Journal of Animal Science, 2021, Vol. 99, No. 1, 1–8

doi:10.1093/jas/skaa383
Advance Access publication November 28, 2020
Received: 31 July 2020 and **Accepted:** 23 November 2020
Ruminant Nutrition

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Characterization of dietary protein in *Brassica carinata* meal when used as a protein supplement for beef cattle consuming a forage-based diet

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Abstract

As a novel oilseed crop in Florida, *Brassica carinata* has the capacity of producing high-quality jet biofuel, with a protein-dense meal (~40% crude protein; **CP**) obtained as a by-product of oil extraction. Characterization of the meal protein is limited, yet necessary for formulation of beef cattle diets; therefore, the objective of this experiment was to determine ruminal and postruminal digestibility of protein from *B. carinata*. Eight ruminally cannulated Angus crossbred steers (473 ± 119 kg) were used in a duplicated 4 × 4 Latin square design, in which in situ ruminal and postruminal degradability of nutrients were evaluated. The three-step in vitro procedure was used to compare CP and amino acid (**AA**) degradation in *B. carinata* meal pellets (**BCM**) with that of cottonseed meal (**CSM**), dry distillers grains with solubles (**DDGS**), and soybean meal (**SBM**). In situ bags were incubated in the rumen for 0 to 96 hr, with the undegraded supplement remaining after 16 hr subjected to serial in vitro enzymatic solutions. Data were analyzed using the MIXED procedure of SAS. Ruminal rate of degradation of dry matter, organic matter, and CP was greatest ($P < 0.01$; 10.9, 11.3, and 11.5 %/h, respectively) for SBM. Rumen degradable protein (**RDP**) content did not differ ($P = 0.20$; 47.8% and 55.1%, respectively) between CSM and DDGS, but was decreased ($P < 0.01$) compared with SBM and BCM, which did not differ ($P = 0.99$; 72.3% and 71.8% RDP, respectively). Compared with DDGS, SBM had greater ($P < 0.01$) intestinal digestibility of rumen undegradable protein (**RUP**). Intestinally absorbable digestible protein (**IADP**) was greatest ($P < 0.01$) for CSM, with SBM and BCM having the least IADP. Total tract digestibility of CP (**TTDP**) was greater ($P < 0.01$) for SBM compared with CSM and DDGS. The contribution of RUP to intestinally absorbable AA was 7.2 and 3.1 g of lysine and methionine per kilogram of CP in BCM, respectively. The evaluation of *B. carinata* meal as protein supplemented for cattle consuming a forage-based diet resulted in 71.8% RDP and 97.1% TTDP, thus indicating its viability as a high-quality protein supplement for beef cattle.

Key words: amino acid, beef cattle, *Brassica carinata*, protein

Abbreviations

AA	amino acid
ADF	acid detergent fiber
aNDF	neutral detergent fiber analyzed using heat stable α-amylase
BCM	*Brassica carinata* meal pellets
BW	body weight
CP	crude protein
CSM	cottonseed meal
DDGS	dry distillers grains plus solubles
DM	dry matter
IADP	intestinally absorbable digestible protein
IDP	estimated intestinal protein digestibility
NFC	non-fibrous carbohydrate
OM	organic matter
RDP	rumen degradable protein
RUP	rumen undegradable protein
TDN	total digestible nutrients
TTDP	apparent total tract digestibility of dietary proteins
SBM	soybean meal

Introduction

In the southeastern United States, the use of carinata (*Brassica carinata*) in crop rotation and as a cover crop is increasing due to its heat and drought tolerance, and cold and disease resistance (AAFC, 2015; Seepaul et al., 2016). As a nonfood oilseed crop, carinata possesses a favorable very long chain fatty acid composition for conversion to biofuel (Marillia et al., 2013) and has been successfully utilized as a 100% drop-in jet biofuel. The meal obtained as a byproduct of oil extraction would be considered waste, yet the protein-dense meal (~40% crude protein; **CP**) of carinata has the potential to be utilized as a protein supplement in beef cattle operations. Utilization of the plant, oil from the seed, and residual meal promotes the use of carinata as a renewable, and potentially sustainable, resource (AAFC, 2015).

Data regarding supplementation of carinata meal as a protein source to cattle are limited. A comparison of ruminal degradation kinetics, and intestinal and total digestion of nutrients between canola and carinata was evaluated in dry Holstein cows, resulting in carinata having a similar profile to canola (Xin and Yu, 2014). When supplementing carinata, compared with soybean meal (**SBM**), cottonseed meal, or dried distillers grains plus solubles, to beef steers consuming bahiagrass hay (*Paspalum notatum*), it was observed that carinata performed similarly to SBM in ruminal metabolism and apparent total tract digestibility of nutrients (Schulmeister et al., 2019a). Beef heifers consuming bermudagrass hay (*Cynodon dactylon*) supplemented with carinata had increased average daily gain compared with heifers consuming hay only, and no differences in attainment of puberty or treatment effects on concentrations of thyroid hormones or acute phase proteins were detected (Schulmeister et al., 2019b).

The southeastern United States 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 forages with limited protein content (Stewart et al., 2007), necessitating the supplementation of high-quality protein and amino acids (**AA**). It was hypothesized that carinata would have a similar protein profile to SBM, based upon previous research. The objective of this experiment was to characterize the ruminal fractionation of dietary protein, and subsequent postruminal degradation of dietary protein in carinata meal compared with protein supplements common to the southeastern United States, and to determine the AA profile of carinata upon ruminal and postruminal degradation.

Materials and Methods

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

Experimental design, animals, and treatments

The experiment was conducted at the University of Florida, Feed Efficiency Facility (**FEF**) in Marianna, FL. Eight ruminally cannulated Angus crossbred steers (473 ± 119 kg of initial body weight; **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: 1.39 kg/d *B. carinata* meal pellets (**BCM**), 1.62 kg/d cottonseed meal (**CSM**), 2.15 kg/d dry distillers grains plus solubles (**DDGS**), or 1.17 kg/d SBM. Treatments were provided daily in a feed tub within each pen and were consumed entirely, shortly after being offered. Supplementation of BCM was provided at 0.3% of initial BW, based on required CP values for growing steers (NASEM, 2016). Treatments were calculated to be isonitrogenous based on total nitrogen (**N**) provided by supplementation of 1.39 kg/d of BCM. On day 0, steers were weighed, after being fasted from feed and water for 16 hr, and housed individually in pens at the FEF with ad libitum access to water and bahiagrass hay (*P. notatum*). Each pen at the FEF was equipped with 2 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 treatment supplements from days 0 to 14, and a ruminal in situ degradability procedure was conducted from days 21 to 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 hr. Following ruminal incubation, the undegraded supplement, after 16 hr of incubation, was subjected to serial solutions simulating postruminal digestion (Calsamiglia and Stern, 1995; Gargallo et al., 2006), with subsequent analysis of concentration of CP and determination of the BCM AA profile.

Laboratory analyses

Ruminal in situ dry matter (**DM**) disappearance of treatments was determined using duplicate bags within steer. Supplement samples were collected at the beginning of each period, dried for 48 hr at 55 °C, and weighed (5.0 g) into 10 × 20 cm Ankom in situ bags (R1020, Ankom Technology, 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 (Bar Diamond, Parma, ID) after soaking in warm (39 °C) water for 15 min. All bags were placed in the rumen simultaneously, and incubated for 0, 3, 6, 9, 12, 16, 24, 48, 72, and 96 hr, except for the 0 hr bag, which was soaked in 39 °C water for 15 min to determine the soluble fraction of protein. Bags were removed at the predetermined

times, placed in ice water for transport, 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 hr at 55 °C and weighed. Residues from the in situ incubation were composited by incubation time within steer and composite samples were analyzed for DM, organic matter (**OM**), and CP. The 16 hr bag was removed and analyzed separately to determine intestinally absorbable CP by the three-step procedure (Calsamiglia and Stern, 1995; Gargallo et al. 2006).

In situ samples were weighed (0.50 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 hr to determine OM. To characterize the treatments offered, carinata meal pellets and bahiagrass hay subsamples were composited within treatment, dried, ground, and sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY.) to be analyzed for nutrient composition using the procedures described in their January 2020 update (https://dairyone.com/download/forage-forage-lab-analytical-procedures). Total digestible nutrients (**TDN**) were calculated by Dairy One Forage Laboratory, and non-fibrous carbohydrate (**NFC**, %) was calculated as: NFC = 100 – (CP, % + Neutral Detergent Fiber, % + Ash, % + ether extract, %).

Determination of intestinally absorbable CP was analyzed according to the three-step procedure (Calsamiglia and Stern, 1995) with the modifications suggested by Gargallo et al. (2006) to be able to analyze the undigested sample to determine AA composition. Briefly, the 16 hr bag was removed from the rumen, rinsed with tap water until runoff was clear, and dried in a forced-air oven at 55 °C for 48 hr. Contents of ruminal in situ residue bags were composited and analyzed for AA profile (University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO), DM, OM, and CP. Contents were then weighed into 5 × 10 cm nylon bags (Ankom R510, pore size 50 µm; Ankom Technology; Boucher et al., 2009) in duplicate, heat sealed, and suspended in a Daisy^{II} incubator (Ankom Technology) with a 2-L solution of prewarmed 0.1 N HCl solution (pH 1.8) containing 1 g/L of pepsin (P-3000, Sigma, St. Louis, MO) at 39 °C for 1 hr, 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 prewarmed pancreatin solution (0.5 M KH_2PO_4 buffer, pH 7.7, containing 50 mg/L of thymol and 3 g/L of pancreatin; Sigma P-7545) for 24 hr at 39 °C, under constant rotation. After incubation, bags were removed from solution, rinsed with tap water until runoff was clear, and dried in a forced-air oven at 55 °C for 48 hr. 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 Micro Cube, Elementar Analysensysteme GmbH Langenselbold, Germany) following official method 992.15 (AOAC, 1995)] and sent for AA profile analysis (University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO) following official method 982.30 (AOAC, 2005).

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, NY). The model used was:

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

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

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

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

where x = nutrient evaluated, SF = soluble fraction, which is the proportion of material that washed out from the bags without rumen incubation (0 hr), and K_p = fractional rate of passage, assumed to be 5%/hr in this experiment (Foster et al., 2011).

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

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

where A = soluble fraction of CP that disappeared at 0 hr after the rinsing procedure, B = potentially degradable CP, K_d and K_p are degradation constants described previously. Estimated rumen undegradable protein (**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 hr) × % 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):

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

where IS (N) = N content of the rumen-exposed in situ residue, P:P (N) = N content of the pepsin-pancreatin residue, and S (N) = N content of the sample.

In situ digestibility and three-step procedure data were analyzed as a duplicated 4 × 4 Latin square using the MIXED procedure of SAS. 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 1. As an oilseed crop, carinata contains glucosinolates which have the potential to affect palatability (van Doorn et al., 1998), intake, and AA absorption (Barry, 2013) when fed at great concentrations (90 to 140 µmol/g; Lardy and Kerley, 1994). The glucosinolate content of the carinata meal is shown in Table 2. Concentrations of nutrients for CSM, SBM, and DDGS were comparable with published values, with exception to a slightly reduced DM content in DDGS (NASEM, 2016). Ruminal in situ degradation rate of the potentially degradable fraction (Table 3) of DM, OM, and CP was greatest ($P < 0.01$) for SBM. The potentially degradable fraction of DM was greater ($P < 0.01$) for SBM and CSM compared

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

		Treatment[2]			
Item, % DM	**Bahiagrass hay[3]**	**BCM**	**CSM**	**DDGS**	**SBM**
DM, % as fed	94.0	89.8	88.9	86.3	90.7
CP	7.2	43.3	49.2	32.8	52.9
NFC	—[4]	21.7	13.2	20.2	28.7
aNDF	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; provided by Agrisoma Biosciences Inc., Gatineau, QC); 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]Bahiagrass hay (*Paspalum notatum*).
[4]—, this item was not analyzed.

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

Glucosinolate	**BCM[2] (μmol/g)**	**SD[3]**
Allyl	26.86	3.542
3-Butenyl	0.51	0.088
4-Pentenyl	0.09	0.026
2-OH-3-Butenyl	0.45	0.086
CH_3-Thiobutenyl	0.09	0.005
Phenylethyl	0.12	0.014
CH_3-Thiopentenyl	0.19	0.077
3-CH_3-Indolyl	0.08	0.007
4-OH-3-CH_3-Indolyl	0.21	0.039

[1]Analysis conducted by POS Bio-Science (Saskatoon, SK, Canada).
[2]BCM: *Brassica carinata* meal pellets provided by Agrisoma Biosciences, Inc. (Gatineau, QC).
[3]Standard deviation of analyzed sample values, $n = 5$.

with DDGS, despite a greater ($P < 0.01$) soluble fraction of DM for DDGS compared with CSM. No differences were observed in the potentially degradable or soluble fraction between SBM and BCM. A delay in lag time of DM was observed ($P < 0.01$) in CSM and SBM, compared with BCM. More time was required ($P < 0.01$) for ruminal OM degradability for CSM and SBM compared with DDGS. This was 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 greater in SBM. While BCM did not differ from other treatments ($P > 0.10$) in both lag time and the potentially degradable fraction of OM, BCM tended ($P = 0.07$) to have a greater soluble fraction. Dietary CP in BCM and SBM required less lag time ($P < 0.01$) than CSM to begin ruminal degradation, and BCM had the greatest ($P < 0.01$) soluble fraction of CP; however, the potentially degradable fraction of CSM was greater ($P < 0.01$) compared with BCM and DDGS. The ruminally undegradable fraction of DM ($P = 0.20$) and CP ($P = 0.24$) did not differ between treatments (Table 4).

RDP content did not differ ($P = 0.20$; 47.8% and 55.1% RDP, respectively) between CSM and DDGS, but was decreased ($P < 0.01$) compared with SBM and BCM, which were similar ($P = 0.99$; 72.3% and 71.8% RDP, respectively). The RDP for CSM was less than published values reported in the NASEM, while the RDP for DDGS was greater than reported values (NASEM, 2016). Protein fractionation for SBM was similar to published values for RDP and RUP (NASEM, 2016). Compared with DDGS, SBM had a greater 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 ($P < 0.01$) for SBM compared with CSM and DDGS. 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; NASEM, 2016), supporting the data observed in the current experiment. Similar in proportions of RDP and RUP, BCM has a decreased rate of degradation compared with SBM, but a greater soluble fraction contributing to an increase in RDP (Table 3).

Metabolizable protein (**MP**) is defined as the true protein digested in the intestine, supplied by microbial protein and RUP (NASEM, 2016). Though MP is the common nomenclature, TTDP has also been utilized in various studies; nonetheless, the concept is the same. 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 (NASEM, 2016). Estimates of RDP and RUP observed in the current experiment resulted from ruminal incubation for 16 hr, considered to be the mean residence time of dietary protein in the rumen (Calsamiglia and Stern, 1995). The NRC (1996, 2001) assumes an 80% intestinal digestibility of RUP as a result of insufficient information regarding digestibility; however, to accurately predict MP, valid estimates are necessary. Consequently, intestinal digestibility values for RUP (IDP) or MP are not available in the NASEM (2016). Erasmus et al. (1994) observed an ~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 experiment for SBM, but further illustrates the variability in intestinal digestibility of substrates.

Determining the protein fractionation of supplements is important in formulating rations for cattle; however, the availability of AA postruminally is of greater interest as these will be available as a portion of the MP (Merchen and Titgemeyer, 1992). Additionally, while dietary protein is important, balancing AA requirements may be more effective in meeting cattle needs and decreasing protein rations (Patton et al., 2014). The AA composition of BCM in the original feed sample, 16 hr rumen incubation sample, and postruminal incubation residue is presented in Table 5. The total tract digestibility of individual AA, ruminally and postruminally, is presented in Table 6, with the contribution of RUP to intestinally absorbable AA (IAAA). Previous research on the fractionation and characterization of

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

	Treatment[1]					P-value[2]
Item[3]	BCM	CSM	DDGS	SBM	SEM[4]	TRT
DM						
K_d, %/hr	6.61[b]	2.85[c]	5.16[bc]	10.87[a]	0.929	<0.01
T0, hr	0.53[b]	2.87[a]	1.20[ab]	2.52[a]	0.530	<0.01
SF, %	42.97[b]	32.25[c]	48.81[a]	40.70[b]	1.580	<0.01
D, %	54.74[ab]	59.78[a]	45.83[b]	59.11[a]	3.223	<0.01
Undeg, %	2.38	7.98	5.36	0.12	3.063	0.20
OM						
K_d, %/hr	6.71[b]	2.54[c]	5.31[bc]	11.27[a]	0.888	<0.01
T0, hr	0.99[ab]	2.64[a]	0.78[b]	2.68[a]	0.613	<0.01
SF, %	7.49	5.28	5.96	2.33	1.608	0.06
D, %	92.48	94.68	93.95	97.65	1.604	0.06
Undeg, %	0.03[ab]	0.04[ab]	0.09[a]	0.01[b]	0.017	0.03
CP						
K_d, %/hr	7.59[b]	3.86[c]	4.68[bc]	11.50[a]	0.877	<0.01
T0, hr	0.87[b]	8.89[a]	3.44[ab]	2.80[b]	1.779	<0.01
SF, %	22.10[a]	0.24[d]	15.66[b]	7.78[c]	1.929	<0.01
D, %	76.80[bc]	98.70[a]	74.67[c]	88.60[ab]	3.771	<0.01
Undeg, %	0.69	1.06	9.67	3.94	3.876	0.24

[1]BCM, *Brassica carinata* meal pellets (1.39 kg/d); CSM, cottonseed meal (1.62 kg/d; provided by Agrisoma Biosciences Inc., Gatineau, QC); 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).
[3]K_d, rate of degradation of fraction *D*, T0, Lag time, SF, soluble fraction, *D*, potentially degradable fraction, and Undeg, undegradable fraction.
[4]Pooled standard error of treatment means, *n* = 8 steers/treatment.
[a–d]Within a row, means with different superscripts differ, $P < 0.05$.

Table 4. Dietary protein characterization of supplements fed to ruminally cannulated Angus crossbred steers fed bahiagrass hay ad libitum

	Treatment[1]					P-value[2]
Item[3]	BCM	CSM	DDGS	SBM	SEM[4]	TRT
RDP, % CP	71.8[a]	47.8[b]	55.1[b]	72.3[a]	3.30	< 0.01
RUP, % CP	28.2[b]	52.29[a]	44.9[a]	27.79[b]	3.30	< 0.01
IDP, % RUP	89.9[ab]	89.9[ab]	85.4[b]	94.5[a]	2.19	< 0.01
IADP, % CP	25.2[c]	46.9[a]	36.5[b]	26.4[c]	2.85	< 0.01
TTDP, % CP	97.1[ab]	94.8[bc]	93.7[bc]	98.7[a]	0.96	< 0.01

[1]BCM, *Brassica carinata* meal pellets (1.39 kg/d; provided by Agrisoma Biosciences Inc., Gatineau, QC); CSM, cottonseed meal (1.62 kg/d); DDGS, dry distillers grain plus solubles (2.15 kg/d); SBM, soybean meal (1.17 kg/d).
[2]Observed significance levels for treatment (TRT).
[3]RDP (rumen degradable protein), $A + B\ [K_d/(K_d + K_p)]$, where K_p is the rate of passage from the rumen, estimated to be 5%/hr and K_d, rate of degradation of fraction *D*; RUP (rumen undegradable protein), 100 – % RDP; IDP, estimated intestinal protein digestibility; IADP, intestinally absorbable digestible protein (IDP × RUP); TTDP, apparent total tract digestibility of digestible protein (TTDP, RDP + IADP).
[4]Pooled standard error of treatment means, *n* = 8 steers/treatment.
[a–c]Within a row, means with different superscripts differ, $P < 0.05$.

protein in *B. carinata* (Xin and Yu, 2014) resulted in values of RUP and TTDP of 123 and 358 g/kg of DM, respectively. Upon initial evaluation, these values seem less than the current values, but the original concentration of CP in carinata meal or the diet used by Xin and Yu (2014) was not presented, the method of oil extraction was not mentioned, dry Holstein ruminally cannulated cows were used, and a total mixed ration (forage:concentrate = 78:22) was fed. The differences in these factors may contribute to the discrepancies in the values presented by Xin and Yu (2014) and those observed in this experiment.

Mjoun et al. (2010) compared fractionation of protein and subsequent AA profiles in distillers grains products and common SBM products, utilizing the in situ technique and modified three-step procedure described by Gargallo et al. (2006). The RDP and RUP values for SBM (67.7% and 32.3% of CP, respectively) and DDGS (47.7% and 52.3% of CP, respectively) in lactating Holstein cows consuming a total mixed ration (forage:concentrate = 45:55) 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 (Mjoun et al., 2010), confirming the values observed in the current experiment.

The total tract digestibility of essential AA of BMP and contribution of RUP to IAAA is presented in Table 7. Production

Table 5. AA composition of *B. carinata* meal pellets in original meal pellets, ruminally incubated residue, and postruminal residue

	AA composition[1] (w/w%)				
AA	BCM[2]	In situ 16 hr residue[3]	SD[4]	Postruminal digestion residue[5]	SD[6]
Taurine	0.10	0.13	0.011	0.15	0.005
Hydroxyproline	0.24	0.53	0.144	0.93	0.068
Aspartic acid	2.39	2.95	0.256	0.78	0.075
Threonine	1.43	1.83	0.147	0.58	0.048
Serine	1.28	1.59	0.126	0.51	0.051
Glutamic acid	6.68	6.36	0.561	1.08	0.127
Proline	2.24	2.18	0.181	0.83	0.070
Glycine	1.80	1.98	0.166	0.57	0.072
Alanine	1.55	1.92	0.175	0.45	0.057
Cysteine	0.97	0.85	0.136	0.35	0.031
Valine	1.83	2.42	0.214	0.72	0.064
Methionine	0.70	0.80	0.072	0.15	0.020
Isoleucine	1.52	1.94	0.178	0.60	0.052
Leucine	2.58	3.10	0.302	0.72	0.082
Tyrosine	0.91	1.27	0.118	0.38	0.032
Phenylalanine	1.49	1.90	0.182	0.49	0.058
Hydroxylysine	0.05	0.03	0.005	0.03	0.007
Ornithine	0.02	0.04	0.005	0.01	0.005
Lysine	1.61	1.88	0.155	0.57	0.048
Histidine	0.98	0.95	0.085	0.19	0.024
Arginine	2.51	2.44	0.235	0.44	0.067

[1]AA profiles analyzed by University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO.
[2]Original *B. carinata* meal pellets (BCM; ground) as supplied by Agrisoma Biosciences, Inc., Gatineau, QC.
[3]Ruminal disappearance (%) at 16 hr of incubation using in situ technique.
[4]Standard deviation of in situ 16 hr residue analyzed sample values, $n = 8$.
[5]Postruminal disappearance (%) using modified three-step procedure.
[6]Standard deviation of postruminal digestion residue analyzed sample values, $n = 8$.

Table 6. Apparent total tract digestibility of AA from *B. carinata* meal pellets[1] and contribution of RUP to IAAA

	AA digestibility[2]					
Total AA composition	In situ 16 hr residue[3] (%)	SD[4]	Postruminal digestion residue[5] (%)	SD[6]	Contribution of RUP to IAAA[7] (g/kg CP)	SD[8]
Taurine	78.80	10.07	68.87	12.74	0.39	0.25
Hydroxyproline	67.99	10.73	50.75	16.53	1.07	0.65
Aspartic acid	79.92	10.97	92.69	3.07	11.29	6.58
Threonine	79.26	10.95	91.22	3.52	6.89	3.93
Serine	79.99	10.41	91.21	3.60	5.95	3.35
Glutamic acid	84.55	8.33	95.33	1.90	24.82	13.94
Proline	84.29	8.24	89.49	4.17	8.04	4.64
Glycine	82.12	9.60	92.19	3.30	7.54	4.33
Alanine	79.85	10.98	93.64	2.53	7.40	4.26
Cysteine	86.09	7.07	88.77	4.09	3.06	1.73
Valine	78.60	11.44	91.87	3.21	9.14	5.25
Methionine	81.56	9.86	94.84	2.16	3.09	1.73
Isoleucine	79.28	11.20	91.50	3.26	7.33	4.26
Leucine	80.52	10.52	93.73	2.36	11.91	6.79
Tyrosine	77.18	12.65	91.75	3.30	4.85	2.89
Phenylalanine	79.36	11.09	93.01	2.78	7.25	4.14
Hydroxylysine	91.80	5.09	60.04	21.96	0.07	0.06
Ornithine	69.54	16.71	89.35	5.61	0.14	0.09
Lysine	80.92	10.54	91.55	3.50	7.16	4.26
Histidine	84.28	8.51	94.64	2.24	3.68	2.09
Arginine	84.19	8.61	95.15	2.09	9.53	5.41
Total					140.61	80.15

[1]*Brassica carinata* meal pellets as supplied by Agrisoma Biosciences, Inc., Gatineau, QC.
[2]AA digestibility was calculated as [((initial sample DM × original BCM AA profile) – (16 hr residue × sample DM remaining))/(initial sample DM × original BCM AA profile)].
[3]Ruminal disappearance (%) at 16 hr of incubation using in situ technique.
[4]Standard deviation of in situ 16 hr residue analyzed sample values, $n = 8$.
[5]Postruminal disappearance (%) using modified -step procedure.
[6]Standard deviation of postruminal digestion residue analyzed sample values, $n = 8$.
[7]Contribution of RUP to IAAA is defined as (100 – % rumen degradability at 16 hr) × (% intestinal disappearance in situ) × AA concentrations in feed/10.
[8]Standard deviation of the contribution of RUP to IAAA analyzed sample values, $n = 8$.

Table 7. Apparent total tract digestibility of essential AA of *B. carinata* meal pellets[1] and contribution of RUP to IAAA

	AA digestibility[2]			
Essential AA composition	In situ 16 hr residue[3] (%)	Postruminal digestion residue[4] (%)	Contribution of RUP to IAAA[5] (g/kg CP)	SD[6]
Arginine	84.19	95.15	9.53	5.41
Histidine	84.28	94.64	3.68	2.09
Isoleucine	79.28	91.50	7.33	4.26
Leucine	80.52	93.73	11.91	6.79
Lysine	80.92	91.55	7.16	4.26
Methionine	81.56	94.84	3.09	1.73
Phenylalanine	79.36	93.01	7.25	4.14
Threonine	79.26	91.22	6.89	3.93
Valine	78.60	91.87	9.14	5.25
Total			65.98	37.87

[1]*Brassica carinata* meal pellets as supplied by Agrisoma Biosciences, Inc., Gatineau, QC.
[2]AA digestibility was calculated as [((initial sample DM × original BCM AA profile) – (16 hr residue × sample DM remaining))/(initial sample DM × original BCM AA profile)].
[3]Ruminal disappearance (%) at 16 hr of incubation using in situ technique.
[4]Postruminal disappearance (%) using modified three-step procedure.
[5]Contribution of RUP to IAAA is defined as (100 – % rumen degradability at 16 hr) × (% intestinal disappearance in situ) × AA concentrations in feed/10.
[6]Standard deviation of the contribution of RUP to IAAA analyzed sample values, $n = 8$.

of microbial crude protein alone, resulting from RDP, may be insufficient in supplying adequate amounts of AA for optimal production (Kung and Rode, 1996), especially during periods of rapid growth in cattle and high rates of production (Klopfenstein et al., 1978). Thus, limiting AA, such as methionine and lysine, are of more concern and should be supplied as RUP in order to meet the dietary requirements of ruminants when production levels necessitate the addition. Depending upon the diet fed, the postruminal 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 carinata is important in order to synchronize the supplementation of energy, protein, and AA when using a variety of feedstuffs.

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, *B. 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 *B. 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.

Acknowledgments

The authors gratefully acknowledge Agrisoma Biosciences, Inc. (Gatineau, QC) for donating the *Brassica carinata* meal pellets used in this experiment. Appreciation also is expressed to J. P. R. Costa, M. Bueno, O. Helms, J. Folsom, M. Foran, D. Jones, C. Nowell, C. Wood, M. Morgan, and D. Thomas (University of Florida) for their assistance with data collection and laboratory analyses.

Funding

Funding for this research was partially provided by the Florida Department of Agriculture and Consumer Services, grant # 00112160, and by the SPARC Coordinated Agricultural Project (grant no. 2016-11231) from the USDA National Institute of Food and Agriculture.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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