Influence of *Brassica* spp. rapeseed and canola meal, and supplementation of bioactive compound (AITC) on growth performance, intestinal-permeability, oocyst shedding, lesion score, histomorphology, and gene expression of broilers challenged with *E. maxima*

S. Yadav, P.-Y. Teng, A. K. Singh, J. Choi, and W. K. Kim[1]

Department of Poultry Science, University of Georgia, Athens, GA, 30602, USA

ABSTRACT This study was performed to investigate the effect of feeding *Brassica* spp. including full-fat rapeseed, canola meal, and allyl isothiocyanate (**AITC**) to broiler chicken challenged with *E. maxima*. A total of 576 one-day old male broiler chicks were completely randomized to 8 treatments with 6 replicated cages and 12 birds per cage. The treatment diets consisted of nonchallenge control (**NC**, corn-SBM based diet), challenge control (**CC**), 10% rapeseed (10RS), 30% rapeseed (30RS), 20% canola (20CLM), 40% canola (40CLM), 500 ppm AITC (500AITC), and 1,000 ppm AITC (1000AITC). At d 14, all birds were challenged, except NC group, with a subclinical dose of *E. maxima*. Intestinal permeability was conducted on 5 d post-infection (**dpi**) and for oocyst shedding 5 to 6 dpi feces were pooled and collected. On 6 dpi, growth performance, lesion score, histomorphology, and gene expression were measured. The growth performance result showed that 10RS and 30RS groups had lower BW, BWG, FI, and higher FCR ($P < 0.0001$). During the challenge and overall periods, NC group had highest BW, BWG, and FI, and lowest FCR. The inclusion of canola meal showed lower performance during prechallenge period but was able to catch up BWG during challenge period. The AITC levels showed similar growth performance to CC group. Intestinal permeability for 20CLM, 40CLM, 500AITC and 1000AITC was similar to NC group, whereas CC, 10RS, and 30RS had higher permeability compared to NC ($P < 0.0001$). Oocyst shedding was significantly lower for 40CLM and NC, whereas all other treatments had higher oocyst shedding ($P < 0.0001$). All the challenged treatment groups had higher lesion score and microscore than NC ($P < 0.0001$). Histomorphology data showed that jejunum villus height (**VH**) for 1000AITC was similar to NC group, whereas CC group had the lowest VH ($P = 0.01$). The 30RS group had lower VH: crypt depth (**CD**) ratio in the jejunum and ileum. The gene expression at 6 dpi for claudin1, occludin, IL2, IL6, GLUT5, EAAT, B^{0}AT, and LAT1 was significantly changed among the treatments. The results suggest that 30RS retards growth performance and deteriorate gut health during coccidiosis and should not be fed to chicken during the starter phase. Canola meal showed decline in growth prechallenge but maintained growth and intestinal health during the challenge period at 40% inclusion. AITC at 1,000 ppm showed similar growth as control group, but with improved gut health during the challenge period. Canola meal could be a good alternative to SBM especially during coccidiosis, whereas AITC needs to be tested vigorously in animal feeding regime.

Key words: *Eimeria maxima*, rapeseed, canola meal, coccidiosis, broilers

2022 Poultry Science 101:101583
https://doi.org/10.1016/j.psj.2021.101583

INTRODUCTION

Poultry is one of the most preferred meat, and its demand have been increasing due to its health benefit, cost per unit, availability, and acceptance by all the community (OECD-FAO, 2020). Global poultry meat consumption is expected to rise more in the developing countries which will ultimately cause rise in feed ingredient cost and demand (OECD-FAO, 2020). Feed security, food safety and disease emergence or re-emergence are considered major hurdles to increasing strategic future of chicken production because of increasing foodborne and pathogenic diseases, antibiotic residues, cost of chicken production, limited resources of feed ingredients, and understanding interconnection of feed,

Received September 20, 2021.
Accepted October 24, 2021.

[1]Corresponding author: wkkim@uga.edu

health, immunity, and welfare of animals (Hafez and Attia, 2020; Hafez et al., 2021).

In poultry feeding programs, protein is one of the most expensive nutrients with very limited sources of protein. Plant sources includes soybean meal (**SBM**), cotton seed meal, canola meal, rapeseed meal, sunflower cake, groundnut cake, macadamia nut cake, and other oil-seed co-products after oil extractions (Canola, 2009; Beski et al., 2015; Yadav and Jha, 2021). Whereas animal sources include fish meal, bone meal, blood meal, feather meal, etc. In comparison, the protein contents or amino acids balance of the animal sources are higher, but their use is limited due to availability issues, residual antibiotics, or pathogens carryover, and expensive for commercial broiler production (Hossain et al., 2015). The plant source protein especially SBM is commonly used in commercial poultry feeding. The poultry industry is heavily dependent on SBM solely; thus, exploration of other alternative protein sources is necessary. Also, unprecedented condition such as COVID-19 increased the grain consumption by humans lowering the share for animal feed (Hafez et al., 2021). Rapeseed and canola meal are promising alternatives to SBM to reduce the cost of production and heavy dependency on SBM.

Rapeseed meal also known as mustard seed (*Brassica napus*) has been used in animal feeding for long time (Hickling, 2001). Its availability and balanced amino acid content make it suitable to be included in chicken diet. Rapeseed is the third largest oilseed crop produced worldwide, and the co-product after oil extraction is used in animal feeding as a rich source of protein (30 −45%) (Shahidi, 1990). Rapeseed contains appropriate amino acid such as lysine and other sulfur containing amino acids which are limiting in chicken; however, its utilization is limited due to the presence of antinutritional factors (**ANF**) such as glucosinolate and erucic acid (Nega and Woldes, 2018). Whole rapeseed contains additional factors such as complex carbohydrates, fiber, phytic acid, and sinapine which potentially cause reduction in feed intake (Tripathi and Mishra, 2007), digestibility (Bellostas et al., 2007), growth (Tripathi and Mishra, 2007), and overall health (Chun Chang and Brian, 2004). Previous studies used different inclusion levels of rapeseed based on the amount of glucosinolate content, animal species, age, and production level (Wondimagegne et al., 2016). Rapeseed meal can replace up to 50% of SBM and can be included up to 10 to 15% in broiler diets (Nega and Woldes, 2018). Another study showed that rapeseed meal can replace 25% of SBM without any negative effects on growth (Georgeta, 2009). Whole full-fat rapeseed in ruminant feeding has been studied due to its high energy concentrate in the oil although it contains high glucosinolate level (Hristov et al., 2011). However, feeding whole rapeseed to chicken or monogastric is not well-studied especially under disease challenged model.

Another *Brassica* species, canola, having low glucosinolate (<30 μmol/g) and erucic acid (<2%) was developed by plant breeding of rapeseed for oil production purpose (Bell, 1993). With the increase in oil production, the leftover canola meal after oil extraction was utilized for the animal feeding. This variety was developed to reduce the ANF from rapeseed so that higher levels could be included in the chicken diet. Moreover, canola meal has better nutrient profiles compared to SBM in terms of essential minerals (calcium, phosphorus, and selenium), B vitamins, and sulfur containing amino acid (Wickramasuriya et al., 2015). Canola also contains ANF in the forms of fiber, phenolics, sinapine, phytate, and glucosinolate. Among these ANF, glucosinolate is the most important one as its metabolites causes reduction in feed intake due to decreased palatability that negatively affects the growth performance of birds, (Bourdon and Aumaitre, 1990). Although glucosinolate is inactive unless it meets an enzyme myrosinase in the presence of water that breaks it down to biologically active compounds such as thiocyanate and isothiocyanate (Tripathi and Mishra, 2007). Although isothiocyanate is considered undesirable, it has beneficial effects as an anticarcinogenic, antioxidant, antimicrobial, and other disease therapeutic and preventive compound (Tripathi and Mishra, 2007; Maina et al., 2020). To authors' knowledge, no study has been conducted to evaluate the effects of feeding chicken with allyl isothiocyanate (**AITC**) supplementation or feed ingredients rapeseed and canola meal during *Eimeria* challenged condition on growth performance and gut health in broilers.

Along with feed issues, another challenge in chicken production is disease outbreak. Coccidiosis is one of the most common enteric diseases caused by *Eimeria* species of which most common are *E. tenella, E. maxima,* and *E. acervulina* (McDougald et al., 1997; Yadav et al., 2020; Teng et al., 2020a,b). Coccidiosis has always been an issue in chicken farms due to huge economic loss as well as long-term impacts in poultry farms as chicken is a major host for *Eimeria* parasites. Poultry coccidiosis has been ranked among the top 3 economically important diseases, the number one disease related issue (*E. maxima*), and among the top 10 impactful diseases in United Kingdom, United States, and South Asia respectively (Perry et al., 2002; Bennett and Ijpelaar, 2005; USAHA, 2019). According to recent recalculation of global cost of coccidiosis in chicken was estimated around £ 10 billion in 2016, which includes prophylaxis, treatment, and losses cost associated with chicken production (Blake et al., 2020). Nutrition plays a major role during susceptibility or protection, during infection or pathogenesis, and lastly during recovery or compensatory phase (Gomez-Osorio et al., 2021). To control the economic losses or to prevent the outbreak of coccidiosis in farms, nutritional strategies for prophylaxis or treatment measures are being explored. Use of natural dietary supplements to enhance innate immunity could effectively reduce the need of treatment for the enteric infection (Lillehoj et al., 2018).

We hypothesized that antinutritional factors present in rapeseed, canola, or AITC supplementation would affect the growth performance and gut health in broilers during *Eimeria* infection. Thus, the objective of the

present study was to evaluate the effects of different levels of rapeseed and canola meal to partially replace SBM as well as supplementation of synthetic AITC in basal diet on growth performance and gut health parameters of broilers challenged with/without *E. maxima*.

MATERIALS AND METHODS

Preparation of Feed Ingredients

The whole full-fat rapeseed was Dwarf Essex variety from Idaho and obtained locally from Athens Seed Co., GA. It was further processed by a roller grinder to split into pieces and included at desired levels to prepare a balanced diet. Solvent-extracted canola meal was obtained in pellet form which was further ground to prepare treatment diets. Synthetic AITC (Sigma-Aldrich, St. Louis, MO) was purchased and included in basal diet at levels of 500 ppm and 1,000 ppm. Control diet was based on corn-SBM. The feed ingredients were analyzed for nutrient profile and glucosinolate level before formulating feed (Table 1).

Preparation and Sporulation of E. maxima Oocyst

Fresh field isolated oocyst of *E. maxima* was obtained from Dr Brian Jordan at Department of Poultry Science, University of Georgia. Further oocyst preparation and sporulation was performed using 2.5% Potassium dichromate following procedure used by Teng et al. 2021.

Poultry Husbandry, Dietary Treatments, and E. maxima Oral Infection

This study was conducted at the Poultry Research Center after the approval of the Institutional Animal Care and Use Committee of University of Georgia, Athens, GA. A total of 576 one-day old male broiler chicks (Cobb 500) were randomly allocated to 8 treatments with 6 replicates cage and 12 birds per cage. The dietary treatments included nonchallenge control (**NC**, corn-SBM based, nonchallenged with *E. maxima*), challenge control (CC, corn-SBM based, challenged with *E. maxima*), 10% rapeseed (10RS), 30% rapeseed (30RS), 20% canola meal (20CLM), 40% canola meal (40CLM), 500 ppm AITC supplemented in basal diet (500AITC), and 1,000 ppm AITC supplemented in basal diet (1000AITC). The reason for using different levels of rapeseed and canola meal was based on previous studies that showed rapeseed can only be included in diet at 5 to 15%; thus, the present study used the average 10% as a lower level (Nega and Woldes, 2018). Similarly, for

Table 1. Composition and calculated nutrient contents of control and treatment diets, as-is basis.

Items	NC/CC[1]	10RS	30RS	20CLM	40CLM	500AITC	1000AITC
Ingredients %							
Corn, grain	63.97	59.22	38.82	58.93	49.90	63.97	63.97
Rapeseed	0.00	10.00	30.00	0.00	0.00	0.00	0.00
Canola meal	0.00	0.00	0.00	20.00	40.00	0.00	0.00
Soybean meal (48%)	30.28	24.51	20.17	13.39	1.16	30.28	30.28
Soybean oil	1.00	1.75	6.74	2.87	4.73	1.00	1.00
L-threonine	0.16	0.23	0.20	0.21	0.14	0.16	0.16
Limestone	1.26	1.12	0.96	0.94	0.77	1.26	1.26
Dicalcium -phosphate	1.68	1.71	1.75	1.64	1.57	1.68	1.68
Common salt	0.35	0.33	0.30	0.30	0.30	0.35	0.35
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.08	0.08	0.08	0.08	0.08	0.08	0.08
DL-methionine	0.34	0.35	0.33	0.60	0.33	0.34	0.34
L-lysine HCl	0.35	0.45	0.41	0.49	0.47	0.35	0.35
product space	0.30	0.00	0.00	0.30	0.30	0.30	0.30
AITC*,[4], ppm	0.00	0.00	0.00	0.00	0.00	115	353
Glucosinolate[4] (μmol/g)	0.00	2.95	9.66	1.30	1.27	-	-
Erucic acid[5], %	N/D	16.14	24.66	N/D	N/D	N/D	N/D
Nutrients							
ME*, kcal/kg	3,000	2,950	2,950	3,000	2,950	3,000	3,000
Crude protein[4]	20.13	19.88	20.19	20.38	20.94	20.13	20.13
Calcium	0.93	0.90	0.90	0.90	0.93	0.93	0.93
Total phosphorus	0.68	0.71	0.78	0.81	0.94	0.68	0.68
Avail. phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine[6]	1.22	1.24	1.26	1.27	1.28	1.22	1.22
Methionine[6]	0.63	0.64	0.62	0.91	0.68	0.63	0.63
Threonine[6]	0.85	0.87	0.88	0.88	0.83	0.85	0.85

[1]NC: nonchallenge control (corn-SBM based diet), CC: challenge (*E. maxima*) control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet.

[2]Provided per kg of DSM Vitamin premix: Vit. A 2,204,586 IU, Vit. D3 200,000 ICU, Vit. E 2,000 IU, Vit. B12 2 mg, biotin 20 mg, menadione 200 mg, thiamine 400 mg, riboflavin 800 mg, d-pantothenic acid 2,000 mg, Vit. B6 400 mg, niacin 8,000 mg, folic acid 100 mg, choline 34,720 mg.

[3]Provided per kg of mineral premix: Ca 0.72 g, Mn 3.04 g, Zn 2.43 g, Mg 0.61 g, Fe 0.59 g, Cu 22.68 g, I 22.68 g, and Se 9.07 g.

[4]Glucosinolate and crude protein analyzed value.

[5]Erucic acid was determined as % of erucic acid out of total fatty acid. N/D = Not detected in the sample.

[6]Digestible amino acid values.

*AITC, allyl isothiocyanate; ME, metabolizable energy.

Table 2. Growth performance of broiler chicken between 1 and 20 d of age fed different treatment diets.

	NC	CC	10RS	30RS	20CLM	40CLM	500AITC	1000AITC	SEM	*P* value
Pre-challenge, 0-14 d										
BW	416.07^{a}	416.15^{a}	356.00^{b}	277.11^{c}	360.33^{b}	366.28^{b}	416.46^{a}	402.73^{a}	6.899	<0.0001
BWG	372.15^{a}	372.39^{a}	312.15^{b}	233.21^{c}	316.56^{b}	322.62^{b}	372.56^{a}	358.76^{a}	6.900	<0.0001
FI	491.40^{a}	497.65^{a}	471.24^{a}	388.00^{c}	462.12^{ab}	417.96^{bc}	485.40^{a}	486.54^{a}	6.338	<0.0001
FCR	1.32^{d}	1.34^{cd}	1.51^{b}	1.67^{a}	1.46^{bc}	1.29^{d}	1.3^{d}	1.36^{cd}	0.020	<0.0001
Challenge, 14−20 d										
BW	816.95^{a}	724.57^{b}	620.05^{c}	482.72^{d}	641.17^{c}	655.62^{c}	727.7^{b}	709.92^{b}	13.980	<0.0001
BWG	444.80^{a}	352.18^{b}	307.90^{c}	249.51^{d}	324.61^{bc}	333.00^{bc}	355.14^{b}	351.16^{b}	7.960	<0.0001
FI	571.47^{a}	530.64^{ab}	505.12^{ab}	389.85^{c}	475.63^{b}	471.44^{bc}	498.4^{ab}	515.33^{ab}	9.460	<0.0001
FCR	1.29^{b}	1.51^{ab}	1.64^{a}	1.57^{ab}	1.47^{ab}	1.42^{ab}	1.40^{ab}	1.48^{ab}	0.030	0.03
Overall, 0−20 d										
BW	816.95^{a}	724.57^{b}	620.05^{c}	482.72^{d}	641.17^{c}	655.62^{c}	727.7^{b}	709.92^{b}	13.980	<0.0001
BWG	773.04^{a}	680.80^{b}	576.2^{c}	438.82^{d}	597.39^{c}	611.97^{c}	683.8^{b}	665.94^{b}	13.980	<0.0001
FI	1062.88^{a}	1028.29^{ab}	976.36^{abc}	777.85^{d}	937.75^{bc}	889.39^{cd}	983.81^{abc}	1001.86^{abc}	15.200	<0.0001
FCR	1.38^{d}	1.51^{bcd}	1.69^{ab}	1.78^{a}	1.57^{bc}	1.45^{cd}	1.44^{cd}	1.51^{bcd}	0.020	<0.0001

NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet.

N = 72 birds/ treatment.

Abbreviations: BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

^{abc}Means followed by different alphabet in the same row indicates statistical significance by the test of Tukey's at 95% confidence interval. Dpi^{1} represent days-post infection.

canola meal, the range of its inclusion based on previous studies was 15 to 25%; thus, 20% CLM was used as lower level (Mushtaq et al., 2007; Min et al., 2011). The higher levels, 30RS, and 40CLM, were used to investigate anti-*Eimeria* effect of their inherent high concentration of glucosinolate or its bioactive compounds. Similar dietary treatments were included in a previous study from our lab (Yadav et al., 2021). Birds in 500AITC and 1000AITC groups received corn-SBM based control diet till d 11 of age and provided AITC treated diet from d 12. On d 14, birds in NC group were gavage with 1 mL water as sham, whereas all other treatments received 1 mL of freshly prepared *E. maxima* sporulated oocysts at a dose of approximately18,000 oocysts/ mL. The challenge dose was determined based on a previous study from our lab (Teng et al., 2020a), and the current study was conducted till d 20 (6 d postinfection, **dpi**). All the birds had access to ad libitum feed and water and were kept under controlled temperature environment following the recommendation of Cobb management guide (Cobb, 2018).

Sample Collection and Analyses

Growth Performance The birds were weighed on d 0 (day-of-hatch) before allocation, and the body weight (**BW**) and feed intake (**FI**) were recorded during pre-challenge period (d 0−d 14), challenge period (d 14−d 20), and overall period (d 0−d 20). The measured BW and FI were used to calculate body weight gain (**BWG**) and feed conversion rate (**FCR**) as shown in Table 2. The mortality was recorded and used to calculate corrected FI.

Intestinal Permeability

On 5 dpi, one bird from each cage (6 birds/ treatment) was orally gavage with 1 mL/ bird (2.2 mg/bird) of the fluorescein isothiocyanate dextran (**FITC-d**; 100 mg, MW 4,000; Sigma-Aldrich) to determine intestinal permeability by following the method from Teng et al. (2020a). In brief, the birds after FITC-d gavage were kept in separate cages by treatment for 2 h. After exact 2 h, birds were euthanized and blood samples (at least 2 mL) were collected and stored in a dark container with blood vials kept in slant position at room temperature for 2 h. All the blood samples were centrifuged (Eppendorf Centrifuge 5430R, Eppendorf, Hamburg, Germany) at 1,000 × g for 15 min to obtain serum. Blood samples were also collected from 10 extra birds (kept in the same room but not involved in experiment) whose serum was pooled, mixed with FITC-d and used to make a standard curve. The obtained serum samples (in duplicate) and standard solution (in triplicate; 100 μL per well) were kept in a dark 96-well plate (Ref. 655077, Greiner Bio-one, Monroe, NC). This plate was read in a spectrophotometer (SpectraMax ABS plus, Softmax Pro 7 software, Molecular devices, San Jose, CA) at excitation and emission wavelength of 485 and 528 nm, respectively. The values obtained were fitted to standard curve equation to obtained intestinal permeability value as shown in Figure 1.

Oocyst Shedding

On 5 dpi, clean paper was kept under each cage, and fecal dropping was collected for 48 h. From each cage approximately 200 g of representative excreta was collected in sample bag and stored at 4°C until further processing following the modified procedure by Teng et al. (2020b). Briefly, 5 g of fecal samples were diluted with 45 mL water. After proper mixing, 1 mL mixture was taken and diluted with 9 mL saturated salt solution. Homogenized mixtures were incubated for 30 s for letting oocysts float. Using a water dropper pipette (Thermo Fisher Scientific, Waltham, MA), the final

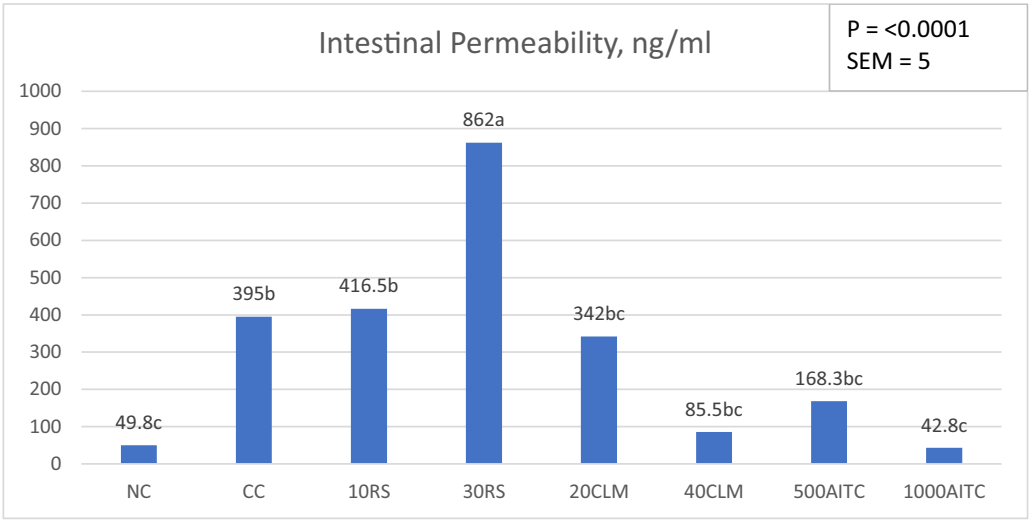

Figure 1. Intestinal permeability of broiler chickens on 5 d post-infection. [abc]Means followed by different alphabet in the bar graph indicates statistical significance by the test of Tukey's at 95% confidence interval. NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet. N = 6 birds/ treatment.

samples were loaded in McMaster chambers (Jorgensen Laboratories, Loveland, CO), and the total oocysts were counted, and the data was calculated as oocysts per gram using formula as shown by Yadav et al. (2020). Data is presented in Figure 2.

Intestinal Gross Lesion Score and Microscore

On 6 dpi, four birds per cage were euthanized by cervical dislocation, and 15 cm of jejunum and ileum tissues with reference to Meckel's diverticulum were evaluated for gross lesion score (**G-LS**) by the 5-score system (Johnson and Reid, 1970) shown in Figure 3. In addition, for microscores (M-LS), slides were prepared by mounting mucosal scraping from same location as G-LS. The slides were kept moist and studied under a microscope on the same day post sampling. Microscores were evaluated following the method by Goodwin et al. (1998) where score 0 indicates no oocyst; score 1, 1−20 oocyst; score 2, 21−50 oocyst; score 3, 51−100 oocyst; score 4, too numerous to count. Around 8 to 10 fields were observed for each slide. The result is shown in Figure 4.

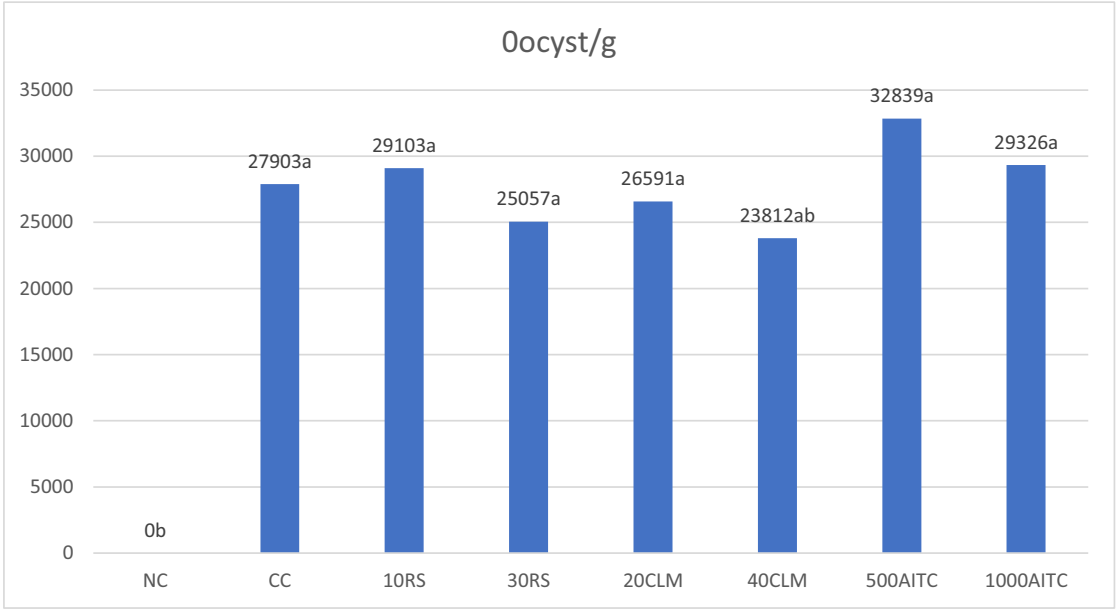

Figure 2. Oocyst shedding of birds challenged with E. maxima on d 14 and feces collected from 5 to 6 d post-infection. [abc]Means followed by different alphabet in the bar graph indicates statistical significance by the test of Tukey's at 95% confidence interval. NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet.

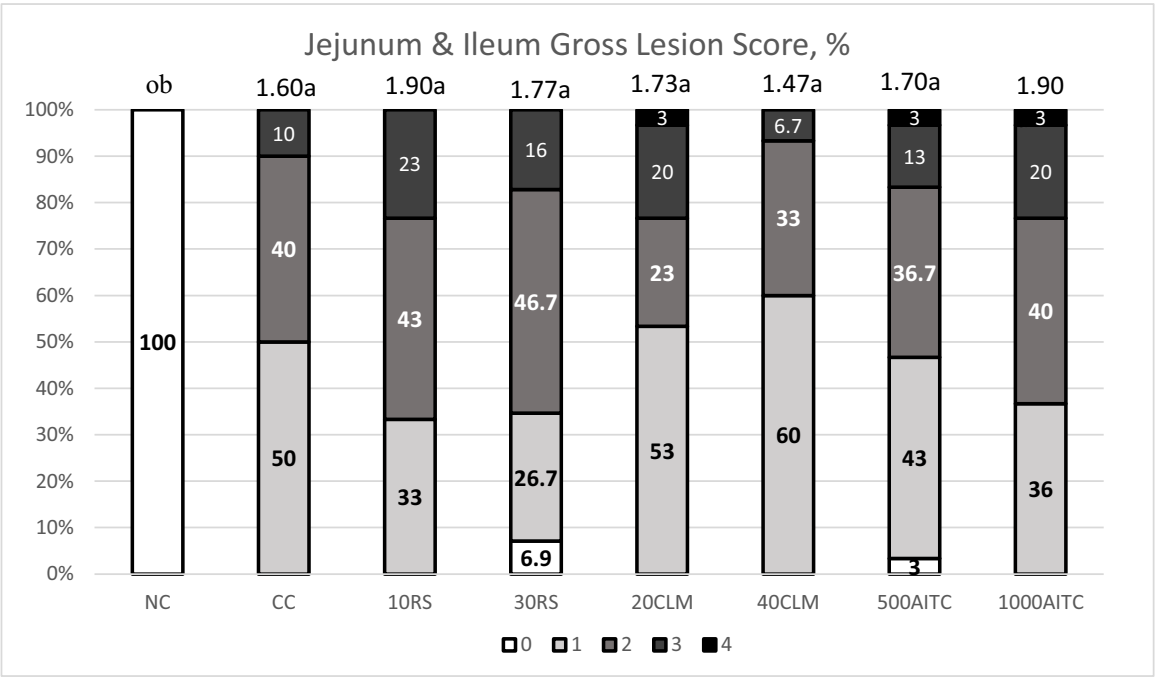

Figure 3. Gross lesion score of birds challenged with E. maxima on d 14 and sampled on 6 d post-infection. [abc]Means scores followed by different alphabet in the bar graph indicates statistical significance by the test of Tukey's at 95% confidence interval. Numbers inside the bar indicates percentage of score out of total (100%) as shown in legend. NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet. N = 30 birds/ treatment.

Histomorphology

On 6 dpi, 1 bird/ cage was euthanized by cervical dislocation, and 5-cm intestinal tissue samples from the duodenum loop, mid-jejunum, and mid-ileum were rinsed with phosphate buffer saline (**PBS**) and kept in 10% buffered formalin. Tissue samples were cut cross-sectionally to 0.5 cm and loaded in tissue embedding cassettes which were sent out for further processing and slide preparation to histology-core at Poultry Diagnostic

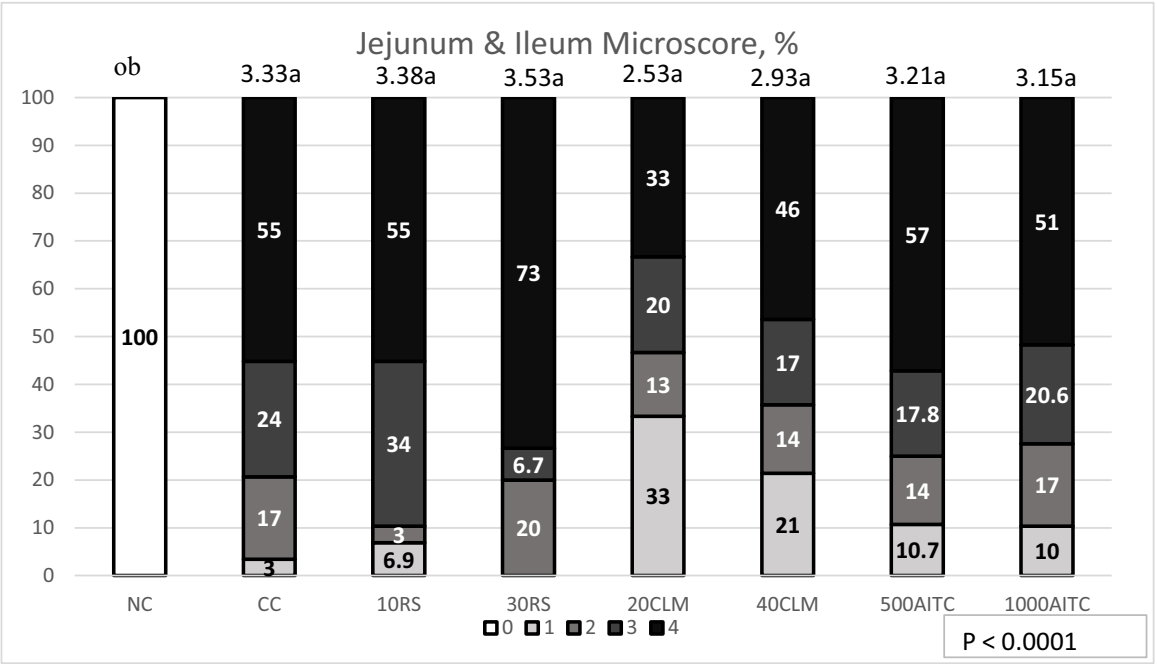

Figure 4. Microscore lesion score of birds challenged with *E. maxima* on d 14 and sampled on 6 d post-infection. [abc]Means scores followed by different alphabet in the bar graph indicates statistical significance by the test of Tukey's at 95% confidence interval. Numbers inside the bar indicates percentage of score out of total (100%) as shown in legend. NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet. N = 30 birds/ treatment.

Table 3. Intestinal histology of broiler chickens on 6 d post-infection fed different treatment diets.

Parameter	NC	CC	10RS	30RS	20CLM	40CLM	500AITC	1000AITC	SEM	*P* value
Duodenum										
VH	2267.59	2166.37	2568.86	2541.48	2315.67	2426.76	2364.88	2526.28	45.994	0.285
CD	262.43	267.88	298.42	280.75	272.75	241.92	287.97	256.75	6.970	0.584
VH: CD	8.70	8.19	8.73	9.47	8.94	10.43	8.35	10.11	0.286	0.439
Jejunum										
VH	1199.70^{a}	913.66^{b}	1060.07^{ab}	1012.10^{ab}	1001.87^{ab}	1043.28^{ab}	1074.08^{ab}	1162.09^{a}	20.973	0.012
CD	217.86	311.78	265.27	285.66	268.72	275.67	286.88	258.58	8.537	0.263
VH: CD	5.88^{a}	3.21^{b}	4.17^{ab}	3.70^{b}	3.84^{ab}	4.02^{ab}	3.77^{ab}	4.56^{ab}	0.187	0.014
Ileum										
VH	800.00	700.14	768.24	621.27	673.97	717.67	729.79	755.90	17.218	0.219
CD	174.07^{b}	230.58^{ab}	253.91^{ab}	282.24^{a}	265.98^{ab}	250.32^{ab}	235.41^{ab}	292.06^{a}	8.715	0.018
VH: CD	4.73^{a}	3.31^{ab}	3.07^{b}	2.32^{b}	2.60^{b}	2.89^{b}	3.28^{ab}	2.75^{b}	0.153	0.001

NC: non-challenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet.

Abbreviations: CD, crypt depth; VH, villus height; VH: CD, villus height to crypt depth ratio.

N = 6 birds/ treatment.

[abc]Means followed by different alphabet in the same row indicates statistical significance by the test of Tukey's at 95% confidence interval.

Research Center (**PDRC**, Athens, GA). The received slides were observed for villus height (**VH**) and crypt depth (**CD**) using a microscope (BZ-Z800, Keyence Inc., Itasca, IL) at 20X magnification. At least 3−5 representative VH, and CD were selected for measurement from each slide. Later ImageJ (version I.X.) program (Abramoff et al., 2004) was used to measure the VH, CD, and their ratios (Table 3).

Intestinal Gene Expression Using Real-Time PCR

On 6 dpi, the mid-jejunum tissues were cut from the same birds for intestinal histomorphology, and digesta were flushed with sterile PBS. The cleaned tissues were collected in aluminum foil and immediately snap frozen in liquid nitrogen. The samples were stored at −80°C until further processing. The total RNA was extracted using QiAzol lysis reagents (Qiagen, Valencia, CA) following manufacturer's instruction. After extraction, a nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) was used to check quality and quantity of RNA. The cDNA was obtained from RNA by reverse transcription using high-capacity cDNA synthesis kits (Applied Biosystems, Foster City, CA). For Real-Time PCR, a Step One thermocycler (Applied Biosystems) was run for each cDNA samples kept in duplicate along with target gene primers using SYBR Green Master mix (Bio-Rad Laboratories, Hercules, CA). The gene expression of target genes was normalized with housekeeping genes and relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method following Teng et al. (2020a) procedure (Table 4) and the primers used are shown in Table 5.

Table 4. The jejunum gene expression of tight junction proteins, immune genes, and nutrient transporter genes in broiler chickens on 6 d post-infection fed different treatment diets.

	NC	CC	10RS	30RS	20CLM	40CLM	500AITC	1000AITC	SEM	*P* value
TJP										
ZO1	1.00	1.04	1.09	1.22	0.97	0.93	1.11	1.22	0.035	0.302
Claudin1	1.00^{c}	2.84^{abc}	3.49^{ab}	3.14^{abc}	1.83^{abc}	1.22^{bc}	2.81^{abc}	3.91^{a}	0.278	0.06
JAM2	1.00	1.24	1.36	1.27	1.16	1.15	1.41	1.49	0.052	0.325
Occludin	1.00^{bc}	0.91^{c}	1.19^{abc}	1.47^{a}	1.21^{abc}	1.16^{abc}	1.15^{abc}	1.33^{ab}	0.037	0.002
IG										
IL2	1.00^{b}	1.26^{ab}	1.44^{ab}	1.57^{a}	1.34^{ab}	1.34^{ab}	1.69^{a}	1.76^{a}	0.064	0.05
IL6	1.00^{b}	2.01^{ab}	6.44^{a}	3.51^{ab}	2.13^{ab}	1.51^{b}	3.37^{ab}	3.03^{ab}	0.425	0.025
NTG										
Muc2	1.00	0.75	0.73	0.80	0.62	0.69	0.65	0.80	0.033	0.114
SGLT1	1.00	0.99	1.06	0.86	1.11	0.88	1.04	1.18	0.032	0.182
GLUT5	1.00^{a}	0.48^{ab}	0.49^{ab}	0.33^{b}	0.69^{ab}	0.54^{ab}	0.45^{b}	0.57^{ab}	0.047	0.013
EAAT	1.00^{a}	0.59^{b}	0.72^{ab}	0.64^{b}	0.58^{b}	0.54^{b}	0.67^{b}	0.66^{b}	0.031	0.003
PEPT1	1.00	0.78	0.78	1.03	0.88	0.83	0.76	1.05	0.033	0.108
B^{o}AT	1.00^{b}	0.98^{b}	1.28^{ab}	1.65^{a}	0.98^{b}	1.00^{b}	1.03^{b}	1.24^{ab}	0.059	0.03
LAT1	1.00^{b}	4.63^{ab}	7.44^{a}	6.49^{a}	4.31^{ab}	2.80^{ab}	6.19^{a}	5.81^{ab}	0.468	0.005

NC: nonchallenge control (corn-SBM based diet), CC: challenge control (corn-SBM based diet), 10RS: 10% Rapeseed included in basal diet, 30RS: 30% Rapeseed included in basal diet, 20CLM: 20% Canola included in basal diet, 40CLM: 40% Canola included in basal diet, 500AITC: 500 ppm allyl-isothiocyanate supplemented in basal diet, 1000AITC: 1,000 ppm allyl-isothiocyanate supplemented in basal diet.

Abbreviations: B^{o}AT, Solute carrier family 6, member 19; EAAT: Excitatory amino acid transporter; GLUT5, Glucose transporter 5; IG, immune genes; IL2, Interleukin 2; IL6, Interleukin 6; JAM2, Junctional adhesion molecule 2; LAT1, L type amino acid transporter 1; Muc2, Mucin 2; NTG, nutrient transporter genes; SGLT1, Sodium glucose transporter 1; PEPT1, Peptide transporter 1; TJP, tight junction proteins; ZO1: Zonula Occludens 1.

N = 6 birds/ treatment.

[abc]Means followed by different alphabet in the same row indicates statistical significance by the test of Tukey's at 95% confidence interval.

Table 5. List of primers used for qPCR of the jejunum tissue samples gene expression of tight junction proteins, immune genes, and nutrient transporter genes in broiler chickens on 6 d post-infection fed different treatment diets.

Gene symbol	Full name	Forward primer	Reverse primer
Housekeeping genes			
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	CCTCTCTGGCAAAGTCCAAG	GGTCACGCTCCTGGAAGATA
β-actin	Beta-actin	CAACACAGTGCTGTCTGGTGGTA	ATCGTACTCCTGCTTGCTGATCC
HMBS	Hydroxymethylbilane synthase	GGCTGGGAGAATCGCATAGG	TCCTGCAGGGCAGATACCAT
TJP[1]			
ZO1	Zonula Occludens 1	CAACTGGTGTGGGTTTCTGAA	TCACTACCAGGAGCTGAGAGGTAA
CLDN1	Claudin 1	TGGAGGATGACCAGGTGAAGA	CGAGCCACTCTGTTGCCATA
JAM2	Junctional adhesion molecule 2	AGCCTCAAATGGGATTGGATT	CATCAACTTGCATTCGCTTCA
OCLN	Occludin	ACGGCAGCACCTACCTCAA	GGCGAAGAAGCAGATGAG
IG[2]			
IL2	Interleukin 2	CGTAAGTGGATGGTTTTCCTCT	GGCTAAAGCTCACCTGGGTC
IL6	Interleukin 6	AAAGCAGAACGTCGAGTC	CTTCAGATTGGCGAGGAG
NTG[3]			
Muc2	Mucin 2	ATGCGATGTTAACACAGGACTC	GTGGAGCACAGCAGACTTTG
SGLT1	Sodium glucose transporter 1	GCCATGGCCAGGGCTTA	CAATAACCTGATCTGTGCACCAGTA
GLUT5	Glucose transporter 5	TCCTCCTGATCAACCGCAAT	TGTGCCCCGGAGCTTCT
EAAT	Excitatory amino acid transporter	TGCTGCTTTGGATTCCAGTGT	AGCAATGACTGTAGTGCAGAAGTAATATATG
PEPT1	Peptide transporter 1	CCCCTGAGGAGGATCACTGTT	CAAAAGAGCAGCAGCAACGA
B^{o}AT	Solute carrier family 6, member 19	GGGTTTTGTGTTGGCTTAGGAA	TCCATGGCTCTGGCAGAGATTT
LAT1	L type amino acid transporter 1	CACACTATGGGCGCATGCT	ATTGTGCCTGGAGGTGTTGGT

[1]TJP, tight junction proteins.
[2]IG, immune gene.
[3]NTG, nutrient transporter gene.

Statistical Analyses

Battery cage was the experimental unit for all the measurements. Data from all the analysis were presented as mean values with standard error of mean (**SEM**) as shown in respective Tables. All the data except lesion score and microscore were analyzed using PROC-GLM of SAS University Edition (Version 9.4, SAS Institute Inc., Cary, NC). When significant difference was observed in one-way ANOVA, the treatments were subjected to multiple comparison using Tukey's test following the procedure by Teng et al. (2020a). For the lesion score and microscore, the Kruskal-Wallis nonparametric analysis was performed. Statistical significances for all analyses were set at $P < 0.05$, and actual P values are presented in the Tables and Figures.

RESULTS

Growth Performance

The growth performance of birds fed SBM, rapeseed and canola were different throughout the study (prechallenge period, challenge period, and overall period: Table 2) ($P < 0.05$). The BW and BWG were significantly lower for rapeseed and canola meal fed birds during the prechallenge period ($P < 0.0001$); rapeseed at the higher inclusion (30RS) had poor BW. Similarly, FI was reduced for 30RS and 40CLM ($P < 0.0001$), whereas 10RS and 20CLM had similar feed intake as control and both AITC fed groups. Due to changes in FI and BWG, there was difference in FCR among different treatment diets fed birds during the prechallenge period. Rapeseed at inclusion of 30% had significantly highest FCR compared to the FCR of other treatments ($P < 0.0001$). The data for the prechallenge period show that rapeseed at higher level in the diet for young chicks is not recommended as it caused deleterious effect on the early growth performance. For the challenge period, the BW and BWG were different between nonchallenge NC group and all other challenged groups ($P < 0.0001$). Within the challenge groups, there was significant decrease in BW and BWG with increasing levels of rapeseed, whereas the inclusion of canola meal caused significantly reduction in BW but not BWG compared to SBM fed CC and AITC fed challenged birds. The FI during the challenge period was reduced for 30RS, 20CLM, and 40CLM compared to NC group ($P < 0.0001$), whereas comparing FI among challenged birds 30RS was significantly lower than all other treatment groups except 40CLM. The FCR during the challenge period was increased for 10RS compared to NC group ($P = 0.03$), whereas all other treatments were in between both NC and 10RS ($P > 0.05$). During the overall period, the BW and BWG were highest for NC group compared to all other challenge treatment groups, and the second highest was CC, followed by 500AITC and 1000AITC group. The 10RS, 30RS, 20CLM, and 40CLM groups had significantly lower BW and BWG compared to the other groups, whereas 30RS group had the lowest BW and BWG among the treatments ($P < 0.0001$). Overall FI was significantly reduced for 30RS, 20CLM, and 40CLM compared to NC group. A similar pattern was observed for overall FCR where 10RS, 30RS, and 20CLM had higher FCR compared to NC group ($P < 0.0001$), but higher canola inclusion had very similar FCR to NC, CC, 500AITC, and 1000AITC. The rapeseed inclusion in the higher level severely caused negative impact on growth performance. Both the 500AITC and 1000AITC groups had similar growth performance to CC group during both challenge and nonchallenge periods.

Histomorphology

In the present study, birds were challenged with *E. maxima* and its site of infection was mainly mid gut in the region of the jejunum. As expected, no differences in duodenum histomorphology were observed for VH, CD, or their ratios (Table 3). Jejunum VH for CC group was significantly decreased compared to NC and 1000AITC groups, whereas all other groups had values between NC and CC group ($P = 0.01$). There was no difference among the treatments for jejunum CD, which caused increased VH: CD ratios for NC group compared to CC and 30RS groups ($P = 0.01$). No difference among the treatments was observed for ileum VH ($P > 0.05$). The ileum CD was increased for 30RS and 1000AITC compared to NC group ($P = 0.02$). The ratio for VH: CD in the ileum was also decreased for 10RS, 30RS, 20CLM, 40CLM, and 1000AITC compared to NC group ($P = 0.001$).

Intestinal Permeability

The intestinal permeability result represents the levels of FITC-d that was recovered in the serum of birds; higher values mean increased permeability (lower gut integrity), whereas lower values represent decreased permeability (higher gut integrity). The result from the present study showed that NC and 1000AITC groups had lower permeability value compared to CC, 10RS, and 30RS ($P < 0.0001$; Figure 1). Interestingly, 20CLM, 40CLM, and 500AITC had significantly reduced permeability compared to 30RS, and no difference compared to NC, CC, 10RS, and 1000AITC.

Oocyst Shedding

The oocyst shedding was measured for 5 to 6 dpi and presented as oocyst per gram (**OPG**) (Figure 2). Usually, oocyst shedding in addition to lesion scoring and body weight gain are commonly suggested as test parameters to measure coccidiosis intervention strategies in farm (Chasser et al., 2020); it is also an indicator of the vaccine intake, development of anticoccidial resistance or the successful challenge by *Eimeria* spp. (Braunius, 1985). The result showed that NC group had no oocyst shedding, whereas all other challenged treatments with *E. maxima* shed higher oocysts ($P < 0.0001$) except for 40CLM which was not significant to both CC and NC. The 40CLM reduced oocyst shedding by almost 15% compared to CC.

Lesion Score and Microscore

On 6 dpi both gross lesion score and microscore were observed in the jejunum and ileum (Figures 3 and 4). Gross lesion score results showed that NC group did not show any intestinal lesion, whereas all other *E. maxima* challenged treatments had significantly higher average score of 1.6, 1.9, 1.77, 1.73, 1.47, 1.7, and 1.9 for CC, 10RS, 30RS, 20CLM, 40CLM, 500AITC, and 1000AITC, respectively. Microscore results for NC, CC, 10RS, 30RS, 20CLM, 40CLM, 500AITC, and 1000AITC showed average score of 0, 3.33, 3.38, 3.53, 2.53, 2.93, 3.21, and 3.15, respectively ($P < 0.0001$). Comparing both the scoring techniques, microscore showed overall higher average score than gross lesion score. This could be because the presence of oocysts does not necessarily means causing damage to the intestine or having higher gross lesion. Comparison among the treatments in gross lesion score showed that although not significant to other challenged groups, 40CLM showed 8% lower score than CC group. Similarly, microscore showed numerical decrease for 20CLM by 24%, and 40CLM by 12% when compared to CC.

Intestinal Gene Expression

In this study, representative genes for tight junction proteins, immunity, and nutrient transporter were also evaluated (Table 4). Gene expression of claudin1 tended to be different among the treatments ($P = 0.06$) where 10RS and 1000AITC had higher claudin1 expression than NC group, and 1000AITC also increased compared to 40CLM. For the occludin gene, relative expression for 30RS was higher than NC and CC, and similarly 1000AITC was higher than CC group ($P = 0.002$). The relative gene expression of interleukin-2 (**IL2**) showed increased expression in 30RS, 500AITC, and 1000AITC compared to NC treatment ($P = 0.05$). Similarly, interleukin-6 (**IL6**) relative expression was upregulated for 10RS compared to 40CLM and NC group ($P = 0.025$). For the nutrient transporter genes, LAT1expression was increased for 10RS, 30RS, and 500AITC compared to NC group ($P = 0.005$). Whereas the excitatory amino acid transporter (**EAAT**) gene expression was significantly decreased for all the treatment groups compared to NC except for 10RS group ($P = 0.003$). The fructose transporter-5 (**GLUT5**) gene expression was also significantly altered by different dietary treatments where expression was lower for 30RS and 500AITC group compared to NC ($P = 0.01$). The Na + -dependent amino acid transporter (B^oAT) relative expression was reduced for NC, PC, 20CLM, 40CLM, and 500AITC when compared with 30RS group ($P = 0.03$). No differences in relative gene expression were observed for ZO1, JAM2, MUC2, SGLT1 and PEPT1 among the treatments.

DISCUSSION

This study investigated the consequences of *E. maxima* challenge to broilers fed SBM in control group, 2 levels of full-fat rapeseed, 2 levels of canola meal, and supplementation with 2 levels of AITC for their potential beneficial effects in broiler chicken. Birds were challenged on d 14 with a dose between mid-low and mid-high to cause subclinical infection as recommended by Teng et al. (2020a).

In the present study, there was difference in growth performance between non-challenged and all other treatment groups challenged with *E. maxima*. This is in accordance with previous studies that showed decrease in growth performance with single or mixed species of *Eimeria* challenge (Hamzic et al., 2015; Dos Santos et al., 2020; Yadav et al., 2020). In a large-scale *E. maxima* challenge study, significant differences in BWG between challenged and nonchallenged group were found similar to the present study (Hamzic et al., 2015). Cocci infection was also characterized by poor growth and feed conversion with high mortality even during subclinical challenge with *Eimeria* (Lee et al., 2013). Although feeding canola meal showed decreased growth performance than control group, no difference was observed between 20 and 40% inclusion, except 40CLM had lowest FCR. The present study is not in agreement with a previous experiment reporting that canola meal can only be included up to 10% for starter chicks and maximum 20% during a grower phase (Canola, 2009). Other studies had different inclusion levels of canola without negative effect on growth: 25% inclusion (Min et al. 2011) and below 20% inclusion (Payvastagan et al., 2012). Throughout the current study, feeding 10RS had similar effect as 20CLM, whereas feeding 30RS had severely affected growth reduction before and after challenge. This could be solely because of the antinutritional factors present in the rapeseed. The antinutritional factors in rapeseed include GLs, erucic acid, sinapine, tannins, phytic acid, and fiber components that limit the use of rapeseed at higher inclusion (Zhu et al., 2018). These factors also reduce nutrient digestibility by limiting nutrient availability to the birds (Zhu et al., 2018, 2019). The 30RS group had least feed intake compared to any other treatments because of the bitter taste of GL component which decreases the palatability along with crude fiber that decrease FI by increasing satiety (Canola, 2009; Zhu et al., 2019). Furthermore, rapeseed used in the present study was high in fat (26% *as-is* basis), and the crude fat digestibility could have been lowered by the erucic acid present in rapeseed (Zhu et al., 2019). Decrease in FI ultimately caused reduction in BW, and BWG compromising overall growth performance with rapeseed inclusion. These limitations were further accompanied by *Eimeria* challenge negatively affecting the birds across challenged treatments compared to NC group.

Because *Eimeria* spp. cause severe intestinal damage, the proper tools/ biomarkers that could identify intestinal entities related to infection would be great help to evaluate mitigation strategy and nutritional manipulation during infection by *Eimeria* or prophylaxis to control before exposure (De Meyer et al., 2019; Criado-Mesas et al., 2021). Some of the most studied parameters are gut permeability, intestinal histomorphology, intestinal lesion score, gene expression related to intestinal barrier proteins, nutrient transporters, and immune genes (Teng et al., 2020a; Yadav et al., 2020).

A single layer of epithelial cells joined together by tight junctions helps maintain gut selective permeability in healthy birds. The gut permeability significantly increases with the infection by *Eimeria* (Teng et al., 2020a; Yadav et al., 2020). The pathological changes in the gut such as altered intestinal histomorphology, lesion score, tight junction proteins, as well as systemic changes all start with the increase in gut permeability caused by the disintegration of mucosal barriers by the presence of *Eimeria* (Belote et al., 2019). In the present study, the gut permeability increased for the CC, 10RS, and 30RS groups compared to NC; this is an indication of reduced tight junction integrity and barrier function. Moreover, reduction of jejunum VH and VH: CD ratio in these treatments also reflects gut health and integrity reduction by *E. maxima* infection.

Although oocyst shedding was not significant among the challenged groups, there was no oocyst detected in the NC group, suggesting that there was no cross contamination between challenged and nonchallenged cages throughout the experiment. Hamzic et al. (2015) found the correlation between BWG and oocyst count where higher BWG has higher oocyst count, and the present study showed numerical decrease in the oocyst shedding of 40CLM compared to CC group. Previous studies also found huge variation between treatments in the challenged groups especially for oocyst shedding and lesion score (Pinard-van der Laan et al., 2009; Hamzic et al., 2015)

Lesion score was observed in the jejunum and ileum of challenged group as mid-gut is the site of action for *E. maxima*. A study by Hamzic et al. (2015) showed that intestinal lesion score is a good indicator of health status in the *Eimeria* infected birds. However, Belote et al. (2019) claimed that the "I See Inside" (**ISI**) methodology is better tool to diagnose even mild alteration caused by subclinical coccidiosis. NC group in the present study did not show any lesion score in both methods of observation (gross lesion scoring and in microscoring). Although not significant within challenged birds there was numerical decrease in lesion for canola fed birds.

Coccidiosis causes damage to the intestinal mucosa, altering their absorptive surface during severe infection, decreasing the digestibility of nutrients, and leading to reduced growth performance (Dalloul and Lillehoj, 2006; Dos Santos et al., 2020; Hafez and Attia, 2020). No difference in histomorphology of the duodenum was noticed when only *E. maxima* was used because *E. maxima* specifically attach and infect jejunum and proximal ilium. The 1000AITC group had similar jejunum VH as NC group although the cell turnover rate was high in the ileum, causing significantly higher CD leading to decreased VH: CD ratio in the ileum for 1000AITC fed birds. According to Criado-Mesas et al. (2021), the higher cell turnover in *Eimeria* challenged birds is due to activation of mTOR complex 1 pathway to reduce the intestinal mucosal disturbance. Previous studies showed no difference in VH and VH: CD ratio when canola was included up to 40% (Figueiredo et al., 2003; Chiang et al., 2009). This agrees with the present study

although there was reduced VH: CD ratio in the ileum, which was supported by a study from Gopinger et al. (2014) showing that 20% canola inclusion decreased VH: CD ratio. This effect on histology of the ileum could also be due to challenge with *E. maxima* which increased the metabolic cost of epithelial cell turnover in the ileum. Teng et al. (2020a) reported that increasing *Eimeria* challenge doses caused linear negative effect on gut histology. The present study had decrease in jejunum VH and VH: CD ratio for challenge control and VH: CD ratio for 30RS, whereas other treatments were able to maintain intestinal morphology in between NC and CC group. The disturbance in intestinal parameters during *Eimeria* infection also causes dysbiosis leading to increase in opportunistic pathogens like *C. perfringens, Enterococcus, Streptococcus* and decrease in non-pathogenic *Lactobacillus* and *Faecalibacterium* (De Meyer et al., 2019; Chen et al., 2020). This dysbiosis further deteriorates the situation in intestinal parameters due to production in toxic materials with the rise in harmful bacteria.

With the damage to gut barrier and epithelium during subclinical doses of *E. maxima* infection, malabsorption of nutrients has been associated with altered gene expression especially nutrient transporter genes, tight junction genes, and immune genes (Su et al., 2018; Teng et al., 2021). The damage of absorption sites and presence of diarrhea ultimately lead to loss of dietary nutrients in excreta. This is one of the pathways leading to decreased growth performance during challenge. Although coccidiosis may damage mucosa, triggering impaired absorption of nutrients (Criado-Mesas et al., 2021), gene expression of MUC2 (component of mucus layer) was numerically reduced in the present study. As AITC are known candidates to reduce oxidative stress, the challenged birds should have coped up with the inflammation associated with oxidative stress which is responsible for changes in gene expression (Criado-Mesas et al., 2021). The gene expression in the intestine also depends on the severity as in case of necrotic enteritis genes are more profoundly expressed compared to mild-coccidiosis (Criado-Mesas et al., 2021). The expression of tight junctions also depends on pathogen as some pathogens utilize these proteins to attach whereas for other pathogens tight junctions act as barrier to enter the underlying cells; thus, the expression of tight junctions should be destroyed or modified for the pathogens to invade intestinal epithelial cells (Awad et al., 2017). Criado-Mesas et al. (2021) found down-regulation of claudin1 and occludin following *Eimeria* challenge; however, it was up-regulated in the present study. The present study also found downregulation of nutrient transport genes, which elucidate the changes to gut histomorphology. There was downregulation of brush border membrane, amino acid transporters (B^oAT) except for rapeseed and higher AITC levels which indicate reduction in uptake of neutral amino acids such as alanine, serine, cysteine, and threonine from gut lumen to epithelial cells which also disrupt other cationic amino acids transport across membrane as explained by Teng et al. (2021).Along with other nutrient transport genes EAAT expression decreased which is responsible for internalization of glutamate and aspartate across membrane (Kanai et al., 2013; Teng et al., 2021). Similar results were obtained by Su et al. (2014) where brush border membrane amino acids transporters are downregulated, whereas the basolateral membrane amino acid transporter like LAT1 was upregulated especially for rapeseed fed birds and low level of AITC in present study. During the acute infection by *Eimeria*, birds respond by decreasing the amino acid pool in the enterocytes limiting the nutrient for cell causing cell death that also respond in decreasing *E. maxima* replication and increase the shedding from the cells as witnessed in the fecal oocyst shedding count increase.

In response to *Eimeria* presence in the intestine, the IL2 gene was significantly upregulated for 30RS, 500AITC and 1000AITC in the present study. Previous study by Li et al. (2002) suggests that duodenal IL2 was upregulated in *Eimeria* resistant birds when compared for susceptibility between different bird lines. IL2 as a proinflammatory cytokines is able to activate T cells and other immune cells as mode for rapid inflammatory response against *Eimeria* (Broom and Kogut, 2019). IL6 acts as both proinflammatory as well as anti-inflammatory at the same time and is upregulated during *Eimeria* infection. In the present study, gene expression of IL6 in 40CLM was very similar to NC group which means that birds were able to resist with the inflammation caused by *Eimeria* whereas upregulation of IL6 in the 10RS group suggest that bird immune cells were fighting against inflammation due to exposure to *E. maxima* in the gut. Thus, immune gene expression of IL2 and IL6 in the jejunum of *Eimeria* infected birds showed more resistance to infection and higher fighting capability of rapeseed and AITC fed birds.

In summary SBM, rapeseed, and canola meal reacted differently to the *Eimeria* infection. Rapeseed at the 30% inclusion had severe negative impact on growth and gut health, whereas a synthetic compound of GL metabolites (AITC) was able to maintain gut integrity and bird performance. This was the first study to evaluate AITC in feed as a supplement, and further in-depth knowledge about this compound will help explore the beneficial role of the natural plant-based compound to control poultry diseases. Canola meal inclusion although decreased body weight of birds, both 20 and 40% were able to maintain feed efficiency and better gut health compared to the other treatments in *Eimeria* challenge groups. The gut parameters including gut permeability, histomorphology, tight junction protein gene expression, lesion scoring, as well as oocyst shedding were positively influenced by canola inclusion in feed even during the acute *E. maxima* infection period. Thus, 40% canola meal and 1,000 ppm of AITC supplementation could be good supplements during coccidiosis outbreak. Further investigation is essential to determine optimum doses of inclusion and elucidate mechanism to alleviate negative effects by *Eimeria* infection.

ACKNOWLEDGMENTS

Authors want to thank Kim's Lab members for helping in sample collection, processing, and suggestions on this manuscript. Special thanks to Yuguo Hou Tompkins and Hanyi Shi for help and generous support throughout the study.

DISCLOSURES

There is no conflict of interest.

REFERENCES

Abramoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics Int. 11:36–42.

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.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: a review. Can. J. Anim. Sci. 73:679–697.

Bellostas, N., A. D. Sørensen, J. C. Sørensen, and H. Sørensen. 2007. Genetic variation and metabolism of glucosinolates. Adv. Bot. Res. 45:369–415.

Belote, B. L., I. Soares, A. Tujimoto-Silva, A. W. Sanches, A. L. Kraieski, and E. Santin. 2019. Applying I see inside histological methodology to evaluate gut health in broilers challenged with Eimeria. Vet. Parasitol. 1:100004.

Bennett, R., and J. Ijpelaar. 2005. Updated estimates of the costs associated with thirty-four endemic livestock diseases in Great Britain: a note. J. Agr. Econ. 56:135–144.

Beski, S. S., R. A. Swick, and P. A. Iji. 2015. Specialized protein products in broiler chicken nutrition: a review. Anim. Nutr. 1:47–53.

Blake, D. P., J. Knox, B. Dehaeck, B. Huntington, T. Rathinam, V. Ravipati, S. Ayoade, W. Gilbert, A. O. Adebambo, I. D. Jatau, and M. Raman. 2020. Re-calculating the cost of coccidiosis in chickens. Vet. Res. 51:1–14.

Bourdon, D., and A. Aumaitre. 1990. Low-glucosinolate rapeseeds and rapeseed meals: effect of technological treatments on chemical composition, digestible energy content and feeding value for growing pigs. Anim. Feed Sci. Technol. 30:175–191.

Braunius, W. W. 1985. Ionophorous anticoccidial drugs in coccidiosis control. Worlds Poult. Sci. J. 41:198–209.

Broom, L. J., and M. H. Kogut. 2019. Deciphering desirable immune responses from disease models with resistant and susceptible chickens. Poult. Sci. 98:1634–1642.

Canola council. 2009. Canola meal Feed Industry Guide. 4th ed. Canola Council of Canada, Winnipeg, Manitoba, Canada. Accessed Jan. 2021. https://cigi.ca/wp-content/uploads/2011/12/2009-Canola_Guide.pdf.

Chasser, K. M., A. F. Duff, K. M. Wilson, W. N. Briggs, J. D. Latorre, J. R. Barta, and L. R. Bielke. 2020. Research Note: Evaluating fecal shedding of oocysts in relation to body weight gain and lesion scores during Eimeria infection. Poult. Sci. 99:886–892.

Chen, H. L., X. Y. Zhao, G. X. Zhao, H. B. Huang, H. R. Li, C. W. Shi, W. T. Yang, Y. L. Jiang, J. Z. Wang, L. P. Ye, and Q. Zhao. 2020. Dissection of the cecal microbial community in chickens after *Eimeria tenella* infection. Parasit. Vectors. 13:56.

Chiang, G., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2009. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian-Austral. J. Anim. Sci. 23:263–271.

Chun Chang, T., and B. Brian. 2004. Isolation of intact glucosinolates from mustard seed meal to increase the sustainability of biodiesel utilization. Soc. Eng. Agric. Food Biol. Syst (2004 ASAE/CSAE Annual International Meeting, Ontario, Canada):1–11.

Criado-Mesas, L., N. Abdelli, A. Noce, M. Farré, J. F. Pérez, D. Solà-Oriol, R. Martin-Venegas, A. Forouzandeh, F. González-Solé, and J. M. Folch. 2021. Transversal gene expression panel to evaluate intestinal health in broiler chickens in different challenging conditions. Sci. Rep. 11:1–14.

Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. Expert Rev. Vaccines. 5:143–163.

De Meyer, F., V. Eeckhaut, R. Ducatelle, M. Dhaenens, S. Daled, A. Dedeurwaerder, M. De Gussem, F. Haesebrouck, D. Deforce, and F. Van Immerseel. 2019. Host intestinal biomarker identification in a gut leakage model in broilers. Vet. Res. 50:1–14.

Dos Santos, T. S., P. Y. Teng, S. Yadav, F. L. de Souza Castro, R. L. Gould, S. W. Craig, C. Chen, A. L. Fuller, R. Pazdro, J. R. Sartori, and W. K. Kim. 2020. Effects of inorganic Zn and Cu supplementation on gut health in broiler chickens challenged with Eimeria spp. Front. Vet. Sci. 7.

Figueiredo, D. F., A. E. Murakami, M. A. D. S. Pereira, A. C. Furlan, and F. L. B. Toral. 2003. Performance and duodenum mucous morphometry of broiler chickens fed canola meal, during the starting period. REV. Bras. De Zootec. 32:1321–1329.

Georgeta, C. 2009. Efficiency of soybean meal replacement by rapeseed meal and/or canola seeds in commercial layer diets. Archiva Zootechnica 12:27–33.

Gómez-Osorio, L. M., J. J. Chaparro-Gutiérrez, and S. López-Osorio. 2021. Nutrition and poultry coccidiosis: causes, consequences and current strategies to modulate the disease. Adv. Poult Nutr. Res. (Intech Open, London, UK).

Goodwin, M. A, J. Brown, and D. I. Bounous. 1998. Use of microscopic lesion scores, gross lesion scores and oocyst count scores to detect Eimeria maxima in chickens. Avian Pathol 27:405–408.

Gopinger, E., E. G. Xavier, M. C. Elias, A. A. Catalan, M. L. Castro, A. P. Nunes, and V. F. Roll. 2014. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult. Sci 93:1130–1136.

Hafez, H. M., and Y. A. Attia. 2020. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Front. Vet. Sci. 7:516.

Hafez, H. M., Y. A. Attia, F. Bovera, M. E. Abd El-Hack, A. F. Khafaga, and M. C. de Oliveira. 2021. Influence of COVID-19 on the poultry production and environment. Environ. Sci. Pollut. Res. Int. 28:44833–44844.

Hamzic, E., B. Bed'Hom, H. Juin, R. Hawken, M. S. Abrahamsen, J. M. Elsen, B. Servin, M. H. Pinard-van der Laan, and O. Demeure. 2015. Large-scale investigation of the parameters in response to Eimeria maxima challenge in broilers. J. Anim. Sci. 93:1830–1840.

Hickling, D. 2001. Canola Meal Feed Industry Guide. 3rd ed. Canola Council of Canada, Winnipeg, MB, Canada.

Hossain, M. A., M. M. Bhuiyan, and P. A. Iji. 2015. Nutritive value of vegetable protein diets for broiler chickens and selection of diets containing different vegetable or animal proteins. Worlds Poult. Sci. J. 71:15–26.

Hristov, A. N., C. Domitrovich, A. Wachter, T. Cassidy, C. Lee, K. J. Shingfield, P. Kairenius, J. Davis, and J. Brown. 2011. Effect of replacing solvent-extracted canola meal with high-oil traditional canola, high-oleic acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 94:4057–4074.

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol 28:30–36.

Kanai, Y., B. Clemencon, A. Simonin, M. Leuenberger, M. Lochner, M. Weisstanner, and M. A. Hediger. 2013. The SLC1 high-affinity glutamate and neutral amino acid transporter family. Mol. Aspects Med. 34:108–120.

Lee, K. W., H. S. Lillehoj, S. I. Jang, S. H. Lee, D. A. Bautista, G. D. Ritter, E. P. Lillehoj, and G. R. Siragusa. 2013. Comparison of live Eimeria vaccination with in-feed salinomycin on growth and immune status in broiler chickens. Res. Vet. Sci. 95:110–114.

Li, G., E. P. Lillehoj, and H. S. Lillehoj. 2002. Interleukin-2 production in SC and TK chickens infected with eimeria tenella. Avian Dis. 46:2–9.

Lillehoj, H., Y. Liu, S. Calsamiglia, M. E. Fernandez-Miyakawa, F. Chi, R. L. Cravens, S. Oh, and C. G. Gay. 2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49:1–18.

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.

McDougald, L. R., A. L. Fuller, and B. L. McMurray. 1997. A survey of coccidian on 43 poultry farms in Argentina. Avian Dis 41:485–487.

Min, Y. N., Z. Wang, C. Coto, F. Yan, S. Cerrate, F. Z. Liu, and W. Waldroup. 2011. Evaluation of canola meal from biodiesel production as a feed ingredient for broilers. Int. J. Poult. Sci. 10:782–785.

Mushtaq, T., M. Sarwar, G. Ahmad, M. A. Mirza, H. Nawaz, M. H. Mushtaq, and U. Noreen. 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. Poult. Sci. 86:2144–2151.

Nega, T., and Y. Woldes. 2018. Review on nutritional limitations and opportunities of using rapeseed meal and other rape seed by-products in animal feeding. J. Nutr. Heal. Food Eng. 8:43–48.

Outlook, O. F. A. 2020. OECD/FAO. Accessed Jan. 2021. https://www.oecd-ilibrary.org/sites/29248f46-en/index.html?itemId=/content/component/29248f46-en.

Payvastagan, S., P. Farhoomand, R. Shahrooze, N. Delfani, and A. Talatapeh. 2012. The effects of different levels of canola meal and copper on performance, susceptibility to ascites and plasma enzyme activities in broiler chickens. Ann. Biol. Res. 3:5252–5258.

Perry, B., T. Randolph, J. McDermott, K. Sones, and P. Thornton. 2002. Investing in Animal Health Research to Alleviate Poverty. ILRI (International Livestock Research Institute), Nairobi, Kenya.

Pinard-van der Laan, M. H., B. Bed'Hom, J. L. Coville, F. Pitel, K. Feve, S. Leroux, H. Legros, A. Thomas, D. Gourichon, J. M. Repérant, and P. Rault. 2009. Microsatellite mapping of QTLs affecting resistance to coccidiosis (*Eimeria tenella*) in a Fayoumi× White Leghorn cross. BMC Genom 10:1–13.

Shahidi, F. 1990. Canola and rapeseed. Page in 355 Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold, New York.

Su, S., K. B. Miska, R. H. Fetterer, M. C. Jenkins, S. J. Lamont, and E. A. Wong. 2018. Differential expression of intestinal nutrient transporters and host defense peptides in *Eimeria maxima*-infected Fayoumi and Ross chickens. Poult. Sci. 97:4392–4400.

Su, S., K. B. Miska, R. H. Fetterer, M. C. Jenkins, and E. A. Wong. 2014. Expression of digestive enzymes and nutrient transporters in Eimeria acervulina-challenged layers and broilers. Poult. Sci. 93:1217–1226.

Teng, P. Y., J. Choi, Y. Tompkins, H. Lillehoj, and W. Kim. 2021. Impacts of increasing challenge with Eimeria maxima on the growth performance and gene expression of biomarkers associated with intestinal integrity and nutrient transporters. Vet. Res. 52:1–12.

Teng, P. Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020a. 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.

Teng, P. Y., S. Yadav, T. S. Dos Santos, A. L. Fuller, and W. K. Kim. 2020b. 2-Nitro-1-propanol improved nutrient digestibility and oocyst shedding but not growth performance of Eimeria-challenged broilers. Poult. Sci. 99:4314–4322.

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

USAHA (2019) Report of the USAHA Committee on poultry and other avian species. United States Animal Health Association. Accessed Feb. 2021. https://www.usaha.org/transmissible-diseases-of-poultry-avian-species.

Wickramasuriya, S. S., Y. J. Yi, J. Yoo, N. K. Kang, and J. M. Heo. 2015. A review of canola meal as an alternative feed ingredient for ducks. J. Anim. Sci. Technol. 57:1–9.

Wondimagegne, B., A. Getnet, and U. Mengistu. 2016. Feeding detoxified Ethiopian mustard (Brassica carinata) seed cake to sheep: effect on intake, digestibility, live weight gain and carcass parameters. Sci. J. Anim. Sci. 5:346–352.

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., P.-Y. Teng, J. Choi, A. K. Singh, S. Vaddu, H. Thippareddi, and W. K. Kim. 2021. Influence of rapeseed, canola meal and glucosinolate metabolite (AITC) as potential antimicrobials: effects on growth performance, and gut health in *Salmonella* Typhimurium challenged broiler chickens. Poult. Sci. 100:101551.

Yadav, S., P. Y. Teng, T. S. Dos Santos, R. L. Gould, S. W. Craig, A. L. Fuller, R. Pazdro, and W. K. Kim. 2020. The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with Eimeria species. Poult. Sci. 99:5936–5945.

Zhu, L. P., J. P. Wang, X. M. Ding, S. P. Bai, Q. F. Zeng, Z. W. Su, Y. Xuan, and K. Y. Zhang. 2018. Effects of dietary rapeseed meal on laying performance, egg quality, apparent metabolic energy, and nutrient digestibility in laying hens. Livest. Sci. 214:265–271.

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.