Effects of feeding broiler breeder hens a coextruded full-fat flaxseed and pulses mixture without or with multienzyme supplement

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ABSTRACT The effects of coextruded full-fat flaxseed and pulses (**FFF**; 1:1 wt/wt) mixture on n-3 polyunsaturated fatty acids (**PUFA**) enrichment in egg yolk, hepatic attributes, apparent retention (**AR**) of components, and ceca metabolites were evaluated in broiler breeder hens. The diets were as follows: 1) corn–soybean control, 2) control diet plus 18% FFF (**FFF−**), and 3) FFF plus enzyme supplement (**FFF+**) containing galactanase, protease, mannanase, glucanase, xylanase, amylase, and cellulase activities. Twenty-six-week-old Cobb 500 broiler breeder hens were allocated to 30 identical cages (2 hens/cage) and given 1-week adaptation period. The 3 diets were assigned to 10 replicate cages based on postadaptation BW and fed based on breeder curve for 30 D. Excreta samples were collected from day 24 to 27 for determination of AR of components, and eggs were collected from day 28 to 30 for yolk polyunsaturated fatty acids analyses. On day 30, birds were weighed, killed via cervical dislocation, liver weighed, and stored for fat analyses. Ceca digesta samples were taken for concentration of short-chain fatty acids. Liver and yolk weights as well as total yolk FA were not influenced by diets ($P > 0.05$). Control birds had lower yolk concentration of α-linolenic acid than birds fed either FFF− or FFF+ ($P < 0.01$) corresponding to 7.5, 36.8, and 37.3 mg/g for the control, FFF−, and FFF+, respectively. Control birds also exhibited lower yolk concentration of docosahexaenoic acid ($P < 0.01$). Control birds had higher hepatic concentration of crude fat and apparent retention of dry matter and crude protein compared with either the FFF− or FFF+ birds ($P < 0.05$). Birds fed FFF- diet had lower ceca digesta concentration of lactic acid than control and FFF+ ($P < 0.05$) birds. Results showed broiler breeder hens enriched egg yolk with n-3 polyunsaturated fatty acids without effects on the liver while the supplemental enzyme did not improve the utilization of FFF.

Key words: broiler breeders, flaxseed, yolk enrichment with omega-3 fatty acids, feed enzymes, hepatic fatty acids

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INTRODUCTION

Broiler breeder (**BB**) nutrition has been shown to influence chick quality and growth performance (Lopez and Leeson, 1995; Peebles et al., 1999a; Kidd, 2003). Of the nutrients available in BB diets, the fat component has the greatest influence on yolk lipid deposition and fatty acid profile which is of considerable importance for embryonic development (Cherian, 2015; Akbari Moghaddam Kakhki et al., 2019a). During embryogenesis, 80% of the lipids available in the yolk are absorbed by the embryo and utilized for energy (Cherian, 2011, 2015). Furthermore, long-chain omega-3 polyunsaturated fatty acids (n-3 **PUFA**) such as docosahexaenoic acid (22:6 n-3; **DHA**) and eicosapentaenoic acid (20:5 n-3; **EPA**) are critical for optimal cell, tissues, and organ development (Koppenol et al., 2014a,b; Yadgary et al., 2014). These fatty acids are needed for prenatal and postnatal development due to their vital role in the synthesis of structural lipids (Mennitti et al., 2015). In this context, n-3 PUFA have received considerable interest in embryo development due to their diverse roles in membrane biogenesis and immune system development (Cherian, 2011; Gonzalez et al., 2011; Akbari Moghaddam Kakhki et al., 2019a). However, current

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BB diets are generally high in n-6 fatty acids due to the high reliance on corn, soybean meal, and animal fat sources as primary lipid sources (Cherian, 2015). Therefore, the incorporation of n-3 PUFA into the egg through enrichment of BB diet could have beneficial effects on broiler chicken long-term health and performance after hatch.

Marine oils, fish meal, and some oilseeds are good sources of n-3 PUFA (Rymer and Givens, 2005). However, owing to reasons such as cost and sustainability, there is need for alternative sources (Kanakri et al., 2017). Flaxseeds are rich in n-3 PUFA and are produced on a wide scale in Canada and may be a suitable alternative to marine sources. Flaxseeds contain 40% crude fat and α-linolenic acid (**ALA**) constitutes about 58% of the total fatty acids (Caston et al., 1994). However, flaxseeds also contain high levels of non-starch polysaccharides, such as mucilage, and several anti-nutritional factors (e.g., phytic acid, anti-pyridoxine, trypsin inhibitors, and hydrocyanic acid), limiting their incorporation in monogastric diets (Rodríguez et al., 2001; Alzueta et al., 2003; Kiarie et al., 2007; 2009). Processing methods such as heating and extrusion of the whole flaxseed have been shown to increase utilization in poultry by destroying or reducing the concentration of anti-nutritional factors (**ANF**) (Caston et al., 1994; Konieczka et al., 2017). The inclusion of exogenous fiber-degrading enzymes may also improve nutrient digestibility (Slominski et al., 2006).

Incorporation of flaxseed in practical poultry diets often results in variable responses and is dependent upon a multitude of factors such as processing, enzymes, and cultivation time. For example, laying hens deposited twice the amount of n-PUFA in the yolk when fed a diet containing free flaxseed oil compared with hens fed milled flaxseed to supply similar level of dietary oil concentration (Ehr et al., 2017). Flaxseed fed in mash vs. crumble resulted in significantly different nitrogen-corrected apparent metabolizable energy (**AMEn**) in broilers, roosters, and laying hens (Leeson and Summers, 2000). However, very little research has been done on the utilization of flaxseed in BB. Leung et al. (2018) reported a decrease in body weight, feed intake, and nutrient retention in BB fed flax meal relative to the control diet. As BB are restricted fed, it is crucial to quantify utilization of flaxseed as it may influence the hepatic fat content, gastrointestinal ecology, as well as deposition of FA in the egg yolk. Therefore, the objective of the study was to evaluate the effects of incorporating a coextruded full-fat flaxseed and pulse mixture (**FFF**, 1:1 wt/wt) on the enrichment of yolk with n-3 PUFA, hepatic attributes, apparent retention (**AR**) of components and ceca metabolites.

MATERIALS AND METHODS

The experimental protocol was approved by the University of Guelph Animal Care Committee, and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009).

Table 1. Analyzed nutrient composition of FFF sample, as fed basis[1].

Item	FFF
Dry matter, %	93.9
Crude protein, %	21.5
Gross energy, kcal/kg	5,152
Neutral detergent fiber, %	7.80
Crude fat, %	18.6
Linolenic acid, %	10.8
Ca, %	0.25
P, %	0.53
Indispensable amino acids, %	
His	0.58
Ile	1.05
Leu	1.61
Lys	1.40
Phe	1.22
Thr	0.95
Val	1.20
Dispensable amino acids, %	
Ala	1.11
Asp	2.48
Cys	0.30
Glu	5.84
Gly	1.40
Pro	1.02
Ser	1.27
(Lys/Crude protein) x 100	6.52

[1]Dry coextruded product consisting of full-fat flaxseed and ground pulses (1:1 wt/wt) (linPRO, O & T Farms, Regina, SK, Canada).

Flaxseed Product and Diets

The flaxseed product used in the present study was a dry extruded product consisting of full fat flaxseed and ground pulses (linPRO, O & T Farms, Regina, SK, Canada). The flaxseed is used as a whole seed and is mixed with the ground pulses before being processed using single-screw dry extrusion (Table 1). In addition to providing nutrients, pulses serve as a carrier of the flaxseed oils through the formation of a protein–fat matrix (Kennelly, 1996).

Three dietary treatments were used: 1) a standard corn-soybean meal control diet (Table 2), 2) a diet containing 18% coextruded full-fat flaxseed without the addition of enzyme supplementation (**FFF−**), and 3) a diet containing 18% coextruded full-fat flaxseed with the addition of FDE (Superzyme-OM, Canadian Bio-Systems, Calgary, Alberta, Canada). The FDE contained galactanase, protease, mannanase, glucanase, xylanase, amylase, and cellulase activities at 50, 200, 400, 600, 1,000, 2,500, 2,800 U/g of product, respectively, and was previously validated to be effective in increasing utilization of flaxseed in broilers and roosters (Slominski et al., 2006). Control diet was formulated to meet BB nutrient specifications (Cobb, 2016) and FFF diets were balanced to ensure the ratio of energy and AA contributing ingredients were similar to the control diet (Fan and Sauer, 1995; Adeola et al., 2016; Kiarie et al., 2016). All diets contained titanium dioxide as an indigestible marker and were fed as mash.

Birds, Housing and Management

A total of sixty 26-week-old Cobbs 500 BB pullets were procured from Ontario Broiler Hatching Egg and

Table 2. Composition of control and experimental diet, as fed basis.

Item	Control	FFF
Corn	61.7	45.0
Soybean meal	25.9	25.2
FFF[1]	-	18.0
Limestone	7.92	7.81
Soy oil	1.61	1.15
Vitamin-trace mineral premix[2]	1.30	1.30
Mono calcium phosphate	0.66	0.64
Titanium dioxide	0.50	0.50
Salt	0.30	0.30
Sodium bicarbonate	0.11	0.12
DL Methionine	0.07	0.05
Choline chloride, 60%	0.01	0.01
Calculated provisions		
AME, kcal/kg	2,800	2,846
Crude protein, %	16.9	19.1
Calcium, %	2.89	2.89
Analyzed provisions		
Dry matter, %	90.0	92.4
Organic matter, %	77.7	79.7
Crude protein, %	15.5	19.2
Gross energy, kcal/kg	3,644	4,997
Neutral detergent fiber, %	11.5	11.5
Crude fat, %	3.15	6.42
Ash, %	13.7	12.7
Starch, %	39.2	32.1

[1]Dry coextruded product consisting of full-fat flaxseed and ground pulses (1:1 wt/wt) (linPRO, O & T Farms, Regina, SK, Canada).

[2]Vitamin mineral premix provided per kilogram of premix: vitamin A, 1,144,000 IU; vitamin D3, 429,000 IU; vitamin E, 5,200 IU; vitamin B12, 1,560 mcg; biotin, 28,600 mcg; menadione, 429 mg; thiamine, 520 mg; riboflavin, 1,040 mg; pantothenic acid, 1,950 mg; pyridoxine, 390 mg; niacin, 6,500 mg; folic acid, 130 mg; choline, 78,000 mg; iron, 7,800 mg; and copper, 1,300 mg.

Chick Commission (Guelph, ON, Canada). The birds were reared on a commercial pullet farm up to 26 wk of age and were transported to campus animal holding units at the Department of Animal Biosciences for experimentation. Hens were randomly assigned to 30 cages (65 cm × 30 cm × 45 cm). Cages were housed in environmentally controlled rooms kept at temperatures of 20°C and received 16 h of fluorescent illumination. All birds were fed the control diet for 1-week adaptation period. The 3 diets were randomly assigned to 10 replicate cages (n = 10) based on body weight after adaptation. Birds were fed once a day based on breeder curve for 30 D (Cobb, 2016) and allowed free access to water throughout the experimental period. Excreta was collected from day 24 to 27, pooled per cage and stored −20°C. Eggs were collected from day 28 to 30 consecutively, and yolks were separated, weighed, pooled by cage, and frozen (−20°C) until required for fatty acids analyses. Birds were euthanized by cervical dislocation on day 30. Whole livers were weighed and along with ceca digesta samples stored frozen at −20°C.

Sample Processing and Laboratory Analyses

Excreta samples were thawed, pooled by cage, and along with liver samples subsequently freeze dried. Samples of FFF, experimental diets, freeze-dried excreta and liver samples were finely ground using a coffee grinder. Liver samples were analyzed for crude fat content. The FFF, diets, and excreta samples were analyzed for dry matter, crude fat, crude protein, neutral detergent fiber (**NDF**), and gross energy. The FFF samples were further analyzed for amino acids content. Dry matter was determined according to standard procedure method 930.15 (AOAC, 2005). Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Nitrogen was analyzed by macro-Kjeldahl method (AOAC 1995) using a Kjeltec protein analyzer (Model #8200, Tecator, Hoganas, Sweden). Crude protein content was calculated by multiply determined nitrogen values by 6.25. Gross energy was determined via bomb calorimetry (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC). Neutral detergent fiber concentrations were determined using ANKOM 200 Fibre Analyzer (ANKOM Technology, Fairport, NY) using methodology described by Van Soest et al., 1991. Titanium concentration in the diets and excreta was determined as described by Myers et al. (2004). The recovery of enzyme in feed was performed by CBS laboratories (Calgary, Alberta) and only xylanase was analyzed using a modified method based on the Megazyme xylanase assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

Frozen yolks were lyophilized to determine dry yolk weight. Dried yolk samples (0.5 g) were extracted for FA determination using methods described by O'Fallon et al. (2007). Freeze-dried yolk samples were aliquoted in 16 × 125 mm screw cap PYREX culture tubes in which 1 mL internal standard C 13:0, 0.7 mL of 10 N KOH and 5.3 mL of methanol were added. Tubes were capped, vortexed, and incubated at 55°C for 1.5 h. Tubes were cooled below room temperature using cold tap water. Then 0.58 mL 24 N H_2SO_4 was added to the tubes before vortexing and placement into a 55°C-water bath for 1.5 h. Tubes were cooled using cold tap water, and afterward, 3.0 mL of hexane was added before hand mixing and vortexing. Tubes were centrifuged for 5 min with the hexane layer transferred to a gas chromatograph vial that was stored at −20°C until analysis with a gas chromatograph. Fatty acid methyl esters were determined using a Shimadzu 2014 gas chromatograph equipped with a Shimadzu AOC-20 auto sampler and a 120 m × 0.25 mm × 0.25 μm BPX-70 capillary column (Mandel Scientific, Guelph, ON.). Helium was used as the carrier gas with a 20:1 split ratio. Injector temperature was 250°C while flame ionization detector temperature was 280°C. Initial oven temperature was 150°C which was held for 1 min, then increased to 180°C at a rate of 10°C/min, from 180°C to 200°C at 2°C/min and from 200°C to 240°C at 1°C/min and held for 2 min. Fatty acid methyl esters of samples were identified by comparison of retention times to that of gas chromatography reference standards, C:13(Nu-Check-Prep, Elysian, MN). Chromatograms were integrated using Shimadzu GC solutions software. Crude fat content in the liver was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY).

Table 3. Yolk and liver weight of broiler breeders fed control corn soybean meal diet or control plus FFF[1] without or with multienzyme supplement[2].

Item	Control	FFF-	FFF+	SEM	*P*-value
Yolk					
Wet weight, g	57.3	56.0	57.0	0.95	0.607
Dry weight, g	33.7	30.0	27.1	4.10	0.520
Liver					
DM content (%)	58.6	53.3	47.2	6.86	0.514
Relative weight, g DM:g BW	16.9	17.9	17.2	0.57	0.510
Crude fat content, %	29.5[a]	16.5[b]	17.4[b]	0.52	<0.001

[a,b]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1]Dry coextruded product consisting of full-fat flaxseed and ground pulses (1:1 wt/wt) (linPRO, O & T Farms, Regina, SK, Canada).

[2]FFF diets fed with fiber-degrading enzyme composite. Supplied galactanase, protease, mannanase, glucanase, xylanase, amylase, and cellulase activities at 50, 200, 400, 600, 1,000, 2,500, 2,800 U/g of product.

Short-chain fatty acid concentrations (**SCFA**; lactic, acetic, propionic, and butyric) were assayed in thawed ceca digesta according to Leung et al., 2018. Briefly, approximately 0.1 g of the sample was resuspended with 1 mL of 0.005 N H_2SO_4 in a microcentrifuge tube. This liquid was then vortexed until the digesta was dissolved completely. Tubes were then centrifuged at 14,500 rpm for 15 min, 400 µL of the supernatant was then transferred to an HPLC vial and topped with 400 µL of 0.005 N H_2SO_4. The fluid was then assayed for SCFA using HPLC (Hewlett Packard 1,100, Waldbronn, Germany) with Rezex, ROA-Organic Acid column, 300 × 7.8 mm from Phenomenex and Refractive Index detector at 400°C (Agilent 1,260 Infinity RID from Agilent Technologies, Waldbronn, Germany).

Calculations and Statistical Analysis

The AR of components was calculated using the following equation (Adeola et al., 2016).

AR, % = [1 − (T in diet/T in excreta) × (N in excreta/N in diet)] × 100.

Where T is the concentrations of titanium dioxide in the diet and excreta, and N is the concentration of any component (crude protein, gross energy, NDF) in the diet and excreta.

Liver weight was expressed on live BB body weight before sacrifice, whereas FA concentration in the yolk was expressed as a function of yolk weight. The cage was the experimental unit. Data were tested for normality with UNIVARIATE plot normal procedure of SAS and then subjected to one-way ANOVA using GLIMMIX procedures of SAS with diet as the fixed effect. Treatment differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The analyzed xylanase concentration in experimental diets was higher in FFF + diet than either control or FFF- diets at 1,482, 185, and 170 xylanase units/kg of feed, respectively. Xylanase was the only enzyme assayed as a way of confirming accurate feed mixing. In general, the enzyme had no effects on measured parameters and therefore only FFF- results will be further discussed.

Yolk Weight, Liver Weight, and Crude Fat Content

The dry yolk weight was not ($P = 0.52$) affected by diets, and the values were 33.7 and 30.0 g for the control and FFF- diets, respectively (Table 3). There were no ($P = 0.51$) treatment effects on liver weight (Table 3). Liver crude fat content in birds fed the control diet (29.59%) was higher ($P < 0.001$) than in birds fed FFF- diet (16.49%). The export of large amounts of protein and lipid into eggs during the laying period is a metabolic challenge for reproducing fowl (Bain et al., 2016; Akbari Moghaddam Kakhki et al., 2019a,b). Although none of the treatments surpassed the threshold (>35% liver crude fat) required to be classified as fatty liver hemorrhagic syndrome, the potential risk with feeding high energy diets is still a concern (Squires and Leeson, 1988; Robinson and Kiarie, 2019). Fatty liver hemorrhagic syndrome in both laying hens and BB leads to excessive lipid peroxidation in the liver causing the weakening and breakdown of lipid membranes by overwhelming antioxidant defence mechanisms, ultimately resulting in hemorrhaging (Wu and Squires, 1997; Gonzalez-Esquerra and Leeson, 2000). As noted in Table 2, the control diet had higher starch levels than FFF- diet at 39.2 and 32.1%, respectively. High levels of starch content in corn–soy–based diets have been shown to induce occurrences of fatty liver in laying hens (Rozenboim et al., 2016). Furthermore, despite the higher crude fat content, the FFF- diet also had a higher level of crude protein compared with the control diet (Table 2). Studies have shown that the balance between energy, protein, and fat is essential for reducing the occurrence of the fatty liver (Rozenboim et al., 2016; Robinson and Kiarie, 2019). Results of the present study agree with the findings of Caston et al. (1994) and Schumann et al. (2000) who reported a reduction in hepatic fat content in birds fed ground flaxseed compared

Table 4. Fatty acid concentration in egg yolk of broiler breeders fed control corn soybean meal diet or control plus FFF[1] without or with multienzyme supplement[2].

Item	Control	FFF -	FFF +	SEM	*P*-value
Fatty acid, mg/g of dry yolk					
Myristate (C14)	2.1[a]	1.6[b]	1.6[b]	0.05	<0.001
Palmitate (C16)	155.7[a]	135.1[b]	134.4[b]	1.69	<0.001
Palmitoleate (C16:1)	20.0[a]	14.1[b]	12.0[b]	1.94	<0.001
Heptadecanoate (C17)	0.9[a]	1.1[a]	2.3[a]	1.02	0.342
Stearate (C18)	47.0[a]	44.6[a]	46.0[a]	1.22	0.176
Oleate (C18:1)	214.8[a]	203.6[b]	202.0[b]	4.16	0.001
Linoleic acid (C18:2n-6)	84.0[a]	89.7[a]	89.5[a]	2.36	0.040
Alpha-linolenic acid (C18:3n-3)	7.5[b]	36.8[a]	37.3[a]	3.34	<0.001
11-14 eicosadienoate (C20:2)	0.06[a]	0.04[a]	0.03[a]	0.01	0.080
Homogamma linolenate (C20:3)	0.1[a]	0.07[b]	0.06[b]	0.02	<0.001
Arachidonic acid (C20:4n-6)	9.4[a]	6.9[b]	6.5[b]	0.40	<0.001
Docosatetraenoic (C22:4)	0.9[a]	0.6[b]	0.5[b]	0.08	<0.001
Docosapentaenoic (C22:5)	1.1[b]	2.1[a]	1.7[a,b]	0.25	0.002
Docosahexaenoic acid (C22:6n-3)	4.1[b]	9.1[a]	8.5[a]	0.49	<0.001
Total fatty acids	547.8	545.3	542.4	2.70	0.377
Total omega -6	93.5	96.6	96.0	1.64	0.378
Total omega - 3	11.6[b]	45.9[a]	45.8[a]	2.62	<0.001
Ratio omega 6:3	14.1[a]	2.1[b]	2.1[b]	1.10	<0.001
Omega 6 to total %	0.2	0.2	0.2	0.003	0.240
Omega 3 to total %	0.02[b]	0.08[a]	0.08[a]	0.005	<0.001

[a,b]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1]Dry coextruded product consisting of full-fat flaxseed and ground pulses (1:1 wt/wt) (linPRO, O & T Farms, Regina, SK, Canada).

[2]FFF diets fed with fiber-degrading enzyme composite. Supplied galactanase, protease, mannanase, glucanase, xylanase, amylase, and cellulase activities at 50, 200, 400, 600, 1,000, 2,500, 2,800 U/g of product.

to a conventional corn–soy diet. Although the mechanism of reduction in liver fat is not understood, Schumann et al. (2000) hypothesized that high intakes of n-3 FA might reduce the concentration of arachidonic acid by downregulating Δ6-desaturase activity.

Egg Yolk Fatty Acid Enrichment

There was no treatment effect ($P > 0.05$) on the total FA content in the egg yolk (Table 4). These results agreed with those reported by Koppenal et al. (2015) and Cherian et al. (2008) where feeding flaxseed oil did not change the total fat content but did manipulate the FA profile of the yolk itself. Feeding increased concentration of flax oil as free oil or milled flaxseed linearly increased deposition of EPA and DHA in the yolk (Ehr et al., 2017). Cherian and Sim (1991) reported that egg yolk fat content is limited to 10% of the total yolk—this plateau is influenced by the total ALA, EPA, and DHA composition in the diet. Therefore, any differences in FA composition were achieved through the manipulation of FA deposition. As expected, feeding the FFF- diet ($P < 0.01$) increased the levels of ALA (averaging 38.8 mg/g) in the egg yolk compared with the control diet (7.5 mg/g; Table 4). Owing to the widely understood competition between ALA and LA for the same desaturase enzymes, FA deposition is directly related to the relative ratios of n-6:n-3 in the diet (Brenner, 1971; Neijat et al., 2016a). The reduced ratio between n-6:n-3 has been reported to enhance the deposition of ALA as well as reduced efficiency of endogenous conversion of ALA to EPA (Neijat et al., 2016a,b) The n-6:n-3 ratio in the FA composition of the egg yolk was reflective of the diet composition, where the control egg yolks had a significantly higher n-6:n-3 ratio. Furthermore, owing to this reduced ratio and subsequent competition for enzymes, the conversion of ALA to DHA was higher in birds fed the experimental diets ($P < 0.001$). As DHA is the active metabolite used for brain and cellular development in chicks, the increased availability in the embryo could provide beneficial effects for the chick (Cherian, 2015; Koppenol et al., 2014a,b).

Apparent Retention of Components and Ceca Digesta Short-Chain Fatty Acids

In comparison with control corn–soybean meal–based diet, BB fed the FFF- diet retained less dry matter ($P < 0.001$) and crude protein ($P = 0.006$) (Table 5). Mucilage, a water-soluble polysaccharide component found in flaxseed, increases the water-holding capacity of digesta, leading to lower retention of dry matter (Slominski et al., 2006). Although extrusion physically breaks down ANF in flaxseed, mucilage presence may impede nutrient absorption by increasing the viscosity of intestinal contents (Slominski et al., 2006; Kiarie et al., 2007; Jia et al., 2008). Increased digesta viscosity has been reported to decrease the diffusion rate of digestive secretions, subsequently reducing absorption and digestion of nutrients such as crude protein and fat (Rodríguez et al., 2001). Birds fed FFF- diet had a higher ($P = 0.007$) flow of NDF than the control diet translating to a lower retention of fiber. The results of the

Table 5. Apparent retention (**AR**) of components, NDF flow, AME, and concentration of short-chain fatty acids in ceca digesta of broiler breeders fed either control corn–soybean meal diet or control plus FFF[1] without or with multienzyme supplement[2].

Item	Control	FFF-	FFF+	SEM	*P*-value
Apparent retention, %					
Dry matter	67.9[a]	56.5[b]	54.1[b]	2.83	<0.001
Crude protein	41.6[a]	18.4[b]	15.8[b]	5.96	0.006
Crude fat	79.0	82.5	77.0	3.90	0.630
Gross energy	79.4	76.3	74.3	1.78	0.139
AME, kcal/kg	3,242[b]	4,126[a]	4,015[a]	72.6	<0.001
NDF flow (diet), g/kg DM	91.7[b]	117.7[a]	129.8[a]	8.04	<0.001
Short-chain fatty acids, μmol/L					
Lactic	11.8[a]	8.8[b]	10.4[a]	0.44	0.001
Acetic	79.1	79.5	80.0	3.64	0.995
Propionic	25.7	22.4	24.7	1.60	0.942
Butyric	19.6	16.1	17.3	1.05	0.077
Sugars, μmol/L					
Lactose	5.6	5.5	5.3	0.52	0.944
Glucose	28.5	20.3	23.7	6.85	0.498
Xylose	11.2	5.2	8.2	2.05	0.133
Arabinose	7.5[a]	5.6[b]	5.6[b]	0.40	0.003

[a,b]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

[1]Dry coextruded product consisting of full-fat flaxseed and ground pulses (1:1 wt/wt) (linPRO, O & T Farms, Regina, SK, Canada).

[2]FFF diets fed with fiber-degrading enzyme composite. Supplied galactanase, protease, mannanase, glucanase, xylanase, amylase, and cellulase activities at 50, 200, 400, 600, 1,000, 2,500, 2,800 U/g of product.

present study agree with the well-understood concept wherein high-fiber diets reduce nutrient digestibility. Mateos et al. (2012) reported reduced nutrient and energy retention by fibrous feed due to the negative effects they have on the digestive processes, such as reduced absorption due to the irritation of the mucosal layer of the gut. Residual sugars are reflective of the composition of diets, where control diets displayed a significantly higher level of arabinose than the FFF diet ($P = 0.003$).

Broiler studies reported adverse effects of feeding flaxseed on fat digestion through interference with emulsification, micelles formation, and transport to the epithelial surface (Jia and Slominski, 2010). However, there were no significant dietary effects on AR of fat in the present study. Leung et al. (2018) observed higher fat retention in BB hens fed flax meal compared with a control corn–soybean meal diet. These findings suggest that BB may have gastrointestinal capacity to overcome deleterious effects of flaxseed soluble fiber on dietary fat utilization. As retention is normalized based on diet over excreted components, it is possible that the lack of difference in fat retention between treatments may be due to diet composition, where despite the FFF- having twice as much fat compared with control (averaging 6.42 vs. 3.15%), the relative amount excreted may have remained constant between the 2 groups (Table 2).

There were no ($P > 0.05$) enzyme effects on AR of measured components in FFF + diet compared to control or FFF- (Table 5). We hypothesize that adequate amounts of enzyme may not have been ingested as the dosage used was recommended for *ad libitum*–fed broilers. As well, owing to their restricted feeding regimen, BB have become extremely effective at utilizing available nutrients and therefore enzyme inclusion may make no difference. Furthermore, the composite used may not have been most suitable for reducing the ANF of flaxseeds. As previously mentioned, increased digesta viscosity due to mucilage is one of the main ANF inhibiting nutrient absorption and digestion. Previous research showed that the use of supplemental enzyme containing 300 U cellulase, 3,950 U mannanase, and 5,000 U pectinase can significantly reduced digesta viscosity in broilers fed mash flaxseed diets (Jia and Slominski, 2010). However, the enzyme tested in the present study did not contain pectinase and had low mannanase relative to supplement used in previous study.

Th AME values were higher ($P < 0.001$) in FFF diets than the control diet with values of 4,015, 4,126, and 3,242 kcal/kg, respectively. It is possible that despite lower retention of protein and no difference in retention of fat, the higher GE content of the FFF- diet translated to a higher AME than the control. Although not analyzed in the present study, it is possible that the fatty acid profile of the diet may have indicated a higher quantity of long-chain fatty acids, resulting in more energy utilization. High soluble fiber digestibility through cecal microbial fermentation has been widely reported in mature layer hens, BB, and could be a factor in the increased AME (Mateos et al., 2012). However, there was no significant difference in the quantity of butyric, acetic, and propionic acid between the 3 diets. Perhaps, confirming that the energy derived from volatile fatty acids does not represent a large portion of metabolizable energy.

In conclusions, increased levels of ALA and DHA were observed in egg yolks of birds fed diets containing FFF, indicating a higher conversion capacity by the BB. There was no effect of feeding FFF on the total fat of

the yolk and liver. The BB fed diets containing FFF had a higher AME but retained lower DM and protein compared to control diets. The addition of a fiber-degrading enzyme supplement did not influence utilization of nutrients and/or the subsequent deposition of fatty acids into the yolk in BB fed diets containing FFF.

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