Nutrient profile and effects of carinata meal as alternative feed ingredient on broiler performance, tight junction gene expression and intestinal morphology

Sudhir Yadav, Po-Yun Teng, Janghan Choi, Amit Kumar Singh, and Woo Kyun Kim[1]

Department of Poultry Science, University of Georgia, Athens, GA, USA

ABSTRACT Two studies were conducted to establish carinata meal as a partial replacement of conventional protein sources. Study I was conducted to determine the nutrient profile, nitrogen-corrected true metabolizable energy (**TME_n**), and amino acid (**AA**) digestibility of 2 groups: low glucosinolate carinata meal (**LGCM**) and high glucosinolate carinata meal (**HGCM**) using rooster assays. The LGCM contained 28 μmol/g glucosinolate, 11.5% moisture, 39.2% crude protein (**CP**), whereas the HGCM had 100 μmol/g glucosinolate, 10.1% moisture, 39.5% CP on as is basis. The precision-fed rooster assays were conducted to determine TME_n and AA digestibility. The TME_n levels of LGCM and HGCM were 1,814 and 1,690 kcal/kg on as is basis, respectively. Standardized digestibility for lysine, methionine, cysteine, threonine, and valine were 72, 88, 69, 75, and 79% for LGCM and 80, 89, 71, 76, and 80% for HGCM, respectively. Based on the nutrient profiles from study I, study II was conducted to evaluate the effects of LGCM and HGCM in broilers. A total of 504 one-day-old Cobb500 male broiler chickens were randomly divided into 42 battery cages with 6 replicates of 12 birds per cage. The seven dietary treatments were control diet, 3 inclusion levels of LGCM (4, 8, and 12%), and 3 of HGCM (4, 8, and12%) in a corn-SBM based diet fed for 21 d. No significant differences in BW, BWG, and FI were observed except for significantly lower BWG in 12% HGCM group compared to control for 14-21 days ($P < 0.05$). The FCR for 12% HGCM increased significantly compared to 4 and 8% of both LGCM and HGCM groups during wk 3 (14−21 d). Based on these studies, carinata meal could be recommended to partially replace conventional feed ingredients at a rate of 12% when LGCM is used and 8% when HGCM is used with no deleterious effects on growth performance, gut histology, and tight junction proteins.

Key words: broiler chickens, carinata meal, digestibility, glucosinolate, growth performance

2022 Poultry Science 101:101411
https://doi.org/10.1016/j.psj.2021.101411

INTRODUCTION

Poultry is one of the largest agricultural sectors in the world, and poultry meat represents a major source of animal protein for most countries (Kearney, 2010). Feed alone contributes for around 70% of the total expenditures in poultry production (Ravindran, 2013), with corn, wheat, and soybean meal (**SBM**) being the most widely used conventional feedstuffs in chicken diets. However, price and supply of these energy/protein sources have fluctuated substantially in the last decade (Yadav and Jha, 2021). Global market availability and competition among food, feed, and fuel for conventional feedstuffs make necessary to explore and evaluate alternatives for chicken diets (Yadav et al., 2019). Alternative feedstuffs such as cassava, macadamia nut cake, wheat millrun, taro, distiller's dried grains with solubles (**DDGS**), palm kernel meal, and oilseed cakes, although quite variable in their nutrient profiles, are able to reduce feed cost, enhance intestinal health, and improve carcass quality of chicken (Yadav, 2017; Vaddu et al., 2021). In addition, alternative feedstuffs can bridge the gap between supply and demand of the conventional feed stuffs in poultry production. These alternative feedstuffs used in animal feed also help to mitigate feed scarcity and decrease the environmental problem as millions of tons of byproducts/co-products can be converted into valuable animal feed.

Brassica carinata, also known as Ethiopian mustard, is an oilseed crop grown for jet biofuel (Hagos et al., 2020). The co-product produced after extraction of oil is *Brassica carinata* meal (**BCM**), which is of increasing interest to animal nutritionists. The use of agriculture co-product from *B. carinata* as an animal feed ingredient has become increasingly important in achieving cost-effective animal production (Ban et al., 2018;

Received March 2, 2021.
Accepted July 23, 2021.

[1]Corresponding author: wkkim@uga.edu

Hong et al., 2019; Schulmeister et al., 2019). *Brassica carinata* is non-GMO and produced sustainably in Ethiopia, Canada, South America, and North America (Xin and Yu, 2013). Production and price of BCM largely depend on the demand of its oil by the jet plane and aviation industry. It is suitable to grow as a rotation crop along with another main crop. It can be grown in clay- and sandy-type soils in semiarid temperate climate and is also resistant and self-adapting to cold, water stresses, and diseases (Cardone et al., 2003). Hence, semiarid climate regions, in particular the southern prairies of Canada and the Northern plains of the United States, are more suitable for this crop (Xin and Yu, 2013). For biofuel purpose carinata is produced and processed in huge quantity in United States and Canada which ultimately resulted in increased amount of carinata co-products such as carinata meal (Xin and Yu, 2013; Hong et al., 2019). The meal of *Brassica carinata* is high in protein and is obtained after the hexane-solvent oil extraction method for animal feeding.

Previous studies have reported that inclusion of feedstuffs high in glucosinolates, such as BCM or rapeseed cake (**RSC**), can have negative impacts on both animal health as well as overall animal production (Tadelle et al., 2003). Glucosinolates are anti-nutrient factors which have been demonstrated to cause feed intake reduction, iodine deficiency, and hypertrophy of organs such as liver, kidney, and thyroid gland (Tripathi and Mishra, 2007). Glucosinolate metabolites, such as thiocyanates, thiourea and oxazolidithione, have been shown to disrupt iodine availability to thyroid and thus can affect thyroid function (Wallig et al., 2002). The reduced feed intake of glucosinolate-rich food/feed is due to the presence of sinigrin and pro-goitrin which are associated with pungent odor and bitter taste (Fenwick et al., 1983). Severe adverse effects have been reported in laying hens compared to broilers (Fenwick, 1982), and a high-glucosinolate rapeseed meal diet (132.83 μmol/g) in laying hens caused lower egg weight, nutrient digestibility, intestinal absorptive area, and egg internal quality compared to those fed low glucosinolate rapeseed meal (22.67 μmol/g) (Zhu et al., 2019). This might be due to a longer rearing period of laying hens compared to the broilers along with feeding cultivars with varying level of glucosinolate and erucic acid. Previous chicken studies with *Brassica* products have recommended inclusion levels ranging from 5 to 25%, largely because of variable nutrient profiles and different glucosinolate levels present in different *Brassica* products (Oryschak et al., 2020). Ashnie et al. (2015) found that including 7.5 to 15% of *B. carinata* during starter and finisher phase, respectively does not influenced growth performance of broiler chicks. However, various processing techniques have been developed to reduce the glucosinolates in feed and efficiently utilize the feed inclusion levels as has been demonstrated with canola meal, also known as double-zero or double-low (Tripathi and Mishra, 2007).

Despite the inherent disadvantages of high glucosinolate carinata meal, its availability is increasing, with a strong potential as a protein substitute in poultry feedstuffs. Subsequently, glucosinolate metabolites such as isothiocyanates are biologically active and have many beneficial roles. Some of the reported roles of isothiocyanates include regulatory functions in inflammation, stress response, metabolism, antioxidant activities, and broad-spectrum antimicrobial property (Bischoff, 2019). Overall, however, there is limited information to support the nutritional values of BCM as a feed ingredient for poultry, nor is there substantial literature evaluating the optimum inclusion level and effects of BCM on growth performance of chicken.

In an effort to better establish BCM as a viable alternative feed ingredient, we sought to evaluate the growth performance, digestibility, and gut health parameters of birds in response to carinata meal. For this, 2 studies were conducted with low glucosinolate carinata meal (**LGCM**) and high glucosinolate carinata meal (**HGCM**). We hypothesized that the nutrient profile and digestibility of LGCM and HGCM are comparable to other protein sources including soybean meal and canola meal. As such, the objective of the first study was to determine the nutrient profile, nitrogen corrected true metabolizable energy ($\mathbf{TME_n}$) and amino acid (**AA**) digestibility of LGCM and HGCM. Upon completion of the first study, we hypothesized that LGCM and HGCM could partially replace soybean meal in chicken diets without negatively impacting growth. Thus, the objective of the second study was to evaluate the effects of dietary inclusion of LGCM and HGCM at different levels on the growth performance and gut health parameters of broiler chicken. Together, these studies provide an important step toward utilization of BCM as a viable alternative feedstuff.

MATERIALS AND METHODS

Two independent studies for digestibility and growth performance were carried out at the poultry research facility at the University of Georgia. Both studies were conducted after approval by the Institutional Animal Care and Use Committee (**IACUC**) of the University of Georgia.

Preparation of Carinata Meal

Two types of carinata meal containing low glucosinolate (28 μmol/g) and high glucosinolate (100 μmol/g) were obtained from Agrisoma Biosciences, Inc., Gatlineau, Quebec. Each of the feed ingredients was later ground and sieved through 3/16- inch mesh. The proximate nutrient profile was determined for both type of carinata meal (Table 1) before conducting studies. Study I was conducted to determine the TME_n and AA digestibility. Based on the profile of nutrients from study I, study II treatment diets were formulated to include LGCM and HGCM at varying labels (Table 2).

Bird Husbandry

Study I For this study, precision-fed conventional roosters and caecectomized roosters were used for TME_n and

AA digestibility, respectively. A total of 40 Single Comb White Leghorn roosters were individually kept in wire cages where 20 conventional roosters were used for TME_n and 20 caecectomized roosters were used for AA digestibility (Parsons, 1985). Among those 20 birds in each group, 10 birds were fed HGCM, and remaining 10 birds were fed LGCM. All the roosters were fasted for 30 h before transfer to individual wire cage. The roosters were precision-fed 35 g of either 100% HGCM or LGCM feed ingredient (35 g/rooster). Total excreta samples were collected post feeding for 48 h from individual trays that were kept underneath each bird cage. The LGCM and HGCM feed ingredients and excreta samples were dried, weighed, and analyzed for moisture, crude protein, and gross energy as performed by Jones et al. (2018) and Wang et al. (2021). The final TME_n and AA digestibility (Table 3) were obtained from average of 10 bird's samples for each LGCM and HGCM.

Study II A total of 504 one-day-old male chicks (Cobb500) were obtained from a Cobb hatchery and were randomly divided into 7 treatment groups with 6 replicates of 12 birds each in battery cages in a completely randomized design. Diet 1 was a corn-SBM based control diet without BCM (Table 2). In diets 2 to 4, LGCM was included at levels of 4, 8, and 12%, and in diets 5 to 7, HGCM was included at levels of 4, 8, and 12% in the diets at the expense of soybean meal, a common source of protein in the poultry diet. The birds had ad libitum access to feed and water and were fed the test diets in mash form for 21 d to meet or exceed the nutrient requirement of Cobb broilers. The birds were kept in controlled environment as per recommendation of Cobb Broiler Management Guide (Cobb, 2018a,b).

Sample Analysis

Study I The moisture content, proximate analysis, and total mineral analysis of the diet and feces were performed by Agricultural and Environmental Services Laboratories at University of Georgia following standard procedures of AOAC (AOAC, 2006) whereas, the gross energy of feed and feces was obtained using bomb calorimeter (IKA C1 Compact Bomb Calorimeter, IKA-Werke, Staufen, Germany). The TME_n (kcal/kg) was obtained by the difference in the gross energy of feed and excreta along with consideration of nitrogen (Latshaw and Freeland, 2008). The energy calculation used for this study was as follows:

$$AME(kcal/g) = (A - B)/35$$

$$TME(kcal/g) = (A - B + C)/35$$

$$AME_n(kcal/g) = (A - B - D)/35,$$

$$TME_n(kcal/g) = (A - B + C - D)/35$$

where A is total energy in feed; B = total energy in excreta; C = total excreta energy of fasted roosters; D = 8.22 × nitrogen balance (g); and 35 used in calculation is the amount of test feed fed to each rooster.

Both ingredients (LGCM and HGCM) AAs were measured, and later AAs present in excreta samples of caecectomized roosters were also quantified to calculate average digestibility of AAs present in both HGCM and LGCM (Latshaw and Freeland, 2008; Jones et al., 2018; Wang et al., 2021).

Study II All 7 feed groups were formulated to be isocaloric and isonitrogenous. The diet formulation and their calculated nutrient profiles are shown in Table 2.

Growth Performance

Feed was offered to birds on an as needed basis to each cage and recorded. Any leftover feed in the feeder were weighed back and recorded weekly. The data generated were used to calculate weekly body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), and feed conversion rate (**FCR**) as shown in Table 4.

Histomorphology

At the end of feeding trial (d 21), duodenum, jejunum, and ileum tissues were collected from one bird randomly selected from each cage for morphometric analyses by the method described by Yadav et al. (2019). In brief, 1-cm long sections from the center of duodenum loop, jejunum, and ileum were excised and flushed with phosphate buffer saline (**PBS**) to clear any fecal remains. The samples were quickly fixed in 10% neutral-buffered formalin. Fixed samples were further dehydrated, cleared, and embedded in paraffin and sectioned at 6-μm thickness, placed on glass slides, and stained by hematoxylin and eosin. The slides were examined under a light microscope (1.6X magnification for duodenum and jejunum, and 5X for ileum) with a Leica DC500 camera (Leica Microsystems Inc., Buffalo Groove, IL). Villus height (**VH**), crypt depth (**CD**), and villus height to crypt depth ratio (**VH:CD**) were determined using LAS v4.8 software (Leica Microsystems Inc.) Table 5.

Real-Time PCR Analysis

The jejunum tissue samples were collected (1 bird/cage), flushed with PBS and snap-frozen in liquid nitrogen and stored at −80°C until further analyses. Later, RNA extraction was performed using QIAzol lysis reagents (Qiagen, Valencia, CA) according to a manufacturer's protocol. RNA was quantified, and purity was evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA). The RNA was further reverse-transcribed utilizing high-capacity cDNA synthesis kits to make cDNA (Applied Biosystems, Foster City, CA). Real-time PCR reaction was prepared with SYBR Green Master mix and performed in a StepOnePlus thermocycler (Applied Biosystems). The target genes expression was analyzed using $2^{-\Delta\Delta Ct}$ method (Teng et al., 2020). The target genes were tight junction

protein genes such as Claudin 1, Claudin 2, junctional adhesion molecule 2 (JAM2 set 1), Occludin, Zonula Occludens-1 (**ZO-1**), and Zonula Occludens-2 (**ZO-2**) (Castro et al., 2020) (Table 6). Here, gene expression data were normalized to housekeeping genes with stable expression across treatments (Table 7). The glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) and Beta-actin were used as reference housekeeping genes.

Statistical Analysis

All the data were analyzed by ANOVA using the PROC GLM program of SAS software (SAS 9.4, SAS Institute Inc., 2013). To estimate the effects of increasing levels of LGCM and HGCM on growth performance, histomorphology, and gene expression parameters, the linear and quadratic orthogonal polynomial contrasts were used. Significant differences among treatments and their multiple comparisons were assessed by Tukey's test. A significance level of P less than or equal to 0.05 was used to declare differences.

RESULTS AND DISCUSSION

Study I

Nutrient Profile and Digestibility Carinata meal used in this study had approximately 39% crude protein, which was comparable to canola meal (36%), and 28 and 100 μmol/g glucosinolate in LGCM and HGCM, respectively (Table 1). Although canola meal initially had higher glucosinolate content and its use was limited, later on genetic selection of plants against glucosinolate as well as updated and improved extraction methods made it possible to intensively use canola meal as a feed ingredient (USDA ERS, 2017). At present, the higher glucosinolate in carinata meal could be utilized for beneficial effects of glucosinolate or later the glucosinolate levels could be reduced similar to that of canola meal to ultimately establish as an alternative protein source. Carinata meal was reported to contain approximately 48.2 to 53% CP by Paula et al. (2019), whereas values reported by Rodriguez-Hernandez and Anderson (2018) were similar to the CP value of the current study. Overall, it can be a good protein source in terms of quantity of protein and quality of AAs and present with CP values in between SBM and canola meal. LGCM and HGCM were low in crude fiber (7.9 and 8%) (Table 1) as compared to canola meal (11.7%) and higher than SBM (3.63%) (Guide, 2009; Ravindran et al., 2014). The total fat in LGCM (1.1%) was lower than in HGCM (1.32%); however, both carinata meals had lower fat contents than canola meal (3.5%) and SBM (1.63%) (Guide, 2009; Ravindran et al., 2014). While many aspects of the nutritional profiles between BCM and other protein feedstuffs were similar, it is unsurprising that some variability would occur. Even within a single species, differences in environment, extraction method, and even specific cultivars contribute to some degree of nutritional variability (Woyengo et al., 2016; Ndou et al., 2018).

Table 1. Analyzed proximate composition, gross energy and nitrogen corrected true metabolizable energy (TME_n) (% of as-is basis, unless otherwise indicated) of low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) used in study I to perform rooster essay.

Nutrient, %	LGCM (as is basis)	HGCM (as is basis)
Moisture	11.5	10.1
Gross energy, kcal/kg	4,097.57	4,274.93
TME_n[1], kcal/kg	1,814	1,690
Crude protein	39.2	39.5
Crude fiber	7.9	8
Total fat	1.1	1.32
Ash	7.41	6.96
Glucosinolates[2] (μmol/g)	28	100
Phosphorus	1.16	1.11
Potassium	1.58	1.71
Calcium	0.43	0.37
Magnesium	0.59	0.63
Sulfur	0.21	0.26
Manganese, mg/kg	50	43
Iron, mg/kg	121	142
Aluminum, ppm	56	22
Copper, ppm	<5	<5
Zinc, ppm	69	63
Sodium, ppm	302	94
Calcium: Phosphorus ratio	0.37	0.33

[1]Nitrogen corrected true metabolizable energy (TME_n) of low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM).

[2]LGCM and HGCM were sourced, and glucosinolates values obtained from Schulmeister et al. (2019).

The TME_n levels of LGCM and HGCM in chickens were 1,814 kcal/kg and 1,690 kcal/kg in as is basis, whereas in DM basis it was 2,046 and 1,878 kcal/kg (Table 1), respectively. This is lower than canola meal, which has TME_n of 2,070 kcal/kg on as is basis (NRC, 1994). This lower value for carinata meal can be attributed to the lower fat content (1.1% for LGCM and 1.32% for HGCM) in the carinata meal compared to canola meal (3.5%). Although HGCM has slightly higher fat content still lower TME_n which could be due to differences in other nutrients, digestibility, and the antinutritional factor between HGCM and LGCM. A similar comparison was discussed in a study by Ndou and Woyengo (2020) where cold-pressed canola expellers have higher oil content compared to carinata meal. From an AA standpoint, the present study clearly showed that carinata meals contain comparable or higher digestible AAs in both types of carinata meal (Table 4) to canola meal containing digestibility values of arginine (86%), methionine (79%), isoleucine (72%), leucine (76%), threonine (69%), tryptophan (78%), and tyrosine (58%) (Newkirk et al., 2003; Guide, 2009). Ndou and Woyengo (2020) found similar results with higher AA digestibility of carinata than that of canola meal. A possible reason for better AA digestibility in carinata meal may be attributed to the internal molecular structure (α-helix, β-sheets and their ratio) of the secondary protein structures, as previous studies have shown that secondary protein structure influences protein quality, availability, and digestibility (Ban et al., 2017). Specifically, the authors suggested that an increase in β-sheets, of which carinata meal has a lower

proportion when compared to canola meal, is associated with decreased digestibility (Ban et al., 2017). An additional contributing factor for increased digestibility of AAs in carinata meal may be due to higher proportion of soluble proteins out of total proteins in comparison to canola meal, as solubility of AA and digestibility are directly proportional (Ban, 2016). Together, these results suggest that carinata meal is a suitable protein source for poultry but would necessitate determining proper supplementation or substitution levels prior to implementation.

Study II

Growth Performance For this 3-wk study, the birds were fed with three levels of LGCM (4, 8, and 12%) and 3 levels of HGCM (4, 8, and 12%) to compare the growth performance of birds fed control diet based on SBM as a protein source. The growth performance of birds in first 2 wk showed that there was no significant difference in the BW, BWG, FI, and FCR (Table 5). During wk 3, the BWG decreased significantly for 12% HGCM ($P_{lin} = 0.015$) along with linear decrease in feed intake ($P_{lin} = 0.051$), leading to significant increase in FCR for the same treatment ($P_{lin} = 0.056$; $P_{quad} = 0.004$). Also, there was significant difference in BWG between LGCM and HGCM during third week of age ($P = 0.05$) whereas average FI tends to be decreasing for HGCM during same period when compared to LGCM ($P = 0.085$). Within the LGCM group during 14 to 21 d rearing period, there was decrease in BWG ($P_{quad} = 0.036$) whereas tends to increase in FCR ($P_{quad} = 0.084$). No differences were found between control vs. LGCM and control versus HGCM groups for growth parameters throughout the study ($P > 0.05$). Thus, birds fed different levels of low and high glucosinolate carinata meal grew and gained similar body weight to birds fed corn-SBM based control diets. When compared to the control group, FCR for 4% BCM (includes both LGCM and HGCM) was the lowest among all treatments. Together, this result suggests that LGCM can be supplemented in broiler diets up to 12%, whereas HGCM can be added up to 8% without any retardation in growth or feed conversion.

Few oilseeds from the *Brassica* genus such as rapeseed/canola meal have been previously reported as potential protein alternatives to traditional SBM

Table 2. Ingredients and calculated nutrient compositions of the treatment diets including basal, low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) for study II using broiler chicken.

Ingredient, %	Control	4% LGCM	8% LGCM	12% LGCM	4% HGCM	8% HGCM	12% HGCM
Corn, grain	60.01	57.35	54.71	52.80	57.75	55.20	52.55
Soybean meal (48%)	34.15	31.44	29.44	27.56	31.11	28.81	27.62
Soybean oil	1.53	2.95	3.71	3.69	2.86	3.81	3.87
LGCM	0	4	8	12	0	0	0
HGCM	0	0	0	0	4	8	12
L-Threonine	0.07	0.07	0.05	0.02	0.08	0.06	0.02
Limestone	1.17	1.19	1.21	1.23	1.19	1.20	1.22
Dicalcium phosphate	1.58	1.51	1.43	1.34	1.53	1.46	1.38
Common salt	0.35	0.35	0.35	0.30	0.35	0.35	0.33
Premix[1]	0.33	0.33	0.33	0.33	0.33	0.33	0.33
DL-Methionine	0.29	0.27	0.23	0.19	0.26	0.23	0.18
L-Lysine HCl	0.22	0.25	0.25	0.24	0.25	0.25	0.21
Sand	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100
Calculated nutrient, %							
Dry matter	86.36	86.50	86.60	86.66	86.52	86.68	86.82
M.E.[2], kcal/kg	3,010	3,050	3,050	3,010	3,040	3,050	3,000
Crude fiber	2.17	2.35	2.56	2.78	2.35	2.55	2.79
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45
L-Lysine HCl	1.32	1.32	1.32	1.32	1.32	1.32	1.32
DL-Methionine	0.63	0.61	0.59	0.56	0.61	0.58	0.55
TSAA[3]	0.98	0.98	0.98	0.98	0.98	0.98	0.98
L-Threonine	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Analyzed nutrient, %							
Crude protein	20.6	20.5	20.0	20.8	19.6	20.1	21.1
Calcium	1.27	1.11	1.05	1.10	1.20	1.25	1.22
Total phosphorus	0.68	0.70	0.64	0.65	0.64	0.68	0.70
Calcium: phosphorus	1.86	1.59	1.63	1.69	1.86	1.84	1.74
Iron, ppm	292	248	224	244	234	237	279
Zinc, ppm	178	134	131	138	131	128	146

[1]Premix provided the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol-acetate), 30 mg; vitamin B1, 2 mg; vitamin B2, 8mg; vitaminB6, 4mg; vitaminB12 (cyanocobalamin), 0.025 mg; vitamin K3 (bisulphatemenadione complex), 3 mg; choline (choline chloride), 250 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; butane anhydrous, 80 mg; D-biotin, 0.15 mg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg iron ($FeCO_3$), 60 mg; copper ($CuSO_4 \bullet 5H_2O$), 8 mg; iodine (KI), 2 mg; selenium (Na_2SeO_3), 0.2 mg.

[2]M.E., metabolizable energy.

[3]TSAA, total sulfur amino acids.

Table 3. Calculated amino acid (AA) content and its digestibility (%, as is basis) of low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) using rooster essay in study I.

Amino acid	LGCM, AA (%)	LGCM, AA digestibility (%)	HGCM, AA (%)	HGCM, AA digestibility (%)
Alanine	1.61	79.17	1.63	81.16
Arginine	2.51	92.27	2.74	92.80
Aspartic acid	2.44	76.54	2.46	79.36
Cysteine	1.05	69.34	1.15	70.68
Glutamic acid	6.69	87.11	7.02	88.52
Glycine	1.87	32.22	1.86	19.02
Histidine	0.99	85.72	0.99	85.88
Isoleucine	1.53	82.66	1.53	83.82
Leucine	2.57	85.22	2.60	86.25
Lysine	1.64	72.41	1.86	79.52
Methionine	0.72	87.84	0.75	89.29
Phenylalanine	1.45	87.46	1.51	88.45
Proline	2.22	77.49	2.35	79.72
Serine	1.35	75.56	1.39	76.67
Threonine	1.51	74.94	1.50	75.51
Tryptophan	0.44	92.66	0.50	93.04
Tyrosine	0.90	80.70	0.92	81.07
Valine	1.83	78.58	1.87	80.04

W/W% = grams per 100 grams of sample. Results are expressed on an "as is" basis unless otherwise indicated.

Table 4. Effects of the low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) on the growth performance of the broiler chickens used in study II.

	BW[1], g				BWG[2], g			FI[3], g			FCR[4]		
Items	D 0	D 0−7	D 7−14	D 14−21	D 0−7	D 7−14	D 14−21	D 0−7	D 7−14	D 14−21	D 0−7	D 7−14	D 14−21
Control	46	157	411	619	111	254	209	131	347	425	1.17	1.37	2.04
4% LGCM	46	145	381	614	99	235	234	116	319	437	1.16	1.36	1.88
8% LGCM	46	161	402	628	114	242	226	127	332	426	1.11	1.39	1.9
12% LGCM	46	156	404	605	110	249	201	126	334	406	1.15	1.35	2.03
4% HGCM	46	153	404	632	107	251	228	128	331	424	1.2	1.33	1.88
8% HGCM	46	156	392	598	110	235	206	126	325	391	1.14	1.39	1.91
12% HGCM	46	152	385	556	106	233	171	125	317	386	1.17	1.36	2.27
SEM	.	1.635	4.746	7.522	1.633	4.218	5.25	1.856	4.877	6.264	0.009	0.009	0.036
P-value													
LGCM linear	.	0.597	0.980	0.754	0.605	0.881	0.579	0.948	0.691	0.371	0.318	0.733	0.980
LGCM quadratic	.	0.421	0.240	0.623	0.408	0.295	0.036	0.181	0.293	0.312	0.310	0.574	0.084
HGCM linear	.	0.561	0.155	0.014	0.561	0.167	0.015	0.430	0.144	0.051	0.704	0.647	0.056
HGCM quadratic	.	0.959	0.982	0.158	0.959	0.989	0.024	0.817	0.807	0.882	0.884	0.751	0.004
Control vs. LGCM	.	0.514	0.356	0.896	0.506	0.425	0.397	0.233	0.286	0.944	0.331	0.945	0.319
Control vs. HGCM	.	0.517	0.297	0.305	0.518	0.349	0.630	0.473	0.199	0.224	0.991	0.844	0.855
LGCM vs. HGCM	.	1.000	0.842	0.175	0.984	0.823	0.050	0.468	0.723	0.085	0.143	0.846	0.222

[1]BW: Body weight represents the average weight of all the birds per cage.
[2]BWG: Body weight gain represents average weight gain during particular period of 0−7, 7−14, or 14−21 days of age.
[3]FI: Feed intake is the average of feed consumed by birds per cage.
[4]FCR: Feed conversion rate is the ratio of average feed to average gain for particular cage.

diets. A study used RSC and considered it as *B. carinata* although it is not same but closely related to carinata meal coming from same genus with different species (Tadelle et al., 2003). In this trial, the authors performed a 7-wk feeding regimen with chicks of the Hubbard genotype, and RSC was included at levels of 0, 7, 14, 21, 28, and 35% in broiler rations as a protein source. Results of this study suggested that the high levels of RSC in diet formulation may predispose birds towards colloid goiter, however, it was also determined that up to 28% RSC inclusion yielded the greatest economic benefits. This higher level of inclusion in Tadelle et al. (2003) could be due to lower glucosinolate content in RSC (12−20 μmol/g) compared to carinata meal used in the present study (LGCM has 28 μmol/g and 100 μmol/g glucosinolate in HGCM). Another study suggested that rapeseed meal can replace 25% of SBM without any significant negative impact on the growth performance and is more economically profitable compared to using SBM as a sole protein source (Urge and Ashnie, 2012). In the present study, LGCM successfully replaced SBM by 19% (calculated from Table 2; % replacement of 12% LGCM to SBM), whereas HGCM can only replace SBM up to 15% (at inclusion level of 8% HGCM) and higher inclusion caused a significant increase in FCR. Prior studies by Schloffel et al. (1993) and Thomas et al. (1983) concluded that inclusion of high glucosinolate rapeseed

Table 5. Histomorphological measurements (μm) of duodenum, jejunum, and ileum tissues from broiler fed low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) in study II.

	Duodenum			Jejunum			Ileum		
Items	VH[1]	CD[2]	VH:CD[3]	VH	CD	VH:CD	VH	CD	VH:CD
Control	1,736	219	7.99	941	158	5.98	599	114	5.34
4% LGCM	2,017	223	9.4	1,108	167	6.62	817	131	6.26
8% LGCM	1,942	256	7.87	897	171	5.36	876	154	5.98
12% LGCM	1,921	208	9.63	1,092	175	6.56	729	129	5.89
4% HGCM	1,905	221	8.67	798	185	4.34	732	132	5.54
8% HGCM	1,793	230	7.89	1,002	154	6.66	628	122	5.28
12% HGCM	1,860	221	8.51	880	145	6.14	602	100	5.98
SEM	40.067	5.931	0.262	40.01	6.731	0.249	30.906	6.029	0.179
P-values									
LGCM linear	0.350	0.996	0.303	0.596	0.507	0.860	0.204	0.355	0.567
LGCM quadratic	0.174	0.122	0.804	0.898	0.914	0.655	0.019	0.187	0.308
HGCM linear	0.614	0.833	0.806	0.961	0.413	0.335	0.790	0.489	0.484
HGCM quadratic	0.657	0.746	0.965	0.926	0.362	0.393	0.305	0.217	0.624
Control vs LGCM	0.106	0.621	0.249	0.457	0.566	0.784	0.026	0.211	0.249
Control vs HGCM	0.398	0.805	0.663	0.708	0.890	0.719	0.541	0.817	0.674
LGCM vs HGCM	0.233	0.711	0.280	0.121	0.541	0.371	0.017	0.135	0.280

[1]VH, villus height.
[2]CD, crypt depth.
[3]VH:CD, villus height to crypt depth ratio. Here, VH and CD were measured in μm.

ranging from 5 to 10% was possible. Our recommendation of 8% inclusion of HGCM is in agreement with these findings. In a thesis report by Mendes (2018), different levels carinata were fed to marketable size pigs and concluded that carinata can replace SBM by as much as 50%. This discrepancy in potential inclusions may simply reflect different species' susceptibility to the negative effects of glucosinolates, with young birds being more susceptible than pigs.

Gut Histomorphology Tissues samples from duodenum, jejunum, and ileum were collected and histological slides were prepared to measure the villus height, crypt depth, and their ratios. The villus height indicated the absorptive capacity of the gut mucosa (Yadav et al., 2019), and because all the birds in the current study grew well, it was expected that all treatments would have similar villus height and crypt depth. There were no significant differences between treatments for villus height, crypt depth, or their ratio at duodenum, and jejunum levels ($P > 0.05$). Whereas ileum VH within different levels of LGCM was different ($P_{quad} = 0.019$) with highest for 8% LGCM. LGCM group ileum VH was also significantly increased compared to control group ($P = 0.026$) and compared to HGCM group ($P = 0.017$) as presented in Table 6. The result of the present study was similar to a study by Chiang et al. (2010), which also found no differences in the histology parameters among birds fed solid-state fermented rapeseed meal. The present study showed that the highest inclusion of HGCM (12%) had numerically decreased crypt depth. This result is somewhat related to findings by Figueiredo et al. (2003), where increase in the level of canola meal caused linear decrease in crypt depth. Overall, no negative differences were observed for histological parameters when SBM was replaced at different levels with BCM.

Gene Expression The gene expression of tight junction proteins was evaluated in the jejunum part of the small intestine. These proteins play an important role in controlling intestinal permeability and maintaining gut health barrier in such way that solutes and ions can flow intracellularly while the entry of pathogens and toxins are blocked (Awad et al., 2017). Tight junction proteins were of interest in this study, as glucosinolate metabolites are known to cause anti-inflammatory function by enhancing these junction proteins in the gut and preventing the negative effect of glucosinolate (Maina et al., 2020). The results in the present study (Table 7) showed that there was no significant upregulation or downregulation of tight junction protein genes among the treatments ($P > 0.05$) except for Zonula Occludin-1 which tends to be significant for different levels of LGCM ($P_{quad} = 0.071$).

In conclusion, the results of the current study suggest that carinata meal has high potential to be included in poultry diet due to higher AA digestibility and TME_n comparable to existing protein sources. Carinata meal with low glucosinolate and high glucosinolate can successfully replace 19 and 15% of soybean meal, respectively. Incorporating carinata meal either with high or low glucosinolate can be beneficial in terms of broiler production as partial substitution of SBM can be incorporated in areas where carinata is produced and SBM is expensive or not available. Except for 12% HGCM treatment group, all other groups had similar growth performance, gut histomorphology development, and regulations of tight junction proteins. Thus, based on these data, 12% LGCM can be included in diet of broiler chicken, and 8% of HGCM could be included without any negative impact on the broiler chickens. Further processing of HGCM either to decrease the glucosinolate or utilize the beneficial role of glucosinolate can be investigated.

Table 6. Primers used for quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) analysis for study II using broiler's samples.

Gene[1]	Gene bank identification	Primer sequence, sense/antisense
Housekeeping genes		
GAPDH	NM_204305.1	GCTAAGGCTGTGGGGAAAGT/ TCAGCAGCAGCCTTCACTAC
Beta-actin	NM_205518.1	CAACACAGTGCTGTCTGGTGGTA/ ATCGTACTCCTGCTTGCTGATCC
Tight junction protein genes		
Cla-1	NM_001013611.2	TGGAGGATGACCAGGTGAAGA/ CGAGCCACTCTGTTGCCATA
Cla-2	NM_001277622.1	CCTGCTCACCCTCATTGGAG/ GCTGAACTCACTCTTGGGCT
ZO-1	XM_015278981.2	CAACTGGTGTGGGTTTCTGAA/ TCACTACCAGGAGCTGAGAGGTAA
ZO-2	XM_025144669.1	ATCCAAGAAGGCACCTCAGC/ CATCCTCCCGAACAATGC
Ocln	XM_026041453.1	ACGGCAGCACCTACCTCAA/ GGCGAAGAAGCAGATGAG
JAM2	XM_025149444.1	AGCCTCAAATGGGATTGGATT/ CATCAACTTGCATTCGCTTCA

[1]Abbreviations: Cla-1, Claudin-1; Cla-2, Claudin-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ocln, Occludin; JAM2, junctional adhesion molecule 2; ZO-1, Zonula Occludens-1; ZO-2, Zonula Occludens-2.

Table 7. Relative mRNA expression of genes related to tight junction proteins in jejunum of broiler chickens fed low glucosinolate carinata meal (LGCM) and high glucosinolate carinata meal (HGCM) from study II.

Items/genes[1]	Cla-1	Cla-2	JAM-2	Ocln	ZO-1	ZO-2
Control	1	1	1	1	1	1
4% LGCM	0.93	0.88	0.98	0.97	0.89	1.05
8% LGCM	0.83	0.63	0.93	1.02	0.91	0.96
12% LGCM	1.14	0.73	0.96	1.21	1.13	1.04
4% HGCM	1	0.83	0.79	1.16	0.86	0.94
8% HGCM	0.88	0.78	0.82	1.11	0.91	0.97
12% HGCM	1.41	0.71	0.98	1.12	1.05	1.16
SEM	0.085	0.057	0.049	0.034	0.033	0.029
P-values						
LGCM linear	0.773	0.158	0.794	0.103	0.313	0.889
LGCM quadratic	0.416	0.479	0.824	0.230	0.071	0.859
HGCM linear	0.315	0.221	0.974	0.472	0.611	0.170
HGCM quadratic	0.279	0.763	0.211	0.418	0.120	0.135
Control vs LGCM	0.898	0.198	0.793	0.539	0.819	0.817
Control vs HGCM	0.741	0.260	0.439	0.257	0.577	0.794
LGCM vs HGCM	0.487	0.812	0.434	0.416	0.613	0.962

[1]Genes: Cla-1: Claudin-1, Cla-2: Claudin-2, JAM2: junctional adhesion molecule-2, Ocln: Occludin, ZO-1: Zonula Occludens-1, ZO-2: Zonula Occludens-2.

ACKNOWLEDGMENTS

This study was financed in part by USDA-NIFA (#2017-68005-26807). We would like to thank Brett Marshall for helping with the manuscript preparation and technical assistance. Appreciation to all the graduate students in Dr. Kim's lab group for help in sample collection.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

AOAC Int. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem, Arlington, VA.

Ashnie, E., M. Urge, and A. Asres. 2015. Effects of different levels of solvent extracted rapeseed (*Brassica carinata*) meal replacement to soybean meal on the performance of broiler chicks. Food Sci. Qual. Manage. 36:78–87.

Awad, W. A., C. Hess, and M. Hess. 2017. Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins 9:60.

Ban, Y., N. A. Khan, and P. Yu. 2017. Nutritional and metabolic characteristics of *Brassica* carinata co-products from biofuel processing in dairy cows. J. Agric. Food. Chem. 65:5994–6001.

Ban, Y., L. L. Prates, and P. Yu. 2018. Biodegradation characteristics and nutrient availability of newly developed carinata seeds in comparison with canola seeds in dairy cattle. Anim. Feed. Sci. Technol. 240:88–101.

Ban, Y. 2016. Page 151 in Molecular structural, physiochemical and nutritional characterization of new lines of Brassica Carinata and the co-products. MSC Thesis. Univ. Saskatchewan, Saskatoon, SK, Canada.

Bischoff, K. 2019. Glucosinolates and organosulfur compounds. Nutraceut. Vet. Med. 1:113–119. 9. Springer.

Cardone, M., M. Mazzoncini, S. Menini, V. Rocco, A. Senatore, M. Seggiani, and S. Vitolo. 2003. *Brassica* carinata as an alternative oil crop for the production of biodiesel in Italy: agronomic evaluation, fuel production by transesterification and characterization. Biomass Bioenergy 25:623–636.

Castro, F. L., P. Y. Teng, S. Yadav, R. L. Gould, S. Craig, R. Pazdro, and W. K. Kim. 2020. The effects of L-Arginine supplementation on growth performance and intestinal health of broiler chickens challenged with Eimeria spp. Poult. Sci. 99:5844–5857.

Chiang, G., W. Lu, X. Piao, J. Hu, L. Gong, and P. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian Australas. J. Anim. Sci. 23:263–271.

Guide, Canola Meal Feeding. 2009. Canola Meal Feeding Industry Guide. 4th ed. Canadian International Grain Institute. Accessed Feb. 2021. https://cigi.ca/wp-content/uploads/2011/12/2009-Canola_Guide.pdf.

Cobb Vantress, 2018a. Broiler management guide. Accessed Jan. 2020. https://cobbstorage.blob.core.windows.net/guides/5fc96620-0aba-11e9-9c88-c51e407c53ab.

Cobb Vantress 2018b. Cobb 500 broiler performance and nutrition supplement. Accessed Jan. 2020. https://cobbstorage.blob.core.windows.net/guides/5a171aa0-6994-11e8-9f14-bdc382f8d47e.

Fenwick, G. R. 1982. The assessment of a new protein source—rapeseed. Proc. Nutr. Soc. 41:277–288.

Fenwick, G. R., N. M. Griffiths, and R. K. Heaney. 1983. Bitterness in Brussels sprouts (*Brassica oleracea* L. var. gemmifera): the role of glucosinolates and their breakdown products. J. Sci. Food Agric. 34:73–80.

Figueiredo, D. F., A. E. Murakami, M. A. D. S. Pereira, A. C. Furlan, and F. L. B. Toral. 2003. Desempenho e morfometria da mucosa de duodeno de frangos de corte alimentados com farelo de canola, durante o período inicial. R. Bras. Zootec. 32:1321–1329.

Hagos, R., A. S. Shaibu, L. Zhang, X. Cai, J. Liang, J. Wu, R. Lin, and X. Wang. 2020. Ethiopian mustard (*Brassica* carinata A. Braun) as an alternative energy source and sustainable crop. Sustain 12:7492.

Hong, J., J. W. Lee, D. Pettersson, and T. A. Woyengo. 2019. Nutritive value of enzyme-supplemented carinata meal for growing pigs. Transl. Anim. Sci. 3:1359–1368.

Jones, M. K., K. E. Richardson, C. W. Starkey, N. M. Dale, and A. J. Davis. 2018. Impact of extended heat treatment on the amino acid digestibility and TMEn content of a formaldehyde-treated diet. J. Appl. Poult. Res. 27:550–554.

Kearney, J. 2010. Food consumption trends and drivers. Philos. Trans. R. Soc. B Biol. Sci. 365:2793–2807.

Latshaw, J. D., and K. Freeland. 2008. Metabolizable energy values determined with intact and cecectomized roosters. Poult. Sci. 87:101–103.

Maina, S., G. Misinzo, G. Bakari, and H. Y. Kim. 2020. Human, animal and plant health benefits of glucosinolates and strategies for enhanced bioactivity: a systematic review. Molecules 25:3682.

Mendes, K. M. 2018. The impact of elevated levels of Brassica carinata on the growth, feed efficiency, carcass merit, and lean quality of finishing pigs. MS Thesis. Univ. Florida, Gainesville.

Ndou, S. P., and T. A. Woyengo. 2020. Nutritive value of carinata meal and cold-pressed canola expellers for pigs. Anim. Feed Sci. Technol. 265:114496.

Ndou, S. P., E. Kiarie, M. C. Walsh, and C. M. Nyachoti. 2018. Nutritive value of flaxseed meal fed to growing pigs. Anim. Feed Sci. Technol. 238:123–129.

Newkirk, R. W., H. L. Classen, T. A. Scott, and M. J. Edney. 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83:131–139.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Oryschak, M. A., M. N. Smit, and E. Beltranena. 2020. *Brassica napus* and *Brassica juncea* extruded-expelled cake and solvent-extracted meal as feedstuffs for laying hens: lay performance, egg quality, and nutrient digestibility. Poult. Sci. 99:350–363.

Parsons, C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agric. Sci. Camb. 104:469–472.

Paula, E. M., L. G. da Silva, V. L. N. Brandao, X. Dai, and A. P. Faciola. 2019. Feeding canola, camelina, and carinata meals to ruminants. Animals 9:704.

Ravindran, V., M. R. Abdollahi, and S. M. Bootwalla. 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci. 93:2567–2577.

Ravindran, V. 2013. Main ingredients used in poultry feed formulations. Poult. Dev. Rev. 22:67–69. Rome, Italy. Accessed Dec. 2021. http://www.fao.org/3/a-al705e.pdf.

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. 101:1206–1215.

SAS Institute. 2013. SAS Proprietary Software Release 9.4. SAS Inst. Inc., Cary, NC.

Schloffel, J., H. Jeroch, W. Seffner, and G. Jahreis. 1993. Toasted rapeseed meal in broiler fattening diet. Arch. Tierernahr. 45:79–87.

Schulmeister, T. M., M. Ruiz-Moreno, G. M. Silva, M. Garcia-Ascolani, F. M. Ciriaco, D. D. Henry, G. C. Lamb, J. C. Dubeux Jr, and N. Dilorenzo. 2019. Evaluation of *Brassica* carinata meal as a protein supplement for growing beef heifers. J. Anim. Sci. 97:4334–4340.

Tadelle, D., Y. Alemu, H. M. Moges, and K. Fasil. 2003. Effect of level of rapeseed (*Brassica carinata*) cake in rations on broiler performance. Livest. Res. Rural Dev. 15:1–5.

Teng, P. Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020. Graded Eimeria challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. Poult. Sci. 99:4203–4216.

Thomas, V. M., R. J. Katz, D. A. Auld, S. F. Peterson, E. A. Sauter, and E. E. Steel. 1983. Nutritive value of expeller extracted rape and safflower oil seed meals for poultry. Poult. Sci. 62:882.

Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132:1–27.

Urge, M., and E. Ashnie. 2012. Effects of different levels of solvent extracted rapeseed (Brassica Carinata) meal replacement to soybean meal on the performance of broiler chicks. Doctoral Diss., Haramaya Univ., Ethiopia.

USDA-ERS. United States Department of Agriculture, Economic Research Service. 2017. Canola production. Accessed Dec. 2020. https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola/

Vaddu, S., J. Kataria, E. N. Rama, A. E. Moller, A. Gouru, M. Singh, and H. Thippareddi. 2021. Impact of pH on efficacy of peroxy acetic acid against Salmonella, Campylobacter, and Escherichia coli on chicken wings. Poult. Sci. 100:256–262.

Wallig, M. A., R. L. Belyea, and M. E. Tumbleson. 2002. Effect of pelleting on glucosinolate content of Crambe meal. Anim. Feed Sci. Technol. 99:205–214.

Wang, J., A. K. Singh, F. Kong, and W. K. Kim. 2021. Effect of almond hulls as an alternative ingredient on broiler performance, nutrient digestibility, and cecal microbiota diversity. Poult. Sci. 100:100853.

Woyengo, T. A., J. E. Sánchez, J. Yáñez, E. Beltranena, M. Cervantes, A. Morales, and R. T. Zijlstra. 2016. Nutrient digestibility of canola co-products for grower pigs. Anim. Feed Sci. Technol. 222:7–16.

Xin, H., and P. Yu. 2013. Chemical profile, energy values, and protein molecular structure characteristics of biofuel/bio-oil co-products (carinata meal) in comparison with canola meal. J. Agric. Food Chem. 61:3926–3933.

Yadav, S., and R. Jha. 2021. Macadamia nut cake as an alternative feedstuff for broilers: effect on growth performance. Anim. Feed Sci. Technol. 275:114873.

Yadav, S., B. Mishra, and R. Jha. 2019. Cassava (*Manihot esculenta*) root chips inclusion in the diets of broiler chickens: effects on growth performance, ileal histomorphology, and cecal volatile fatty acid production. Poult. Sci. 98:4008–4015.

Yadav, S. 2017. Effect of high fiber and high starch alternative feedstuffs on the growth performance and gut health of broiler chickens. MS Diss., Univ. Hawai'i, Mānoa. Accessed Jan. 2021. http://hdl.handle.net/10125/62078

Zhu, L. P., J. P. Wang, X. M. Ding, S. P. Bai, Q. F. Zeng, Z. W. Su, Y. Xuan, T. J. Applegate, and K. Y. Zhang. 2019. The effects of varieties and levels of rapeseed expeller cake on egg production performance, egg quality, nutrient digestibility, and duodenum morphology in laying hens. Poult. Sci. 98:4942–4953.