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Ruminant Nutrition

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Evaluation of *Brassica carinata* meal as a protein supplement for growing beef heifers[1,2]

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Abstract

Brassica carinata is a new oilseed crop in Florida with the potential of producing high-quality jet biofuel. A high-protein meal (~40% crude protein; CP) is obtained as a byproduct of oil extraction; however, limited research is available on the utilization of this meal as a protein supplement for beef cattle. A generalized randomized block design was used to evaluate the effects of supplementation with *B. carinata* meal pellets on performance and attainment of puberty in growing beef heifers consuming bermudagrass hay (*Cynodon dactylon*) ad libitum. Sixty-four Angus crossbred heifers (240 ± 39 kg initial body weight; BW) were stratified and blocked (2 blocks: light and heavy) by initial BW and randomly allocated into 18 pens over 2 consecutive years (10 in year 1 and 8 in year 2). Within block, pens were randomly assigned to 1 of 2 treatments: 0 (**CTL**) or 0.3% of BW/d (as fed) of *B. carinata* meal pellets (**BCM**). Blood samples and BW were collected weekly for 70 d, before daily supplementation. Data were analyzed using PROC MIXED of SAS with repeated measures. Model included the fixed effects of treatment, day, treatment × day interactions, block, and block × treatment interactions, with the random effect of year. Plasma was analyzed for concentrations of progesterone, triiodothyronine ($\mathbf{T_3}$), thyroxine ($\mathbf{T_4}$), ceruloplasmin (**Cp**), and haptoglobin (**Hp**). An effect of treatment was observed ($P < 0.01$) for ADG between CTL (0.14 kg) and BCM (0.42 kg). There was no treatment or block ($P > 0.05$) effect for 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 Cp, with CTL having greater concentrations compared with BCM. Time to attainment of puberty did not differ ($P = 0.93$) between treatments. Feeding *B. carinata* meal as a protein supplement at 0.3% of BW/d is a viable option for increasing ADG of growing beef heifers, without affecting attainment of puberty, thyroid hormone status, or eliciting an acute phase response.

Key words: acute phase proteins, beef heifers, *Brassica carinata*, puberty

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Introduction

Brassica carinata is a nonfood oilseed crop with a favorable very long-chain fatty acid composition for conversion to biofuel (Marillia et al., 2013), with extracted oil 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 United States, *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% crude protein; CP) is obtained as a byproduct of oil extraction. Analysis of the meal yields low concentrations of sinigrin and progoitrin, byproducts of ruminal degradation of glucosinolates (EFSA, 2008), which have been associated with decreased intake, interference of thyroid hormone metabolism, and reduced fertility and reproductive performance in cattle.

Cattle in the southeastern United States often graze warm-season C4 grass pastures of limited nutritive value which are not adequate to support high levels of production. During critical periods, such as winter and various stages of growth and development in beef cattle, protein supplementation is necessary (Hersom et al., 2011). Protein supplements in this region, such as cottonseed and soybean meal, are byproducts of various industries and in conjunction with the poor-quality warm-season hay fed during the winter, provide an opportunity to meet the nutritional requirements of growing heifers (Schulmeister et al., 2019).

Brassica carinata meal has been evaluated as a high-quality source of CP for ruminants utilizing in situ and ruminal metabolism procedures (Xin and Yu, 2014; Schulmeister et al., 2019), and as a feeding trial utilizing Holstein heifers fed cold-pressed carinata meal (Rodriguez-Hernandez and Anderson, 2018); however, research in feeding hexane-extracted *B. carinata* meal to beef heifers is limited. It was hypothesized that feeding low glucosinolate *B. carinata* meal to beef heifers would improve performance without negatively affecting attainment of puberty. Thus, the objective of this study was to determine the effects of supplementation with *B. carinata* meal on performance, attainment of puberty, and blood metabolites in growing Angus crossbred heifers consuming bermudagrass hay.

Materials and Methods

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee, study #201308011.

Experimental Design, Animals, and Treatments

The 2-yr experiment was conducted at the North Florida Research and Education Center in Marianna, FL. A total of 64 Angus crossbred heifers (240 ± 39 kg initial body weight; BW; 367 ± 0.09 d of age) were used in a generalized randomized block design, with the end of the study coinciding with the beginning of the breeding season. Heifers were stratified and blocked (2 blocks: light and heavy) by initial BW and randomly allocated to 18 pens over 2 consecutive years (10 pens in year 1: 3 heifers/pen, except for 2 control pens with 4 heifers/pen; 8 pens in year 2: 4 heifers/pen). Within block, pens were randomly assigned to 1 of 2 treatments: 1) **CTL** = 0% *B. carinata* meal pellets, or 2) **BCM** = 0.3% of BW/d (as fed) of *B. carinata* meal pellets. The meal was obtained after a hexane extraction process and was provided by Agrisoma Biosciences, Inc. (Gatlineau, Quebec). Heifers were provided ad libitum access to bermudagrass (*Cynodon dactylon*) hay and water, and *B. carinata* meal pellets were delivered to treatment pens each morning. Initial BW was considered as the average of days –1 and 0 BW, and final BW was considered as the average of days 69 and 70. Blood samples were collected on day 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 supplementation.

Blood Sampling

Blood was collected from jugular venipuncture every 7 d, at approximately 0700 h in the morning, immediately before supplementation of the *B. carinata* meal pellets, in 10-mL evacuated tubes containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ), placed on ice, and subsequently centrifuged for 15 min at 4,000 × *g* at 4 °C. After centrifugation, plasma was transferred into polypropylene vials (12 × 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), capped, and stored at –20 °C for further analysis of concentrations of progesterone (**P4**), triiodothyronine (T_3), thyroxine (T_4), ceruloplasmin (**Cp**), and haptoglobin (**Hp**).

Laboratory Analyses

Hay samples were collected every 7 d, composited by pen within year, and analyzed for dry matter (DM), organic matter (**OM**), CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Samples were weighed (0.50 g) into tared beakers, placed in an oven at 100 °C overnight to calculate DM, and subsequently placed in a muffle furnace at 650 °C for 6 h to calculate OM. To determine concentrations of NDF, samples were weighed (0.50 g) into F57 filter bags and analyzed in an Ankom 200 Fiber Analyzer (Ankom Technology) using sodium sulfite and heat-stable α-amylase. Samples were subsequently analyzed for concentrations of ADF. Concentration of nitrogen (**N**) in feed was determined by rapid combustion using a micro elemental N analyzer (Vario Micro cube, Elementar Analysensysteme GmbH., Langenselbold, Germany), 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).

P4, T_3, T_4, Hp, and Cp Analyses

Concentrations of P4 were determined by an immunoassay (Immulite 1000, Siemens Health, Inc., Malvern, PA) according to manufacturer's instructions. Heifers were considered to have attained puberty after the first increase in P4 (evidence of first pubertal ovulation) that exceeded 1.0 ng/mL followed by a P4 pattern consistent with normal estrous cycles. Briefly, 200 µL of plasma were placed in a sample cup, loaded onto a conveyor belt with a kit-specific (kit # LKPW1) test unit directly following the sample, with samples and previously loaded 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 was 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).

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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 (Cooke and Arthington, 2013), with the lowest detectable value of 0.03 mg/mL. Inter- and intra-assay coefficients of variation 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 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). Pen was considered the experimental unit for both performance and repeated measures. The model for performance and attainment of puberty included fixed effects of treatment, day, treatment × day interactions, block, and block × treatment interactions, with the random effect of year. The day of attainment of puberty was calculated using the concentration of P4 and averaged by pen. Repeated measures, with pen within year as subject, were used to analyze T_3, T_4, Cp, and Hp concentrations over time. Covariance structures for repeated measures were first-order autoregressive, based upon the smallest Akaike Information Criterion values (Littell et al., 1998). 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 *B. carinata* meal pellets presented to heifers is presented in Table 1, with glucosinolate content from *B. carinata* meal pellets in Table 2. Bermudagrass hay used in this study had 13.3% CP and depending on expected growth and performance of yearling heifers should be sufficient to meet their nutritional demands (NASEM, 2016). Heifers supplemented with BCM at 0.3% of their BW for 70 d, had greater average daily gain (ADG; $P < 0.01$; Table 3) compared with CTL heifers. Considering the additional ADG of 0.28 kg when supplementing BCM, and an average supplemental amount of 0.69 kg of carinata meal DM/d, the feed conversion ratio of the supplemental carinata meal is of 2.46 kg of feed per kg of BW gain, which was remarkable. 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 microbial crude protein flow to the small intestine. Additionally, the energy provided by carinata meal compared with the provision of bermudagrass hay alone may have resulted in greater weight gain for the BCM heifers.

Differences in initial BW were not observed for treatment ($P = 0.96$; Table 3) or treatment × block interactions ($P = 0.80$); however, an effect of block was observed ($P < 0.01$) for both initial and final BW. This was expected due to the initial stratification and blocking of heifers based on BW. A tendency for a treatment effect was observed on final BW ($P = 0.09$).

Brassica carinata belongs to the mustard family, *Brassicaceae*, which are notorious for high concentrations of glucosinolates.

Table 1. Analyzed[1] chemical and nutrient composition (DM basis) of diet fed to growing Angus crossbred heifers during a 70-d period, over 2 consecutive years

	Treatment[2]	
Item[3]	*Brassica carinata*	Bermudagrass hay
DM, %	89.1 ± 1.06	92.7 ± 1.84
Glucosinolates[4], µmol/g	28.7	–
CP, %	43.6 ± 0.35	13.3 ± 2.12
NFC, %	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, %	2.5 ± 0.10	–
S, %	1.7 ± 0.02	–
Ca, %	0.6	–
P, %	1.3	–
Mg, %	0.6	–
K, %	1.7	–
Na, %	0.0	–
Fe, %	191.0	–
Zn, %	72.0	–
Cu, %	7.0	–
Mn, %	49.0	–
Mo, %	0.7	–
NE_L, Mcal/kg	1.8 ± 0.17	1.1 ± 0.11
NE_M, Mcal/kg	1.8 ± 0.20	1.1 ± 0.06
NE_G, Mcal/kg	1.2 ± 0.17	0.6 ± 0.05
TDN, %	76.0 ± 5.66	55.0 ± 2.83

[1]Dairy One Forage Testing Laboratory, Ithaca, NY.
[2]*Brassica carinata* meal pellets, fed at 0.3% initial BW/d (as fed), were provided by Agrisoma Biosciences, Inc. (Gatlineau, Quebec); Bermudagrass hay (*Cynodon dactylon*) fed ad libitum; values averaged over 2 consecutive years, except for minerals.
[3]DM = dry matter; CP = crude protein; NFC = non-fiber carbohydrates; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; S = sulfur; Ca = calcium; P = phosphorus; Mg = magnesium; K = potassium; Na = sodium; Fe = iron; Zn = zinc; Cu = copper; Mn = manganese; Mo = molybdenum; NE_L = net energy of lactation; NE_M = net energy of maintenance; NE_G = net energy of gain; TDN = total digestible nutrients.
[4]Analyzed by POS Bio-Science (Saskatoon, SK, Canada).

Table 2. Analyzed[1] content of glucosinolates derived from *B. carinata* meal

Glucosinolate	*Brassica carinata*[2], µmol/g	SEM[3]
Allyl	26.86	1.771
3-butenyl	0.51	0.044
4-pentenyl	0.09	0.013
2-OH-3-butenyl	0.45	0.043
CH3-thiobutenyl	0.09	0.032
Phenylethyl	0.12	0.006
CH3-thiopentenyl	0.19	0.046
3-CH3-indolyl	0.08	0.004
4-OH-3-CH3-indolyl	0.21	0.019

[1]Analysis conducted by POS Bio-Science (Saskatoon, SK, Canada).
[2]*Brassica carinata* meal pellets provided by Agrisoma Biosciences, Inc. (Gatlineau, Quebec).
[3]Pooled standard error of means, $n = 5$.

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Lardy and Kerley (1994) suggested 90 to 140 µmol/g as high concentrations of glucosinolates in growing crossbred beef steers. Although the meal in the current study had a decreased concentration of glucosinolates (28 µmol/g), it was imperative to evaluate the 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 *B. carinata*, and upon ruminal degradation produce unstable compounds resulting in formation of isothiocyanate and thiocyanate (EFSA, 2008). Thiocyanate and isothiocyanate are problematic with regard to fertility/reproductive impairment, thyroid metabolism, growth retardation, and inhibition of copper absorption (EFSA, 2008). In the current study, the interval to attainment of puberty was not affected ($P = 0.93$) by supplementation of BCM compared with CTL heifers.

An effect of treatment was not observed ($P > 0.10$; Table 4) on concentrations of T_3 or T_4, though an effect of day ($P < 0.01$; Figure 1) demonstrated an increase in T_3 and T_4 on day 7. An increase in concentration of thyroid hormones may be attributed to environmental factors such as cooler temperatures (Guyton, 1986), potentially explained by the study occurring during the winter. Lardy and Kerley (1994) observed a significant decrease in concentrations of T_4 with increasing inclusion concentrations of glucosinolates, which was not observed in the current study. There was a tendency ($P = 0.09$) for heavy BCM heifers to have an increase in plasma T_4 and a subsequent tendency ($P = 0.08$) for heavy CTL heifers to have increased concentrations of plasma T_4 compared with light CTL heifers. The relationship between these variables is not clear; nonetheless, it is suspected that the weight differences in heifers between the 2 yr may be related. Concentration of plasma T_3 observed for light lactating and nonlactating cows (128.9 to 109.2 vs. 122.7 to 128.7 ng/mL, respectively) was similar to heifers in the current study, yet as BW increased, plasma T_4 and T_3 decreased (488 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 (Walker et al., 2015) and heifers in the present study.

Thiocyanate has the potential to bind iodine, preventing trapping and uptake of iodine by the thyroid gland (Barrett et al., 1997), yet 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 (Whittaker, 1999), which were not observed in the current study. Furthermore, thiocyanate has the potential to interfere with thyroid hormone synthesis (Guyton, 1986), in which case, additional supplementation of iodine is not effective. 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

Table 3. Effect of *Brassica carinata* meal supplementation on average daily gain (ADG), initial and final body weight (BW), and attainment of puberty in Angus crossbred heifers fed bermudagrass hay ad libitum, during a 70-d period, over 2 consecutive years

	Treatment[1]			P-value[2]		
Item	BCM	CTL	SEM[3]	TRT	BLK	TRT × BLK
ADG, kg	0.42[a]	0.14[b]	0.101	<0.01	0.86	0.47
Initial BW, kg	244	243	34.2	0.96	<0.01	0.80
Final BW, kg	273	253	40.9	0.09	<0.01	0.97
Puberty[4], d	399	400	6.7	0.93	0.43	0.16

[1]BCM = *Brassica carinata* meal pellets, fed at 0.3% initial BW/d (as fed), were provided by Agrisoma Biosciences, Inc. (Gatlineau, Quebec); CTL = bermudagrass hay (*Cynodon dactylon*) fed ad libitum; values averaged over 2 consecutive years.
[2]Observed significance levels for treatment (TRT), block (BLK), and their interaction (TRT × BLK).
[3]Pooled standard error of treatment means, $n = 9$ pens/treatment.
[4]Puberty is defined as the first increase in progesterone greater than or equal to 1.0 ng/mL followed by a progesterone pattern consistent with normal estrous cycles; values reported are average age of heifers within pen, in days.
[a,b] Within a row, treatment means with different superscripts differ, $P < 0.05$.

Table 4. Effect of *Brassica carinata* meal supplementation on thyroid hormone[1] metabolism and acute phase protein[2] response in Angus crossbred heifers fed bermudagrass hay ad libitum, during a 70-d period, over 2 consecutive years

	Treatment[4]			P-value[5]				
Item[3]	BCM	CTL	SEM[6]	TRT	DAY	TRT × DAY	BLK	TRT × BLK
T_3, ng/dL	128.64	122.69	3.982	0.31	< 0.01	0.98	0.13	0.60
T_4, µg/dL	4.29	4.30	0.142	0.94	< 0.01	0.78	0.09	0.08
Hp, mg/mL	0.08	0.04	0.019	0.28	0.44	0.39	0.38	0.37
Cp, mg/dL	9.78[b]	11.47[a]	0.267	< 0.01	< 0.01	0.56	0.64	0.66

[1]Thyroid hormones: T_3 = triiodothyronine; T_4 = thyroxine.
[2]Acute phase proteins: Hp = haptoglobin; Cp = ceruloplasmin.
[3]Concentrations of metabolites in plasma.
[4]BCM: *Brassica carinata* meal pellets, fed at 0.3% initial BW/d (as fed), were provided by Agrisoma Biosciences, Inc. (Gatlineau, Quebec); CTL: bermudagrass (*Cynodon dactylon*) hay fed ad libitum; values averaged over 2 years.
[5]Observed significance levels for treatment (TRT), block (BLK), and their interaction (TRT × BLK).
[6]Pooled standard error of treatment means, $n = 9$ pens/treatment.
[a,b] Within a row, treatment means with different superscripts differ, $P < 0.05$.

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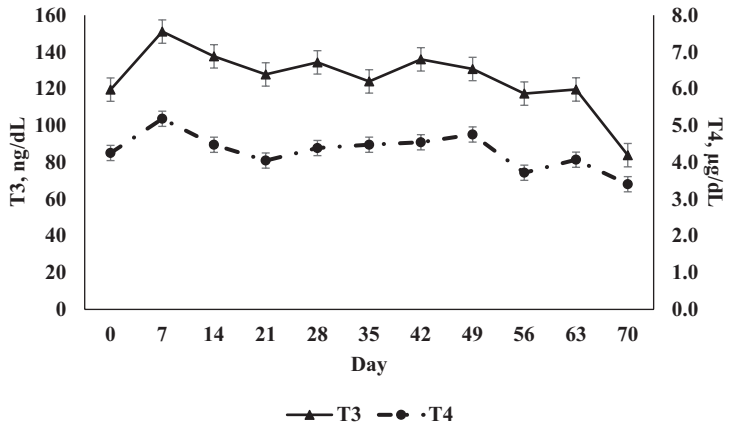

Figure 1. Effect of day on concentrations of plasma thyroid hormones (T_3: $P < 0.01$; SEM = 6.358; T_4: $P < 0.01$; SEM = 0.207; $n = 9$ pens/treatment) in Angus crossbred heifers fed bermudagrass hay ad libitum and BCM at 0.3% initial BW/d (as fed), during a 70-d period, over 2 consecutive years.

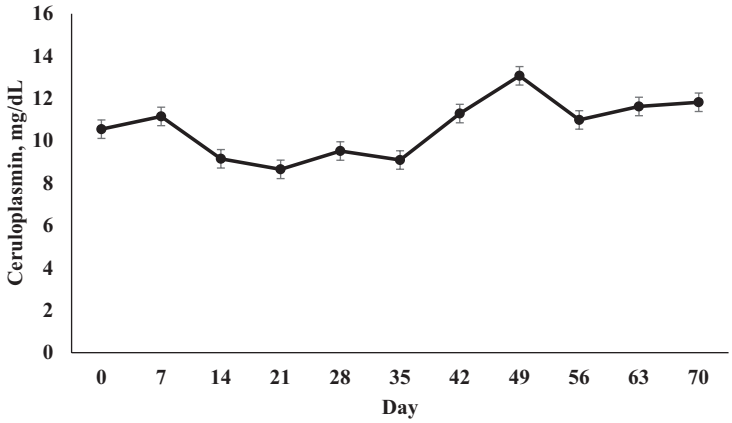

Figure 2. Effect of day on acute phase protein ceruloplasmin ($P < 0.01$; SEM = 0.436; $n = 9$ pens/treatment) in Angus crossbred heifers fed bermudagrass hay ad libitum and BCM at 0.3% initial BW/d (as fed), during a 70-d period, over 2 consecutive years.

and heifers significantly different (8.10 ± 2.78, 9.15 ± 3.67, µg/dL; respectively) and concentrations of T_3 similar (1.91 ± 0.65, 3.92 ± 0.71, ng/mL; respectively). Although these values are not similar to those observed in the current study, thyroid hormones fluctuate depending on age, size, environment, and a host of other factors (Tripathi et al., 2001).

Glucosinolates are sulfur-containing moieties, and elevated concentrations of sulfur (**S**) may inhibit copper (**Cu**) absorption (Yu and Beynen, 1996), subsequently affecting immune function, Cu 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, in which cytokine stimulation of hepatocytes increases production of positive acute phase proteins (**APP**; Carroll and Forsberg, 2007). Previous research indicates that 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). Concentration of plasma Cp was decreased (Table 4; $P < 0.01$) in BCM-supplemented heifers 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, respectively); nevertheless, concentrations were similar at weaning (11.08 mg/dL), which are similar to the concentration of Cp in CTL heifers of the current study. High dietary concentrations of S 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 S content, as BCM contains approximately 1.7%, whereas CTL heifers were not receiving additional S. 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. An effect of day ($P < 0.01$; Figure 2) was observed in concentration of plasma Cp, in which concentrations decreased from days 7 through 14, increased from day 35, peaked at day 49, and stabilized for the duration of the study. Moriel and Arthington (2013) observed a peak of concentrations of positive APP in plasma between days 8 and 14, which coincided with vaccinations, yet concentrations returned to baseline values between days 21 and 29. A similar pattern was observed in the latter part of the current study; however, concentrations were significantly decreased between days 14 and 35, despite treatments. Concentration of plasma Hp was not affected ($P = 0.28$; Table 4) by supplementation of BCM. These results are similar to concentrations observed in "healthy" dairy cows (0.08 mg/mL), compared with cows infected with tickborne intracellular protozoan parasite, *Theilera annulata*,

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in which case plasma Hp ranged from 0.13 to 1.01 mg/mL, indicating a significant increase in Hp synthesis in response to infection (Nazifi et al., 2009).

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 (Elsasser 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. Furthermore, 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). It is concluded that supplementation of BCM in growing beef heifers increased ADG by 0.28 kg/d compared with CTL heifers, without altering the interval to attainment of puberty or thyroid hormone metabolism; thus, it is a viable alternative for protein supplementation in beef cattle. Supplementation of BCM led to variable results on plasma concentrations of APPs: 1) concentration of plasma Hp was not affected and 2) concentration of plasma Cp was decreased in BCM heifers compared with CTL heifers. Future research should address the impact of S in *Brassica carinata* meal in beef cattle, and the potential effects of glucosinolates on hormonal imbalances, reproduction, and subsequent milk production.

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