Evaluation of *Brassica carinata* meal on ruminant metabolism and apparent total tract digestibility of nutrients in beef steers[1,2]

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ABSTRACT: *Brassica carinata* is a new oilseed crop with the potential of producing high-quality jet biofuel. A high-protein meal (~40% crude protein) is obtained as a byproduct of hexane-solvent oil extraction; however, limited research is available on the use of this meal as a protein supplement for beef cattle. 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 metabolites in beef cattle consuming bahiagrass hay (*Paspalum notatum* Flüggé), 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 1 of 4 treatments: 1) 1.62 kg/d cottonseed meal (**CSM**); 2) 2.15 kg/d dry distillers grains plus solubles (**DDGS**); 3) 1.39 kg/d *B. carinata* meal pellets **(BCM)**; or 4) 1.17 kg/d 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 to determine apparent total tract nutrient digestibility using indigestible NDF as an internal marker. Blood and ruminal fluid samples were collected before providing the protein supplements and then every 3 h, during a 24-h period, to analyze urea nitrogen (**PUN**) and glucose in plasma, as well as ruminal pH, ammonia nitrogen (NH_3-N), and VFA concentrations. 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 PUN, with steers receiving SBM having greater concentrations. A treatment × time interaction was observed ($P < 0.05$) for total VFA concentration, acetate to propionate ratio, and molar proportions of acetate, propionate, butyrate, and valerate. Steers consuming SBM had greater molar proportions of branched-chain VFA ($P < 0.01$) compared with CSM and DDGS. There was no effect of treatment ($P > 0.05$) on intake or apparent total tract digestibility of nutrients. *Brassica carinata* performed similarly to commonly used protein supplements indicating its viability as a protein supplement for beef cattle.

Key words: beef cattle, Brassica carinata, digestibility, metabolism

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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). 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 (Agriculture and Agri-Food Canada [AAFC], 2015). In the southeastern United States, *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 residue (~40% crude protein; CP) is obtained as a byproduct of hexane-solvent oil extraction.

As the southeastern United States is typically comprised of cow-calf operations, cattle often graze warm-season C4 grass pastures of limited nutritive value, which are inadequate in supporting high levels of production, especially during critical periods such as winter and various growth and production stages of cattle, requiring supplementation of protein (Hersom et al., 2011; McBride and Matthews, 2011). Common protein supplements in this region, such as cottonseed and soybean meal, result from byproducts of various industries and in conjunction with the poor-quality hay available during 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 CP for ruminants utilizing an in situ procedure (Xin and Yu, 2014), a feeding trial utilizing Holstein heifers (Rodriguez-Hernandez and Anderson, 2018), and a regulatory study utilizing weaned steer calves (McKinnon and Damiran, 2015, unpublished research report); however, research in feeding *B. carinata* to beef cattle is limited. We hypothesized that feeding *B. carinata* would result in similar effects on intake, digestibility, ruminal fermentation parameters, and blood profile as common protein supplements. The objective of this study was to evaluate the effects of supplementation with *B. carinata* meal in comparison with common protein supplements on ruminant metabolism, blood metabolites, and apparent total tract digestibility of nutrients in Angus crossbred steers consuming bahiagrass 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 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 4 consecutive 28-d periods. Steers were randomly allocated to 8 pens, and within each period, steers were randomly assigned to 1 of 4 treatments: 1) 1.39 kg/d *B. carinata* meal pellets [**BCM**; hexane-solvent extracted, low glucosinolate carinata meal (Agrisoma Biosciences, Inc., Gatlineau, Quebec)]; 2) 1.62 kg/d cottonseed meal (**CSM**); 3) 2.15 kg/d dry distillers grain plus solubles (**DDGS**); or 4) 1.17 kg/d soybean meal (**SBM**), supplemented daily. Diets were formulated to meet or exceed nutrient requirements of mature beef steers (National Academies of Sciences, Engineering, and Medicine [NASEM], 2016). Treatments were calculated to be isonitrogenous based on total nitrogen provided by supplementation of 1.39 kg/d of BCM at 0.3% of the steers' average BW. On day 0, after 16 h of feed and water withdrawal, steers were shrunk weighed and housed individually in pens at the FEF with ad libitum access to water and bahiagrass hay. 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 days 0 to 14, and days 14 through 18 consisted of a digestibility measurement period in which hay and fecal samples were collected twice daily for 4 d each. Day 19 involved the collection of ruminal fluid, ruminal pH, and blood every 3 h during a 24-h collection period. All procedures described were performed similarly for each of the 4 periods.

Ruminal Fluid, pH, and Blood Sampling

Ruminal fluid and blood samples were collected before feeding (0 h) and every 3 h postfeeding 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 immediately placed in ice and later 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 × 75 mm; Fisherbrand; Thermo Fisher Scientific, Inc., Waltham, MA) and stored at −20 °C for further analysis.

Beginning on days 14 and 15, hay and fecal samples were collected, respectively, for 4 consecutive days to determine apparent total tract digestibility of DM, OM, CP, NDF, and ADF. Hay samples were collected twice daily immediately after delivery of protein supplement and stored at −20 °C. Fecal samples were collected twice daily at 0800 and 1600 h from the ground, inside the pen, immediately after the animal defecated. After collection, fecal samples were immediately placed in ice and later 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 composition 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 (0.50 g) in duplicate, dried in a forced-air oven at 100 °C overnight to calculate sample DM, and subsequently ashed in a muffle furnace at 650 °C for 6 h to calculate OM. To determine NDF concentration, samples were weighed (0.50 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). Concentration of iNDF in hay and feces was determined as described by Cole et al. (2011) with modifications proposed by Krizsan and Huhtanen (2013). Briefly, samples were weighed (0.50 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 described previously. Concentrations of nitrogen (N) in feed and feces were determined by rapid combustion using a micro elemental N analyzer (Vario Micro cube, Elementar Analysensysteme GmbH., Langenselbold, Germany), following official method 992.15 (Association of Analytical Communities [AOAC], 1995) with CP calculated as concentrations of N multiplied by 6.25. Protein supplements and bahiagrass hay were analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Total digestible nutrients (**TDN**) were calculated by Dairy One Forage Laboratory, and nonfibrous carbohydrate (NFC, %) was calculated as NFC = 100 − (CP, % + NDF, % + Ash, % + EE, %).

Ruminal Fluid and Blood Profile Assay Procedures

Concentration of VFA in ruminal fluid samples was determined in a liquid-liquid solvent extraction using ethyl acetate (Ruiz-Moreno et al., 2015). Samples were centrifuged for 15 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 15 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 and allowing the fractions to separate, 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 ruminal 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 on 200-µL samples at 620 nm in flat-bottom 96-well plates using a plate reader (DU-500, Beckman Coulter, Inc.). Plasma was analyzed for concentration of urea nitrogen (**PUN**) using a quantitative colorimetric kit (B7551-120; Pointe Scientific, Inc., Canton, MI), with the following modification: absorbance was read on 200-µL samples at 500 nm in flat-bottom 96-well plates using a plate reader (DU-500, Beckman Coulter, Inc.). Plasma was analyzed for concentration of glucose using a quantitative colorimetric

kit (G7521-1L; Pointe Scientific, Inc., Canton, MI), with the following modification: absorbance was read on 200-µL samples at 520 nm in flat-bottom 96-well plates using a plate reader (DU-500, Beckman Coulter, Inc.). All assays were conducted in duplicate determinations with subsequent analyses performed when CV were above 5%. Intra- and inter-assay CV were 1.8% and 1.3%, respectively, for NH_3-N, 1.8% and 0.5%, respectively, for plasma concentrations of glucose, and 4.4% and 4.3%, respectively, for PUN.

Statistical Analysis

Data were analyzed as a duplicated 4 × 4 Latin square with repeated measures for the ruminal fermentation parameters (NH_3-N, pH, and VFA) and plasma variables (PUN and glucose) using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC), with steer as the experimental unit (n = 8/treatment). The model for ruminal fermentation parameters included the fixed effects of treatment, time, the treatment × time interactions, square, and period; random effects included effects of steer within square, and steer within treatment. Covariance structures for repeated measures were autoregressive and variance components, based upon the smallest Akaike Information Criterion values (Littell et al., 1998). The model for intake and digestibility included the fixed effects of treatment, square, and period, and the random effects of steer within square. Steer within period was the subject for repeated measures. In the repeated measures analysis, when significant time postfeeding effects or time × treatment interactions were observed, least square means were separated and compared. Differences between treatment means were identified by Tukey's least squares means comparison and significance was declared at $P \leq 0.05$, with tendencies considered at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The chemical and nutrient composition of hay and protein supplements fed to steers is presented in Table 1. Bahiagrass is a common perennial grass grown in Florida and is often utilized for grazing beef cattle or production of hay (Chambliss and Sollenberger, 1991), despite the poor quality; thus, additional supplementation with energy and protein is often necessary to meet the nutritional demands of cattle (Moore et al., 1991). Common protein supplements in the Southeast include cottonseed and soybean meal; yet, the interest in renewable and sustainable energy has introduced *B. carinata* meal as an alternative source of protein for beef cattle. As a member of the mustard family, *Brassicaceae*, which is known for containing high concentrations of glucosinolates, *B. carinata* has been developed to have decreased concentration of glucosinolates (28 µmol/g; Table 2). Lardy and Kerley (1994) suggested 90 to 140 µmol/g as high concentrations of glucosinolates in growing crossbred beef steers, with elevated concentrations potentially resulting in negative effects on growth performance; as upon digestion, bacterial myrosinases will degrade the stable, intact compound possibly resulting in deleterious byproducts (Duncan and Milne, 1992).

The nutritive value of the bahiagrass hay fed to steers in this study was poor as illustrated

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

		Treatment[3]			
Item	Bahiagrass hay[2]	BCM	CSM	DDGS	SBM
DM, %	94.0	89.8	88.9	86.3	90.7
OM, %	94.0	91.6	90.7	91.8	92.3
CP, %	7.2	43.3	49.2	32.8	52.9
NFC[4], %	–	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[5], %	56	80	67	83	79
S, %	0.35	1.75	0.35	0.43	0.39

[1]Dairy One Forage Testing Laboratory, Ithaca, NY.

[2]Bahiagrass hay (*Paspalum notatum* Flüggé).

[3]BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d); composited over 4 periods.

[4]NFC = nonfibrous carbohydrates.

[5]TDN = total digestible nutrients.

by the digestible OM or TDN:CP ratio; however, supplementation with protein would be expected to increase intake (Moore et al., 1995). Neither intake nor apparent total tract digestibility of nutrients was affected ($P > 0.10$) by protein supplementation (Table 3). Rodriguez-Hernandez and Anderson (2018) observed a decrease in intake between heifers consuming 10% of carinata meal and DDGS during the first week of supplementation, with intake for the remainder of the study not affected by treatment. The meal fed to the heifers in the study of Rodriguez-Hernandez and Anderson (2018) was produced through the cold pressed extraction method, whereas the meal from the current study was produced using the hexane-solvent extraction, which may affect palatability and consequently intake, as well as digestibility of nutrients. Rodriguez-Hernandez and Anderson (2018) reported the total tract digestibility of nutrients in heifers receiving carinata meal were decreased for DM, OM, NDF, and ADF, compared with heifers receiving DDGS. Although the digestibility of CP was similar for the treatments, comparing those results with the current study is difficult as the diets fed by Rodriguez-Hernandez and Anderson (2018) included a 10.51% and 12.01% inclusion of SBM for carinata meal and DDGS treatments, respectively, and the current study employed only one source of protein. The 3-step in vitro procedure was conducted by Xin and Yu (2014), using 3 dry Holstein cows consuming a total mixed ration of 78% forage to 22% concentrate and 2 sources of carinata and canola meal, which is not comparable to the current study. A performance study was conducted by McKinnon and Damiran (2015), unpublished research report, in which canola and carinata meal were fed to 360 recently weaned calves, with a similar concentration of glucosinolates in the carinata meal as the current study. Dry matter intake of the recently weaned steers consuming carinata meal was 7.35 to 7.61 kg/d, comparable to the 6.83 kg/d observed in the steers in the current study.

Table 2. The analyzed[1] content of glucosinolates derived from *Brassica carinata* meal

Glucosinolate	BCM[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]BCM= *Brassica carinata* meal pellets.

[3]Pooled standard error of treatment means, $n = 5$.

Table 3. Effect 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[2] utilized as an internal marker

	Treatment[3]					
Item	BCM	CSM	DDGS	SBM	SEM[4]	*P* value[5]
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 $2 \times d^{-1}$ for 4 d; intake of bahiagrass hay was measured using the GrowSafe System Ltd., Airdrie, Alberta, Canada.

[2]iNDF = indigestible NDF after 288 h of ruminal incubation.

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

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

[5]Observed significance levels for treatment.

No effects of treatment ($P = 0.65$) or treatment × time interaction ($P = 0.37$) were observed for ruminal pH (Table 4), with averaged values ranging from 6.61 to 6.67, indicating a favorable ruminal environment for cellulolytic microorganism activity (Russell and Wilson, 1996). An effect of time postfeeding of supplement ($P < 0.01$; Figure 1) was observed for ruminal pH. Steers were provided ad libitum access to bahiagrass hay and water, and as a result, the initial pH was greater at 0 h. Upon consumption of the protein supplements, a decrease in pH was observed between 3 and 9 h, followed by a slow increase leading to an acclimated plateau through 18 h, and an increase through 24 h. It is speculated that the protein supplements decreased the pH partially due to other constituents of the supplements, such as nonfiber carbohydrates, and the inclination of the steers to consume the protein supplements immediately upon arrival. The acclimation 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 were not affected by treatments ($P = 0.37$); however, an effect of time ($P < 0.01$; Figure 1) was observed; yet, no treatment × time interaction ($P = 0.60$) was detected. Concentration of ruminal NH_3-N peaked at 3 h and steadily declined through 21 h and remained unchanged between 21 and 24 h postfeeding. 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. The progression of ruminal pH after protein supplementation is illustrated in Figure 1. Upon consumption of protein supplements, pH decreases and concentration of NH_3-N increases, yet as fermentation continues 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 to maximize microbial protein synthesis. Satter and Slyter (1974) suggest that the "precise limiting concentration is 1.43 m*M*, but use of the higher value gives a slight margin of excess." In this study, ruminal NH_3-N concentration was above the cited threshold of 3.57 m*M* (Satter and Slyter, 1974) only at 3 h postfeeding (Figure 1). Because microbial CP synthesis was not directly measured in the current study, it is uncertain whether the concentrations of NH_3-N throughout the day were limiting in terms of optimal ruminal function.

An effect of treatment ($P < 0.01$) was observed for concentrations of PUN, with steers supplemented with SBM having the greatest and DDGS having the least concentrations. Concentrations of

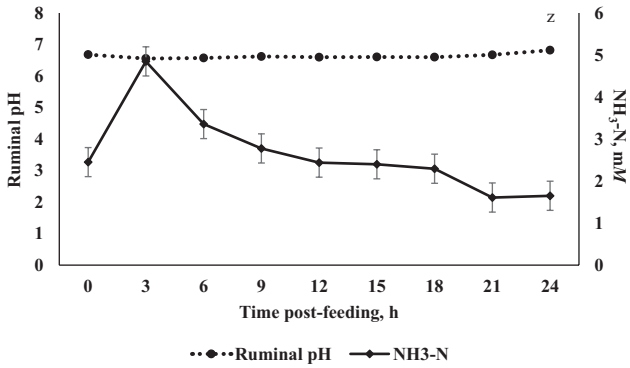

Figure 1. Effect of time postfeeding on ruminal pH ($P = 0.65$; dotted line; SEM = 0.0402; $n = 8$ steers/treatment) and concentration of ruminal ammonia–nitrogen (NH_3-N, m*M*; $P < 0.01$; solid line; SEM = 0.3465; $n = 8$ steers/treatment) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum and supplemented with different sources of protein.

Table 4. Effect of protein supplementation on ruminal fermentation parameters and blood profile of ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum

	Treatment[2]					*P*-value[4]		
Item[1]	BCM	CSM	DDGS	SBM	SEM[3]	TRT	TIME	TRT × TIME
Ruminal pH	6.63	6.63	6.61	6.67	0.044	0.65	<0.01	0.38
NH_3-N, m*M*	2.27	2.75	2.37	3.18	0.458	0.38	<0.01	0.60
PUN, mg/dL	8.87[b]	9.21[b]	6.85[c]	10.86[a]	0.532	<0.01	0.09	0.09
Glucose, m*M*	3.67	3.63	3.63	3.72	0.045	0.37	<0.01	0.99

[a–c]Within a row, means with different superscripts differ, $P \leq 0.05$.

[1]Ruminal fluid and blood samples were collected every 3 h for 24 h; NH_3-N = ruminal ammonia nitrogen, PUN = plasma urea nitrogen, glucose = plasma glucose.

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

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

[4]Observed significance levels for treatment (TRT) and time postfeeding (TIME) effects, and for their interaction (TRT × TIME).

ruminal NH_3-N and blood urea nitrogen (**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, thus indicating a transition range in which concentration of BUN is correlated to ADG and N efficiency (i.e., N utilization was more efficient in steers with concentrations of BUN below the 9 mg/dL value, as evidenced by greater ADG, and less efficient in steers with concentrations above the 12 mg/dL value, as evidenced by decreased ADG; Hammond, 1997). The increase in concentrations of PUN observed 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 suggested by Preston et al. (1978) for finishing steers and are therefore potentially more favorable in terms of maximizing ADG while decreasing N loss.

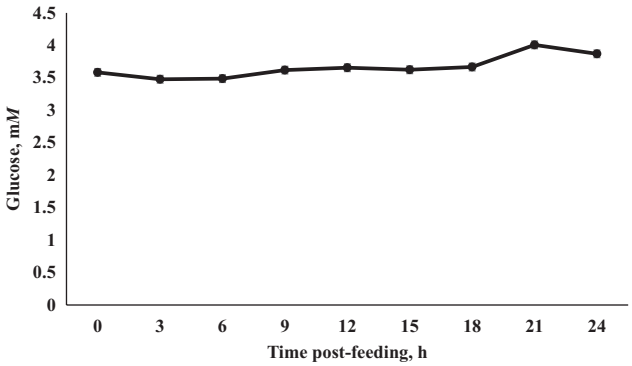

Figure 2. Effect of time postfeeding on concentration of plasma glucose ($P < 0.01$; SEM = 0.0583; $n = 8$ steers/treatment) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum and supplemented with different sources of protein.

Concentrations of plasma glucose ($P = 0.37$) were not different between treatments though an effect of time ($P < 0.01$; Figure 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 ruminal NH_3-N decreases, an increase in glucose is observed (Van Soest, 1994). Both ruminal NH_3-N concentrations and plasma glucose had an effect of time postfeeding ($P < 0.01$); however, the potential connection between these variables is not clear.

Treatment × time interactions ($P < 0.01$) were observed for molar proportions of acetate (Table 5; Figure 3), propionate (Figure 4), and butyrate (Figure 5). A decrease in molar proportions of acetate at 3 h postfeeding 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. At 6 h postfeeding, molar proportions of acetate were greater in steers supplemented with CSM, differing from all treatments except for 18 and 24 h postfeeding. Consequently, an increase in molar proportions of propionate and butyrate at 3 h postfeeding was observed for DDGS supplementation, compared with the remaining protein supplements. As the treatments

Table 5. Effect of protein supplementation on proportions of VFA (mol/100 mol), 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	<0.01	<0.01	<0.01
Propionate	15.75	15.03	16.23	15.37	0.205	<0.01	<0.01	<0.01
Butyrate	7.95	7.57	9.47	8.01	0.144	<0.01	<0.01	<0.01
BCVFA[4]	1.34[ab]	1.20[b]	1.14[b]	1.57[a]	0.063	<0.01	<0.01	0.30
Valerate	0.63	0.59	0.60	0.62	0.016	0.20	<0.01	<0.01
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	0.30
A:P	4.71	5.03	4.49	4.84	0.084	<0.01	<0.01	<0.01

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

[1]BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d); 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.

were provided on an isonitrogenous basis and not isoenergetic, we speculate that the increase in molar proportions of propionate and butyrate together with the simultaneous decrease in molar proportions of acetate at 3 h postfeeding may be related to the additional provision of energy available in DDGS. Molar proportions of propionate gradually declined in DDGS until 12 h, in which no further differences were detected compared with SBM and BCM. Despite the effect of supplementation over time, the increase in molar proportions of propionate did not seem to affect the concentration of plasma glucose, as 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 during certain times of the day may be related to one of the other precursors, however this was not evaluated.

Molar proportions of butyrate were similar in behavior to propionate with an increase observed in DDGS at 3 h and a gradual decline through 15 h postfeeding in which no further differences

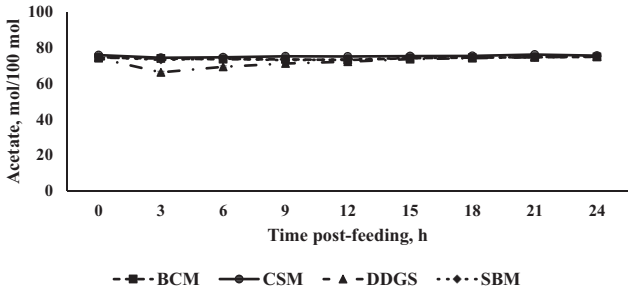

Figure 3. Effect of protein supplementation postfeeding on molar proportions of acetate (mol/100 mol) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.01$; SEM = 0.316; $n = 8$ steers/treatment). BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d).

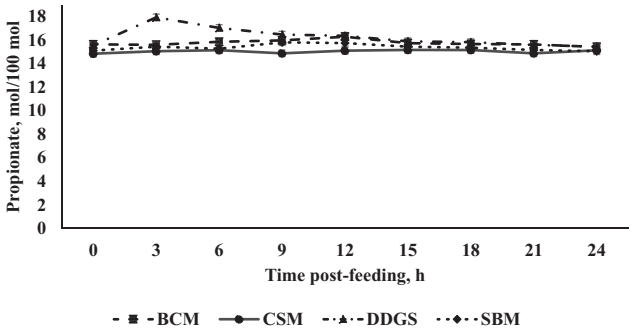

Figure 4. Effect of protein supplementation postfeeding on molar proportions of propionate (mol/100 mol) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.01$; SEM = 0.205; $n = 8$ steers/treatment). BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d).

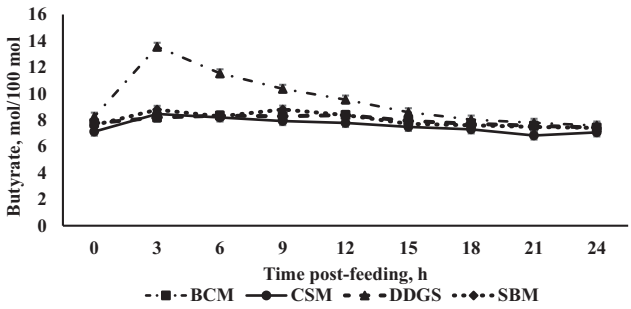

Figure 5. Effect of protein supplementation postfeeding on molar proportions of butyrate (mol/100 mol) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.01$; SEM = 0.144; $n = 8$ steers/treatment). BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d).

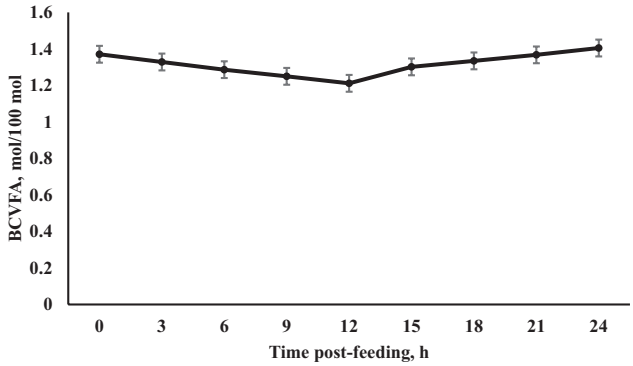

Figure 6. Effect of protein supplementation postfeeding on molar proportions of branched-chain volatile fatty acids (BCVFA; isobutyrate + 2-methylbutyrate; $P < 0.01$; SEM = 0.063; $n = 8$ steers/treatment) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

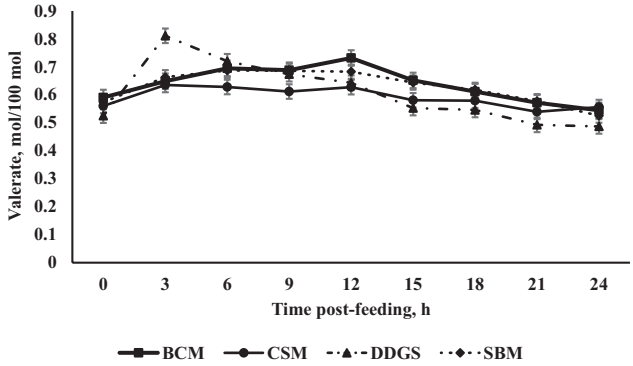

Figure 7. Effect of protein supplementation postfeeding on molar proportions of valerate (mol/100 mol) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.01$; SEM = 0.02; $n = 8$ steers/treatment). BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d).

were detected in treatments. An effect of treatment ($P < 0.01$) and time ($P < 0.01$) was observed for molar proportions of branched-chain volatile fatty acid (BCVFA) while no treatment × time interaction ($P = 0.30$) was detected. Molar proportions of BCVFA were greater ($P < 0.01$) in SBM compared with CSM and DDGS and gradually decreased postfeeding ($P < 0.01$; Figure 6) for all treatments until 12 h. 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 ($P = 0.40$) or time ($P = 0.55$) effects, or treatment × time interaction ($P = 0.15$) was observed for molar proportions of caproate. A treatment × time interaction ($P < 0.01$; Figure 7) was observed for molar proportions of valerate, with a peak at 3 h postfeeding for DDGS, and no further differences detected. Concentrations of total VFA were not affected by treatment ($P = 0.93$), nor was a treatment × time interaction ($P = 0.30$) observed; however, an effect of time was observed ($P = 0.03$; Figure 8) as concentrations of total VFA decreased after feeding through 9 h and increased through 18 h postfeeding. Absorption rates of individual VFA vary with concentrations of VFA or changes in ruminal pH (Dijkstra, 1994); 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 postfeeding, concentration of total VFA did not differ among treatments, indicating similar fermentation patterns, 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 ($P < 0.01$; Figure 9) was observed for the acetate to propionate ratio (A:P), which further reflects the relationship of DDGS and CSM with regards to production of acetate and propionate.

As a novel oilseed crop, *B. carinata* has potential as a renewable and sustainable source of energy due to the favorable fatty acid profile of the oil. The meal would be considered a waste after oil extraction for biofuel production, yet it possesses a high protein concentration, which enables its use as a potential feed byproduct for livestock. There is concern over the adverse effects of glucosinolates when feeding carinata meal, as glucosinolates and subsequent metabolites may negatively affect reproduction, growth, and performance (European Food Safety Authority [EFSA], 2008). The meal used in the current study was low in glucosinolates (28 µmol/g), and as such performed similarly to commonly used protein supplements in the southeastern United States, as it was hypothesized. It is concluded that carinata meal is a viable alternative as a protein supplement when fed daily in forage-fed beef cattle systems. Future studies should determine the fractionation of protein, both ruminally and postruminally, and delineate the amino acid profile of protein reaching the small intestine, contributing to the metabolizable protein available to the ruminant, in an effort to determine the most appropriate use of carinata in various stages of growth and development in cattle.

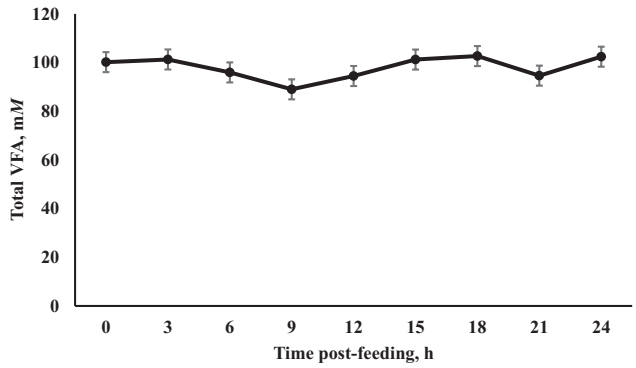

Figure 8. Effect of protein supplementation postfeeding on concentrations of total volatile fatty acids (VFA; $P = 0.03$; SEM = 5.854; $n = 8$ steers/treatment) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum.

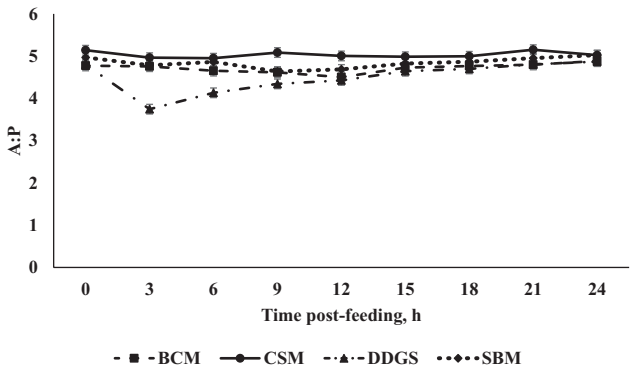

Figure 9. Effect of protein supplementation postfeeding on acetate to propionate ratio (A:P) in ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum. Treatment × time interaction observed ($P < 0.01$; SEM = 0.084; $n = 8$ steers/treatment). BCM = *Brassica carinata* meal pellets (1.39 kg/d); CSM = cottonseed meal (1.62 kg/d); DDGS = dry distillers grain plus solubles (2.15 kg/d); SBM = soybean meal (1.17 kg/d).

LITERATURE CITED

AAFC. Agriculture and Agri-Food Canada. 2015. Carinata: an oilseed ready for lift-off. http://www.agr.gc.ca/eng/news/scientific-achievements-in-agriculture/bioproducts/carinata-an-oilseed-ready-for-lift-off/?id=1389626028671 (Accessed 18 July 2017.)

Allison, M. J. 1978. Production of branched-chain volatile fatty acids by certain anaerobic bacteria. Appl. Environ. Microbiol. 35:872–877.

AOAC. Association of Analytical Communities. 1995. Official methods of analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75. doi:10.3168/jds.S0022-0302(80)82888-8

Chambliss, C. G., and L. E. Sollenberger. 1991. Bahiagrass: the foundation of cow-calf nutrition in Florida. In: Proc. 40th Annual Beef Cattle Short Course. Univ. of Florida, Gainesville, FL. http://animal.ifas.ufl.edu/beef_extension/bcsc/1991/pdf/chambliss.pdf

Cole, N. A., K. McCuistion, L. W. Greene, and F. T. McCollum. 2011. Effects of concentration and source of wet distillers grains on digestibility of steam-flaked corn-based diets fed to finishing steers. Prof. Anim. Sci. 27:302–311. doi:10.15232/S1080-7446(15)30493–9

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39:61–69. doi:10.1016/0301-6226(94)90154–6

Dukes, H. H., M. J. Swenson, and W. O. Reece. 1993. Dukes' physiology of domestic animals. 11th ed. Comstock, Ithaca, NY.

Duncan, A., and J. Milne. 1992. Effect of long-term intraruminal infusion of the glucosinolate metabolite allyl cyanide on the voluntary food-intake and metabolism of lambs. J. Sci. Food Agric. 58:9–14.

EFSA. European Food Safety Authority. 2008. Glucosinolates as undesirable substances in animal feed – scientific opinion of the panel on contaminants in the food chain. EFSA J. 6:590. doi:10.2903/j.efsa.2008.590

Hammond, A. C. 1992. Use of blood urea nitrogen concentration to guide protein supplementation in cattle. In: Proc. 3rd Florida Rumin. Nutr. Symp. Univ. of Florida, Gainesville, FL. p. 9–18.

Hammond, A. C. 1997. Update on BUN and MUN as a guide protein supplementation in cattle. In: Proc. 8th Florida Rumin. Nutr. Symp. Univ. Florida, Gainesville. p. 43–52.

Hersom, M., E. Alva, T. Thrift, and J. Yelich. 2011. Strategic supplementation of beef cows to correct for nutritional imbalances. In: Proc. 22nd Florida Rumin. Nutr. Symp. Univ. Florida, Gainesville, FL. p. 150–163.

Krizsan, S. J., and P. Huhtanen. 2013. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. J. Dairy Sci. 96:1715–1726. doi:10.3168/jds.2012-5752

Lardy, G. P., and M. S. Kerley. 1994. Effect of increasing the dietary level of rapeseed meal on intake by growing beef steers. J. Anim. Sci. 72:1936–1942.

Littell, R. C., P. R. Henry and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Dep. Stat. Anim. Sci., Univ. Florida, Gainesville, FL. p. 42–46

Marillia, E-F., T. Francis, K. C. Falk, M. Smith, and D. C. Taylor. 2013. Palliser's promise: *Brassica carinata*, an emerging western Canadian crop for delivery of new bio-industrial oil feedstocks. Biocatal. Agric. Biotechnol. 3:65–74. doi:10.1016/j.bcab.2013.09.012

McBride, W. D., K. H. Matthews, and United States Department of Agriculture. 2011. The diverse structure and organization of U.S. beef cow-calf farms. U.S. Dept. Agric. Washington, DC.

Moore, J. E. and W. E. Kunkle. 1995. Improving forage supplementation programs for beef cattle. In: Proc. 6th Florida Rumin. Nutr. Symp. Univ. Florida, Gainesville, FL. p. 65–74.

Moore, J. E., W. E. Kunkle, and W. F. Brown. 1991. Forage quality and the need for protein and energy supplements. In: Proc. 40th Florida Beef Cattle Short Course. Univ. Florida, Gainesville, FL.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.

Preston, R. L., F. Byers, and K. R. Stevens. 1978. Estrogenic activity and growth stimulation in steers fed varying protein levels. J. Anim. Sci. 46:541–546. doi:10.2527/jas1978.462541x

Rodriguez-Hernandez, K., and J. L. Anderson. 2018. Evaluation of carinata meal as a feedstuff for growing dairy heifers: effects on growth performance, rumen fermentation, and total-tract digestibility of nutrients. J. Dairy Sci. 2:1206–1215. doi:10.3168/jds.2017-13584

Ruiz-Moreno, M., E. Binversie, S. W. Fessenden, and M. D. Stern. 2015. Mitigation of in vitro hydrogen sulfide production using bismuth subsalicylate with and without monensin in beef feedlot diets. J. Anim. Sci. 93:5346–5354. doi:10.2527/jas.2015-9392

Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low ph? J. Dairy Sci. 79:1503–1509. doi:10.3168/jds.S0022-0302(96)76510-4

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32:199–208. doi:10.1079/BJN19740073

Schulmeister, T. M., M. Ruiz-Moreno, J. Benitez, M. Garcia-Ascolani, F. M. Ciriaco, D. D. Henry, G. C. Lamb, J. C. B. Dubeux and N. DiLorenzo. 2015. Evaluation of *Brassica carinata* as a protein supplement for growing beef heifers. In: 2015 Florida Beef Research Report. p. 137–141.

Seepaul, R., S. George, and D. Wright. 2016. Comparative response of *Brassica carinata* and b-napus vegetative growth, development and photosynthesis to nitrogen nutrition. Ind Crops Prod. 94:872–883. doi:10.1016/j.indcrop.2016.09.054

Van Soest, P. J. 1994. Nutritional ecology of the ruminant, 2nd ed. Comstock Publ., Ithaca, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Xin, H. and P. Yu. 2014. Rumen degradation, intestinal and total digestion characteristics and metabolizable protein supply of carinata meal (a non-conventional feed resource) in comparison with canola meal. Anim. Feed Sci. Technol. 191:106–110. doi:10.1016/j.anifeedsci.2014.01.013