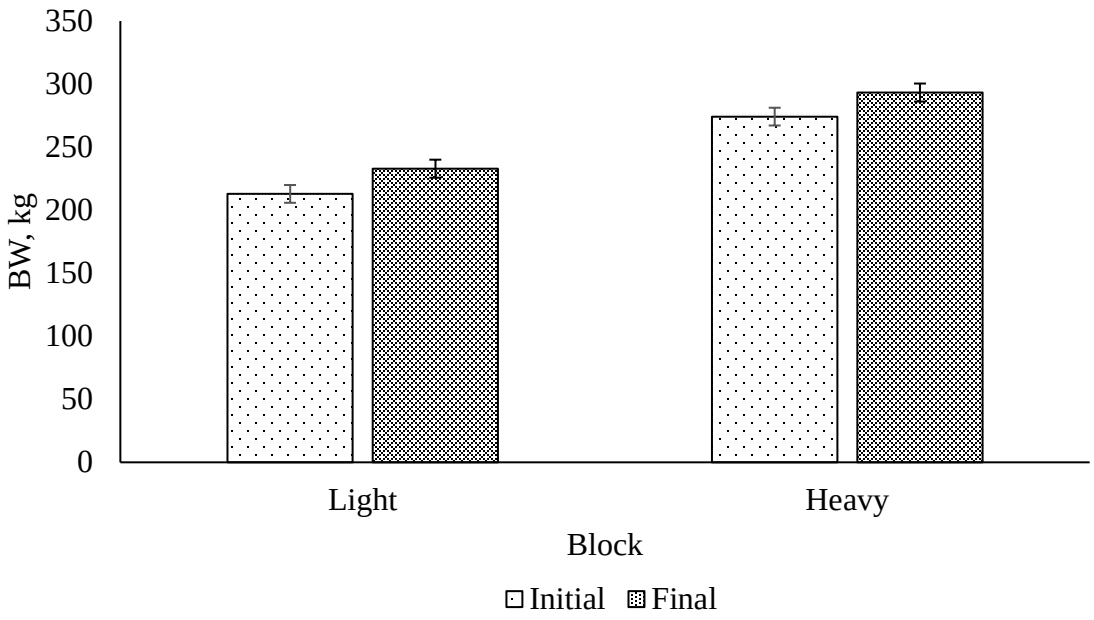

Figure 5-1. Effects of block ($P < 0.0001$) on initial and final BW of Angus crossbred heifers fed bermudagrass hay ad libitum over two consecutive years. No effect of treatment ($P = 0.96$) or treatment × block interaction ($P = 0.80$) was observed for initial BW. No effect of treatment ($P = 0.088$) or treatment × block interaction ($P = 0.97$) was observed for final BW.

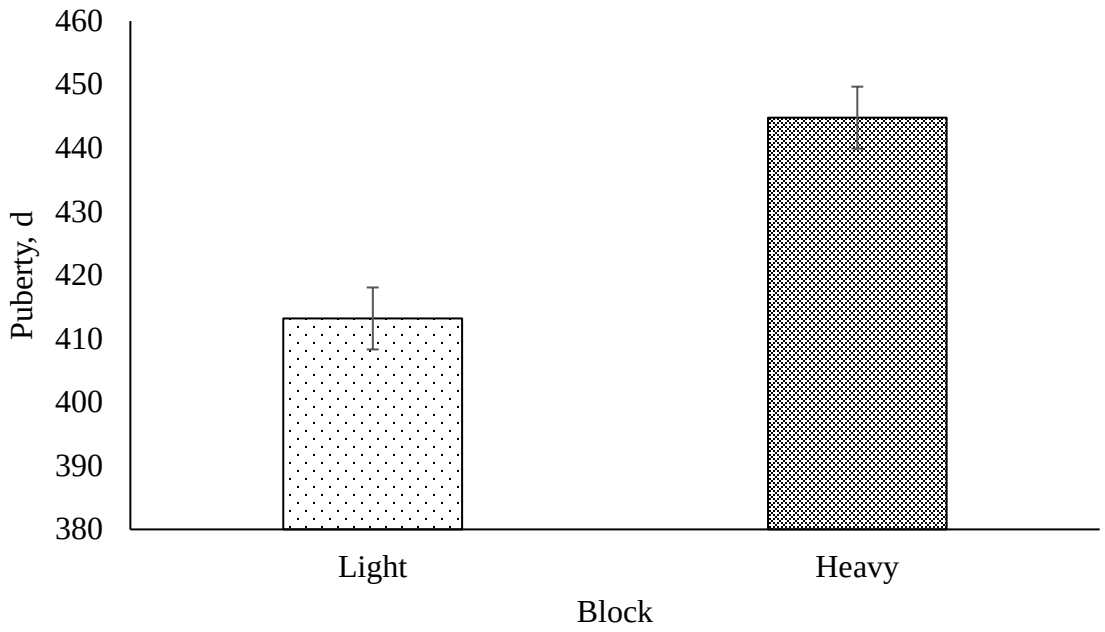

Figure 5-2. Effects of block ($P < 0.0001$) on days to attainment of puberty in Angus crossbred heifers fed bermudagrass hay ad libitum over two consecutive years. No effect of treatment ($P = 0.68$) or treatment × block interaction ($P = 0.49$) was observed.

Table 5-3. Effects of protein supplementation on thyroid hormone[1] metabolism and acute phase protein response in Angus crossbred heifers fed bermudagrass hay ad libitum.

	Treatment[2]			*P*-value[3]				
Item[4]	BCM	CTL	SEM[5]	TRT	DAY	TRT × DAY	BLK	TRT × BLK
T_3, ng dL^{-1}	128.64	122.69	3.982	0.31	< 0.001	0.98	0.13	0.60
T_4, µg dL^{-1}	4.29	4.30	0.142	0.94	< 0.001	0.78	0.087	0.077
Haptoglobin, mg mL^{-1}	0.08	0.04	0.019	0.28	0.44	0.39	0.38	0.37
Ceruloplasmin, mg dL^{-1}	9.78[b]	11.47[a]	0.267	< 0.001	< 0.001	0.56	0.64	0.66

[a,b] Within a row, means with different superscripts differ, $P < 0.05$.

[1]Thyroid hormones: T_3 = triiodothyronine; T_4 = thyroxine.

[2]BCM: *Brassica carinata* meal pellets; CTL: bermudagrass hay (*Cynodon dactylon*) fed ad libitum; values averaged over 2 years.

[3]Observed significance levels for treatment (TRT) and block (BLK) and for their interaction (TRT × BLK).

[4]Concentrations of metabolites in plasma.

[5]Pooled standard error of treatment means, $n = 9$ pens/treatment.

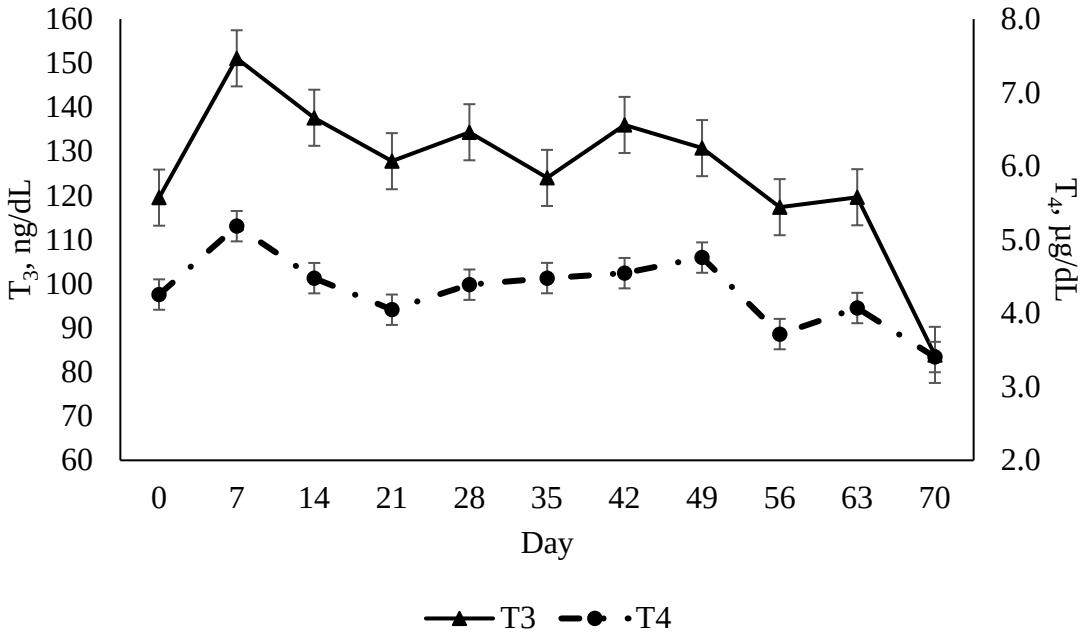

Figure 5-3. Effects of protein supplementation on thyroid hormones concentrations in plasma in Angus crossbred heifers fed bermudagrass hay ad libitum over two consecutive years. No effect of treatment was observed for T_3 ($P = 0.31$) or T_4 ($P = 0.94$), nor was an effect of treatment × day observed for T_3 ($P = 0.98$) or T_4 ($P = 0.78$). Day effect was observed ($P < 0.0001$).

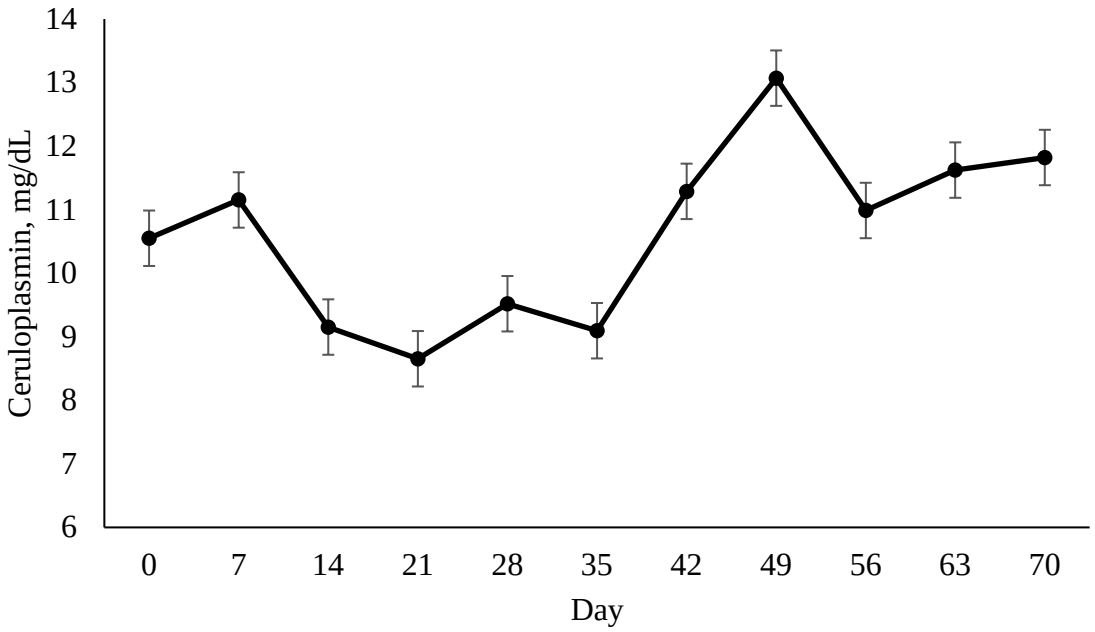

Figure 5-4. Effects of protein supplementation on day ($P < 0.0001$) on acute phase protein ceruloplasmin in Angus crossbred heifers fed bermudagrass hay ad libitum over two consecutive years. An effect of treatment was observed ($P < 0.0001$), however no treatment × day interaction ($P = 0.56$) was observed.

CHAPTER 6
SUMMARY

Brassica carinata meal is a high-protein residue obtained as a byproduct of oil extraction from seeds, which is then further refined to be used as high-quality jet biofuel. This meal has not been extensively tested as a supplement for beef cattle, therefore three experiments were conducted to evaluate the effects of supplementation of *B. carinata* meal on animal performance and metabolism. Experiment 1 involved the assessment of ruminal fermentation parameters, nutrient digestibility, and blood profile in beef steers fed bahiagrass hay (*Paspalum notatum*), compared with frequently used protein supplements. Experiment 2 was designed to characterize ruminal protein fractionation, and subsequent post-ruminal degradation of protein and amino acids. Experiment 3 assessed animal performance, attainment of puberty, and blood profile in growing beef heifers fed bermudagrass hay (*Cynodon dactylon*) over two consecutive years.

In Exp. 1, a duplicated 4 × 4 Latin square design was used to determine the effects of supplementation with *B. carinata* meal on ruminal fermentation, digestibility, and blood profile in beef cattle consuming bahiagrass hay (*Paspalum notatum*), compared with frequently used protein supplements. Eight Angus crossbred steers (473 ± 119 kg initial BW) were randomly allocated to 8 pens, over 4 periods of 28 d each. Within period, steers were assigned to one of four treatments: 1.62 kg d^{-1} cottonseed meal (CSM), 2.15 kg d^{-1} dry distiller's grains plus solubles (DDGS), 1.39 kg d^{-1} *B. carinata* meal pellets (BCM), or 1.17 kg d^{-1} soybean meal (SBM), supplemented daily, on an isonitrogenous basis. Steers had ad libitum access to bahiagrass hay and water. Intake was measured using the GrowSafe system. Following a 14 d adaptation, feed and fecal samples were collected twice daily for 4 d to determine apparent total tract nutrient digestibility using iNDF as an internal marker. Blood and ruminal fluid samples were collected every 3 h, during a 24 h period, to analyze blood urea nitrogen (BUN) and

glucose in plasma, as well as pH, NH_3-N, and VFA concentrations in ruminal fluid. Data were analyzed using PROC MIXED of SAS with repeated measures. Model included the fixed effects of treatment, time, treatment × time, square, and period, and the random effects of steer(square) and steer(treatment). No effect of treatment ($P > 0.05$) was observed for pH, NH_3-N, or glucose concentration. An effect of treatment ($P < 0.01$) was observed for BUN, with steers receiving SBM having greater concentrations. There was no effect of treatment ($P > 0.05$) on total VFA concentrations. Steers consuming CSM had greatest acetate molar proportion, and greater acetate to propionate ratio when compared with DDGS and BCM. Steers consuming DDGS had greatest molar proportions of butyrate and greater molar proportions of propionate compared with SBM and CSM. There was no effect of treatment ($P > 0.05$) on DMI or apparent total tract digestibility of DM, OM, CP, NDF, or ADF.

In Exp. 2, a ruminal in situ degradability study was conducted, utilizing steers from Exp.1, where the undegraded supplement remaining after 16 h of ruminal incubation was subjected to serial solutions simulating post-ruminal digestion, with subsequent analysis of CP content and determination of the BCM AA profile. An effect of treatment ($P < 0.01$) was observed for K_d of DM and CP with SBM having the greatest degradation rate. An effect of treatment ($P < 0.01$) was observed for fractionation of ruminal protein, with CSM and DDGS having nearly equivalent amounts of RDP and RUP (approximately 51 and 49%, respectively), which differed from SBM and BCM having approximately 72 and 28%, respectively. Compared to DDGS, SBM had the greatest IDP ($P < 0.01$), with CSM having the greatest IADP ($P < 0.01$), and similar for BCM and SBM. Total tract digestibility of CP was greatest for SBM compared to CSM and DDGS.

The objective of Exp 3 was to determine the effects of supplementation with *B. carinata* meal (BCM) on performance, attainment of puberty, and blood profile in growing beef heifers consuming bermudagrass hay (*Cynodon dactylon*). Sixty-four Angus crossbred heifers (240 ± 39 kg initial BW) were stratified and blocked (2 blocks: light and heavy) by initial BW and randomly allocated into 18 pens over 2 consecutive yr (10 pens in yr 1 and 8 pens in yr 2). Within block, pens were randomly assigned to one of two treatments: 0 (CTL) or 0.3% of BW d^{-1} (as fed) of BCM pellets, with both treatments having ad libitum access to bermudagrass hay and water, and BCM pellets supplemented daily. Blood samples and BW were collected weekly for 70 d, before daily supplementation. Plasma was analyzed for concentrations of progesterone, triiodothyronine (T_3), thyroxine (T_4), ceruloplasmin (Cp), and haptoglobin (Hp). An effect of treatment was observed ($P < 0.01$) in ADG between CTL (0.14 kg) and BCM (0.42 kg). There was no treatment or block ($P > 0.05$) effect for plasma concentrations of T_3, T_4 or Hp; however, there was an effect of day ($P < 0.01$) for T_3, T_4, and Cp. An effect of treatment ($P < 0.01$) was observed for concentrations of Cp, with CTL having greater concentrations compared with BCM. Time to attainment of puberty did not differ between treatments ($P = 0.68$); however, an effect of block ($P < 0.01$) indicated an earlier attainment of puberty in light BW heifers.

Brassica carinata meal performed similarly to commonly used protein supplements. Increased ADG was observed in growing beef heifers when supplemented daily at 0.3% of BW d^{-1}, without affecting attainment of puberty, thyroid hormone metabolism, and acute phase protein synthesis. As the residual meal has not been previously utilized as feed for cattle, the AA profile of the original meal, after 16 h incubation in the rumen, and post-ruminal residue has been determined, in addition to the essential AA contribution of RUP. *Brassica carinata* meal is a protein source with a CP fraction comprised of 71.8% RDP and a total dietary protein of 97%,

thus indicating its viability as a high-value protein supplement for beef cattle. Further research is necessary to evaluate the implications of feeding *B. carinata* on fetal development, and subsequent production traits, as well as assessing any potential negative effects of glucosinolates and subsequent byproducts related to copper deficiency.

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BIOGRAPHICAL SKETCH

Tessa Marie Schulmeister was born at home, in the small town of Dothan, Alabama in 1983. When Tessa was five years old, she either wanted to become a teacher or a veterinarian due to her love of children and animals. Throughout her childhood Tessa would collect random animals and bring them home, much to her mother's dismay. At the age of sixteen, Tessa was employed in teaching children from the ages of three to five, preparing them for kindergarten, and working at the local mall, while attending high school. Tessa began college courses at the local junior college in 2001, however the terrorist attack of 9-11 compelled Tessa to join the U.S. Navy, where she enlisted for five years. After an honorable discharge from the U. S. Navy, she obtained an associate's degree with emphasis in psychology from Blackhawk Junior College, and a bachelor's degree in biology from Northern Illinois University, where she was an undergraduate research assistant to Dr. Bethia King. After graduation in 2012, Tessa moved to Florida to be closer to family, and was employed by Dr. DiLorenzo in 2013. Under the employment of Dr. DiLorenzo, Tessa was encouraged to further her education, and began the pursuit of a master's degree from the University of Florida in Animal Sciences, under the supervision of Dr. Nicolas DiLorenzo, Dr. Cliff Lamb, and Dr. Jose Dubeux. Tessa's research was focused on the novel crop, *Brassica carinata*, and its potential use as a protein supplement in beef cattle. Tessa is also a full-time mom to two wonderful, not-so-little boys.