

Impact of dietary fat types on expression levels of dopamine and serotonin transporters in the ileum of broiler chickens

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ABSTRACT Various types of dietary fats undergo distinct fermentation processes by gut microbes, potentially leading to the production of neurotransmitters that can influence the gut. Serotonin and dopamine are recognized neurotransmitters with positive effects on gut function. A broiler chicken trial was conducted to evaluate the influence of dietary fat types on protein expression of 2 neurotransmitter transporters, dopamine (DAT) and serotonin (5-HTT). A total of 560 day-old (Ross 708) male broiler chicks were randomly assigned to 7 dietary treatments. The experimental treatments included a basal diet of corn-soybean meal (SBM), supplemented with 3% of various fats: poultry fat (CON), olive oil (OLIV), fish oil (FISH), canola oil (CANO), lard (LARD), coconut oil (COCO), or flaxseed oil (FLAX). Bodyweight (BW) and feed conversion ratio (FCR) were recorded. Ileal tissues were aseptically collected to determine the expression levels of DAT and

5-HTT through western blot analysis. In addition, plasma samples were analyzed for reactive oxygen metabolites (d-ROM) tests on d 55. Results showed that dietary fat type inclusion did not have any detrimental effect on growth performance parameters. The expression levels of DAT were higher ($P < 0.05$) in FLAX treatments compared to CON treatments on d 20 and d 55, respectively. Similarly, with 5-HTT levels, FLAX, CANO, and LARD treatments were higher ($P < 0.05$) than CON treatments on d 20 and d 55. However, higher levels of oxidative stress (d-ROM values) were recorded in COCO (32.75 Carr U), CANO (29 Carr U), and CON treatments (25.5 Carr U) compared to FLAX (18.5 Carr U; $P < 0.05$) treatment. These findings suggest that incorporating dietary flaxseed oil at a 3% level in the diet has significant potential to elevate the expression levels of intestinal DAT and 5-HTT without inducing oxidative stress.

Key words: dietary fat type, dopamine, serotonin, ileum, broiler chicken

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INTRODUCTION

Diet remains a crucial factor affecting the gut microbiota composition and the overall health of chickens throughout its life (Lankelma et al., 2015; Oriach et al., 2016). The gut composition of chicken plays a significant role in the health of the host by shaping the immune system development, metabolizing dietary nutrients including fatty acids and glucose, digesting complex polysaccharides, synthesizing vitamins, and bioactive molecules (Lankelma et al., 2015; Oriach et al., 2016). It has been reported that about 50% of variation

experienced in gut microbiota has been attributed to dietary changes (Zhang et al., 2010) and these changes can manifest early altering the microbiota during the adult phase of life (David et al., 2014).

Dietary fat, which is a significant source of energy in poultry diets, is believed to influence the composition of gut microbiota and studies have shown that different dietary fat types and quantities can modify gut microbiota (Liang et al., 2015). It has been implicated that the type (saturated and unsaturated) and quantity of dietary fat can influence intestinal microbiota composition and its metabolites (Agans et al., 2018). Gut microbiota plays a significant role in the digestion, immune response, and regulation of entero-endocrine systems and interact with the central nervous system through the production of metabolic compounds including nor-epinephrine, dopamine, and serotonin (Dicks, 2022). In the enteric nervous system (ENS), the intestinal barrier is composed of structural cellular components consisting

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of goblet cells, Paneth cells, enterocytes, tight junctions, mucus layer and enterochromaffin cells (**EC**) (Conte et al., 2020). The ECs are the predominant neuroendocrine cells and are often regarded as the primary detectors of the intestinal luminal content and are the primary source of neurotransmitters within the body (Conte et al., 2020; Dicks, 2022).

The fermentation of dietary fat types, resulting in the production of short-chain fatty acids (**SCFA**), influences the production and release of neurotransmitters in the lining of the intestines. When SCFAs bind to specific receptors and neurons on EC cells, they stimulate the release of neurotransmitters and can influence their transporter activity and availability for reuptake into the system (Niyonambaza et al., 2019; Conte et al., 2020; Mhanna et al., 2024). These ECs are stimulated by gut microbiota and are the primary source of serotonin (**5-HT**); which is synthesized by the hydroxylation and decarboxylation of tryptophan in the gut (Dicks, 2022) and has been reported to be present in the gastrointestinal tract (**GIT**) of broiler chickens at varying ages (Lyte et al., 2022). Dopamine (**DA**) is mainly present in the brain but is also produced in the gut through the secretions of gut microbiota whose composition can vary and could modify intestinal dopamine levels (Chen et al., 2021). In broiler chickens, dopamine-producing cells are found in the ENS synthesized by the lining of the GIT and help regulate intestinal functions including secretions and motility (Strandwitz, 2018). The synthesis of these neurotransmitters in the GIT is influenced by the gut microbiota through the microbial fermentation of the dietary fats (Phillips et al., 1961).

5-HT functions primarily as a neurotransmitter both in the gut and in the brain and as a paracrine messenger (Gershon and Tack, 2007), as well as a hormone in the periphery (Gershon, 2013). Serotonin synthesis which is similar in chickens, humans, and other animals is synthesized from its precursor tryptophan (**Tph**) (Tyce, 1990), by the rate-limiting enzyme tryptophan hydroxylase 2 (**Tph2**) in the serotonergic neurons. In the GIT mucosa, it has been reported that 5-HT synthesis and release can be influenced by microbiota, and in addition to its synthesis, can promote or restrict inflammation through 5-HT receptors (Spohn and Mawe, 2017).

DA is involved in the regulation of different physiological processes that helps reinforce behaviors, which are pleasurable or beneficial for survival (Beaulieu and Gainetdinov, 2011) including cognition, feed intake, emotion, and gastrointestinal motility in the periphery (Ben-Jonathan and Hnasko, 2001). It has been reported that the actions of dopamine are mediated by five dopamine receptors (D1–D5), which belong to G Protein-coupled receptor (**GPCR**) (Beaulieu and Gainetdinov, 2011; Pivonello et al., 2007). Studies have shown that in poultry, dopamine and its receptors have been linked with song learning and production (Budzillo et al., 2017), feather pecking (Kops et al., 2017), aggressiveness (Komiya et al., 2014), feed intake (Khodadadi et al., 2017), and neurogenesis and neuronal recovery (Lukacova et al., 2016). Dopamine is usually recognized as the

reward neurotransmitter; however, it also aids the modulation of behavior, cognition, motivation, and mood, which are essential for survival (Ko and Strafella, 2012).

The gut is a complex and dynamic organ that aids in nutrient absorption (Lan et al., 2005) and production of neurotransmitters (Dicks, 2022). Stressors and dietary changes can induce cellular free radicals, resulting in an imbalance in reactive oxygen species in the GIT (Mishra and Jha, 2019). It has been reported that the inclusion of oxidized oils and fats taxes the antioxidants within the intestinal mucosa (Liang et al., 2015). However, the influence of dietary fat types on the expression levels of ileal neurotransmitter transporters in broiler chickens has not been explored. Therefore, the current study was conducted to determine the effects of dietary fat types on the expression levels of ileal dopamine (**DA**) and serotonin (**5-HT**) transporters and pro-oxidant capacity in broiler chickens.

MATERIALS AND METHODS

The animal care and use procedures were approved by the Institutional Animal Care and Use Committee (IACUC 20-004.0) of North Carolina Agricultural and Technical State University.

Experimental Design, Diet, and Bird Management

In an 8-wk experiment, day-old Ross 708 broiler male chicks (560) were commercially sourced and housed at the Poultry Research Unit of the North Carolina Agricultural and Technical State University (Greensboro, NC). Chicks were allocated to seven treatments in a completely randomized design (**CRD**). The experimental treatments (Tables 1-3) included a basal diet of corn-soybean meal (**SBM**) with 3% dietary inclusion of various dietary fats: poultry fat (**CON**), olive oil (**OLIV**), fish oil (**FISH**), canola oil (**CANO**), lard (**LARD**), coconut oil (**COCO**), or flaxseed oil (**FLAX**). Experimental diets were calculated to be equicaloric and were manufactured at the North Carolina State University Feed Education Unit (Raleigh, NC). The oil types used were procured commercially from Jedwards International, Inc. (Braintree, MA). The experimental diets were provided for ad libitum feed consumption as pellet crumbles throughout the experiment. Each treatment group was randomly assigned to 5 replicate pens, containing 16 chicks, and allowed free access to water throughout the experiment. From d 1 to d 21, the chicks were housed in battery cages (Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR). Each battery cage had a nipple drinker to supply water and a feeder tray which was adjusted in height for reach according to the progressive growth of the chicks. At 21 d, the chicks were transferred to representative fresh pine savings litter floor pens that was equipped with a hanging feeder and a bell drinker. The bird housing was set at a temperature of 92 °F from d 1 to d 7, and 87 °F from d 8 to d 21). Subsequently, it was

Table 1. Composition of experimental starter diets (% “as is”).¹

Ingredients	CON	FLAX	CANO	FISH	COCO	OLIV	LARD
Corn	53.22	53.22	53.22	53.22	53.22	53.22	53.22
Soybean Meal	39.40	39.40	39.40	39.40	39.40	39.40	39.40
Fat/Oil*	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mono-Dicalcium Phosphate	1.81	1.81	1.81	1.81	1.81	1.81	1.81
Limestone 37%	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Salt NaCl	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.35
NCSU Poultry Mineral Premix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride 60%	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09
NCSU Poultry Vitamin Premix ³	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium Premix ⁺	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Santoquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed nutrient composition							
Metabolizable Energy (Kcal/kg)	1,433	1,429	1,438	1,423	1,432	1,412	1,411
Crude Protein, %	23.06	23.19	21.88	22.63	22.75	24.38	23.25
Crude Fat, %	5.42	5.12	5.46	5.27	5.25	5.18	5.05
Crude Fiber, %	2.1	2.3	2.3	2.3	2.5	2.3	2.3
Ash, %	5.64	5.55	5.66	5.57	5.30	5.73	5.77
Calculated nutrient composition							
Total Sulfur Amino Acids, %	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Lysine, %	1.44	1.44	1.44	1.44	1.44	1.44	1.44
Calcium, %	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Available phosphorus, %	0.48	0.48	0.48	0.48	0.48	0.48	0.48

¹Diets used in the study included the following: i) conventional Corn-soybean meal (SBM) with the addition of poultry fat as fat type (CON diet); ii) conventional corn-SBM with Flax Seed oil as fat type (FLAX); iii) conventional Corn-SBM with the addition of Canola oil as the fat type (CANO); iv) conventional Corn-SBM with Fish oil as the fat type (FISH); v) consist of conventional Corn-SBM with addition of Coconut oil as the fat type (COCO); vi) conventional Corn-SBM in which Olive oil was incorporated as fat type (OLIV); vii) conventional Corn-SBM with Lard used as the fat type (LARD). Each of these 7 diets were separately formulated for the starter (d 1 to 21) phase of experiment.

*Seven different fat types were added at 3% in each diet.

²Mineral Premix, supplied per kilogram of diet: Manganese (Mn), 60 mg; Zinc (Zn), 60 mg; Iron (Fe), 40 mg; Copper (Cu), 5 mg; Iodine (I), 1.2 mg; Cobalt (Co), 0.5 mg.

³Vitamin Premix, supplied per kilogram of diet: Vitamin A (6,600 IU), Vitamin D (1,980 IU), Vitamin E (33 IU), Vitamin B12 (0.02 mg), Biotin (0.13 mg), Menadione (1.98 mg), Thiamine (1.98 mg), Riboflavin (6.60 mg), d-Pantothenic Acid (11.0 mg), Vitamin B6 (3.96 mg), Niacin (55.0 mg), Folic Acid (1.1 mg).⁴Experimental diets were analyzed for proximate nutrient composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

⁺Selenium Premix provides 0.3 mg Selenium/Kg of feed as sodium selenite.

reduced to 77 °F up to 56 d. Photoperiod consisted of continuous (23L:1D) lighting at 30 lux from placement to 21 d and then 24L:0D from 22 d to 56 d. The feed phases included starter (1–20 d, Table 1), grower (21–41 d, Table 2), and finisher (42–56 d, Table 3) diets, each formulated to meet or slightly exceed nutritional requirements following guidelines outlined in the Ross broiler nutrition specification handbook (Aviagen et al., 2022).

Growth Performance

Bodyweight (BW), body weight gain (BWG), and feed intake (FI) of chicks were recorded on 7, 21, 42, and 56 d of age for the assessment of broiler growth performance. From the resulting data, the feed conversion ratio (FCR) was calculated. Mortality was also recorded daily throughout the 56-d experiment.

Western Blot

On d 20, 41, and 55, distal ileum tissues were aseptically collected and rinsed in cold phosphate buffered saline (PBS) (Fisher Scientific, NJ), dabbed on Kim wipes (Kimberly-Clark Worldwide, Inc.), placed in cryogenic tube, quickly frozen in liquid N₂, and subsequently

stored at –80°C until time to do the extraction of proteins. Protein was extracted from 40 mg ileal full thickness tissues using 200 µL cComplete Lysis-M buffer (04719956001 Roche) containing cComplete Lysis-M EDTA-free (04719964001 Roche). Electrophoresis was carried out by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by electrotransfer to a polyvinylidene fluoride membrane (1620177XTU Bio-rad). The membrane was incubated in 5% Bovine serum albumin (BSA)/Tris-buffered saline and Tween 20 (TBST) at room temperature for 1 h and subsequently incubated with bs-1714R polyclonal rabbit dopamine transporter (DAT) AI04178462 antibody; 1:1,000 (Bioss) and bs-1893R polyclonal rabbit serotonin transporter (5-HTT) BA09035094 antibody; 1:1,000 (Bioss) at 4°C overnight. The rabbit 5-HTT and DAT antibody were initially validated for the feasibility in chicken studies through the immunohistological staining and Western blot by Huang et al (2019). After washing in TBST for 2 h, the membranes were exposed to horseradish peroxidase (HRP) bs-0432R polyclonal rabbit chicken IgY AI12166069 labeled secondary antibody; 1:5,000 for 1 h at 37°C. The GAPDH (MA5-15738-HRP; Invitrogen) monoclonal antibody band (1:1,000) was adopted as the internal control and was exposed to HRP secondary polyclonal antibody (goat

Table 2. Composition of experimental grower diets (% “as is”).¹

Ingredients	CON	FLAX	CANO	FISH	COCO	OLIV	LARD
Corn	53.22	53.22	53.22	53.22	53.22	53.22	53.22
Soybean Meal	39.40	39.40	39.40	39.40	39.40	39.40	39.40
Fat/Oil*	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mono-Dicalcium Phosphate	1.81	1.81	1.81	1.81	1.81	1.81	1.81
Limestone 37%	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Salt NaCl	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.35
NCSU Poultry Mineral Premix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride 60%	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09
NCSU Poultry Vitamin Premix ³	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium Premix ⁺	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Santoquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed nutrient composition							
Metabolizable Energy (Kcal/kg)	1,449	1,447	1,450	1,467	1,445	1,430	1,487
Crude Protein, %	21.31	21.13	21.38	21.44	20.13	21.13	18.81
Crude Fat, %	5.27	5.49	5.32	5.21	5.30	5.29	5.68
Crude Fiber, %	2.1	2.0	2.1	1.9	2.0	2.0	1.9
Ash, %	4.60	4.64	4.39	4.49	4.21	4.48	3.97
Calculated nutrient composition							
Total Sulfur Amino Acids, %	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine, %	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Calcium, %	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Available phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40

¹Diets used in the study included the following: i) conventional Corn-soybean meal (SBM) with the addition of poultry fat as fat type (CON diet); ii) conventional corn-SBM with Flax Seed oil as fat type (FLAX); iii) conventional Corn-SBM with the addition of Canola oil as the fat type (CANO); iv) conventional Corn-SBM with Fish oil was the fat type (FISH); v) consist of conventional Corn-SBM with addition of Coconut oil as the fat type (COCO); vi) conventional Corn-SBM in which Olive oil was incorporated as fat type (OLIV); vii) conventional Corn-SBM with Lard used as the fat type (LARD). Each of these 7 diets were separately formulated for the starter (d 1 to 21) phase of experiment.

*Seven different fat types were added at 3% in each diet.

²Mineral Premix, supplied per kilogram of diet: Manganese (Mn), 60 mg; Zinc (Zn), 60 mg; Iron (Fe), 40 mg; Copper (Cu), 5 mg; Iodine (I), 1.2mg; Cobalt (Co), 0.5 mg.

³Vitamin Premix, supplied per kilogram of diet: Vitamin A (6,600 IU), Vitamin D (1,980 IU), Vitamin E (33 IU), Vitamin B12 (0.02 mg), Biotin (0.13 mg), Menadione (1.98 mg), Thiamine (1.98 mg), Riboflavin (6.60 mg), d-Pantothenic Acid (11.0 mg), Vitamin B6 (3.96 mg), Niacin (55.0 mg), Folic Acid (1.1 mg).⁴Experimental diets were analyzed for proximate nutrient composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

⁺Selenium Premix provides 0.3 mg Selenium/Kg of feed as sodium selenite.

anti-mouse IgG, dilution 1:1,000). The membranes were washed in TBST for 1 h and visualized using an electrochemiluminescence (**ECL**) system (170-5061, Bio-rad) on Chemidoc imaging system (Bio-rad). The bands obtained in the western blot were scanned and analyzed by ImageJ analysis software (version 1.54d). The data quantified were expressed as the integrated optical density (**IOD**) of the bands, normalized to the IOD of the corresponding GAPDH bands. There were differences in the GAPDH expression among treatments.

Blood Collection and Plasma Collection

On d 55 of the experiment, a bird was randomly sampled from each pen (totaling 5 birds/treatment) for blood collected from the brachial (wing) vein using a sterile 23 gauge 1" needle attached to prelabeled sterile Ethylenediaminetetraacetic acid vacutainer tubes. Thereafter, the blood samples were centrifuged at $1,500 \times g$ for 10 min to recover platelet-free plasma. Hemolysis was not observed in the plasma samples. The plasma (supernatant) was collected and stored in 1.5 mL Eppendorf tubes at -80°C until pro-oxidant capacity was determined.

Pro-Oxidant Capacity

A d-ROMs test kit (Diacron International s.r.l., Grosseto, Italy) was used to assess the reactive oxygen metabolites to determine pro-oxidant capacity of the plasma samples for each treatment using the FREE DUO system (DIACRON Research and Diagnostics, Grosseto, Italy). To carry out the reactive oxygen metabolites assay, 20 μL of plasma was pipetted into a thermostated cuvette, and then 20 μL of d-ROM reagent was pipetted into the same cuvette using separate tips (DIACRON, test kits). A little stir was given, then the cuvette was placed in the reading cell, where it was automatically started and incubated for 3 min and 2 min for kinetic reading. Based on the existing reference level, a pro-oxidant capacity $>27.20 \text{ mg H}_2\text{O}_2/\text{dL}$ was considered a high level of oxidative stress, as described by [Morucci et al \(2022\)](#).

Statistical Analysis

Expression levels of DAT, 5-HTT, and pro-oxidant capacity were subjected to 1-way ANOVA (Statistical Analysis Software, 2004, Version 9.2. SAS Institute Inc., Cary, NC). All data are presented as the mean $\pm$ SEM. Duncan's multiple-range test was used to determine

Table 3. Composition of experimental finisher diets (% “as is”).¹

Ingredients	CON	FLAX	CANO	FISH	COCO	OLIV	LARD
Corn	58.84	53.22	53.22	53.22	53.22	53.22	53.22
Soybean Meal	32.75	39.40	39.40	39.40	39.40	39.40	39.40
Fat/Oil*	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mono-Dicalcium Phosphate	1.81	1.81	1.81	1.81	1.81	1.81	1.81
Limestone 37%	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Salt NaCl	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.35
NCSU Poultry Mineral Premix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride 60%	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09
NCSU Poultry Vitamin Premix ³	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium Premix ⁺	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Santoquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed nutrient composition							
Metabolizable Energy (Kcal/kg)	1,443	1,447	1,457	1,446	1,458	1,455	1,436
Crude Protein, %	23.31	22.81	21.81	23.63	22.13	22.81	22.25
Crude Fat, %	5.04	5.37	5.40	5.29	5.44	5.40	5.22
Crude Fiber, %	2.0	2.0	2.0	2.2	2.1	2.1	2.0
Ash, %	4.85	4.95	5.03	4.98	4.66	5.17	4.99
Calculated nutrient composition							
Total Sulfur Amino Acids, %	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Lysine, %	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Calcium, %	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44	0.44	0.44

¹Diets used in the study included the following: i) conventional Corn-soybean meal (SBM) with the addition of poultry fat as fat type (CON diet); ii) conventional corn-SBM with Flax Seed oil as fat type (FLAX); iii) conventional Corn-SBM with the addition of Canola oil as the fat type (CANO); iv) conventional Corn-SBM with Fish oil was the fat type (FISH); v) consist of conventional Corn-SBM with addition of Coconut oil as the fat type (COCO); vi) conventional Corn-SBM in which Olive oil was incorporated as fat type (OLIV); vii) conventional Corn-SBM with Lard used as the fat type (LARD). Each of these 7 diets were separately formulated for the starter (d 1 to 21) phase of experiment.

*Seven different fat types were added at 3% in each diet.

²Mineral Premix, supplied per kilogram of diet: Manganese (Mn), 60 mg; Zinc (Zn), 60 mg; Iron (Fe), 40 mg; Copper (Cu), 5 mg; Iodine (I), 1.2mg; Cobalt (Co), 0.5 mg.

³Vitamin Premix, supplied per kilogram of diet: Vitamin A (6,600 IU), Vitamin D (1,980 IU), Vitamin E (33 IU), Vitamin B12 (0.02 mg), Biotin (0.13 mg), Menadione (1.98 mg), Thiamine (1.98 mg), Riboflavin (6.60 mg), d-Pantothenic Acid (11.0 mg), Vitamin B6 (3.96 mg), Niacin (55.0 mg), Folic Acid (1.1 mg).⁴Experimental diets were analyzed for proximate nutrient composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

⁺Selenium Premix provides 0.3 mg Selenium/Kg of feed as sodium selenite.

significant differences among means. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Fatty acid Composition of Experimental Diets

The fatty acid composition of the experimental diets, starter, grower, and finisher are presented in Tables 4, 5, and 6 respectively. Total Omega 3 Isomers (Total n3; Table 4, 5, and 6) were increased in FLAX diets compared to other treatment diets. Lauric acid (C12:0) and SFAs were higher in COCO diets compared to other treatment diets. Omega 9 (Oleic acid, C18:1) was increased in CANO and OLIV diets. Across each phase of diets, the composition of PUFAs in FLAX, FISH, and CANO; the SFAs in LARD and COCO and MUFAs in OLIV are presented in Tables 4, 5, and 6.

Growth Performance

From 1 to 7 d, there were no differences in BW, BWG, FI, and FCR among treatments (Table 7).

From 8 to 21 d, BWG was different among treatments (Table 7). Compared to the CON treatment, CANO, COCO, OLIV, and LARD treatments were not different, but FLAX and FISH treatments had the highest BWG (Table 7). There was no difference ($P > 0.05$) in BW and FI among all treatments. Furthermore, only FLAX treatment had lower FCR (1.163) than the CON treatment (Table 7). From 22 to 42 d, BWG, FI and FCR were different among treatments (Table 8). OLIV treatment had higher ($P < 0.05$) BWG than the CON treatment, while FI was lower ($P < 0.05$) in CON treatment than all other treatments. The OLIV treatment had lower FCR than the other treatments (1.407 vs. 1.483–1.552; Table 8). From 43 to 56 d, CON treatment had the lowest ($P < 0.05$) BW (4.919 kg) compared to other treatments, while OLIV (5.325 kg) treatment had the highest ($P < 0.05$); the values for other treatments were in-between (Table 8). However, there were no differences in BWG, FI, and FCR. Cumulative growth performance (1–56 d; Table 9) showed that CON treatment had lower BWG than the other treatments (4.793 kg vs. 4.808–5.172 kg, $P < 0.05$). Although there were no differences in FI, the FISH treatment had a higher FCR than the other treatments (1.568 vs. 1.468–1.555, $P < 0.05$).

Table 4. Fatty acid composition of experimental diets.¹

Fatty acids ²	Starter Diets						
	CON (%)	FLAX (%)	CANO (%)	FISH (%)	COCO (%)	OLIV (%)	LARD (%)
C4:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C6:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C8:0	< 0.02	< 0.02	< 0.02	< 0.02	0.19	< 0.02	< 0.02
C10:0	< 0.02	< 0.02	< 0.02	< 0.02	0.13	< 0.02	< 0.02
C11:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C12:0	< 0.02	< 0.02	< 0.02	< 0.02	1.02	< 0.02	< 0.02
C14:0	0.02	0.03	< 0.02	0.16	0.39	< 0.02	0.04
C14:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C16:0	1.12	0.60	0.58	0.80	0.67	0.74	0.96
C16:1	0.19	< 0.04	< 0.04	0.20	< 0.04	0.05	0.06
C16:2	< 0.02	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02
C16:3	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	< 0.02
C16:4	< 0.02	< 0.02	< 0.02	0.05	< 0.02	< 0.02	< 0.02
C17:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C17:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:0	0.25	0.17	0.12	0.16	0.17	0.16	0.36
C18:1	1.75	1.21	2.21	1.10	0.91	2.39	1.51
C18:2	1.95	1.75	1.85	1.51	1.47	1.58	1.73
C18:3 n-3	0.12	1.10	0.25	0.13	0.12	0.11	0.19
C18:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:4	< 0.02	< 0.02	< 0.02	< 0.05	< 0.02	< 0.02	< 0.02
C20:0	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:1	< 0.02	< 0.02	0.03	0.02	< 0.02	< 0.02	< 0.02
C20:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:5 n-3	< 0.02	0.05	< 0.02	0.33	< 0.02	< 0.02	< 0.02
C21:5	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:4	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:5 n-3	< 0.02	< 0.02	< 0.02	0.04	< 0.02	< 0.02	< 0.02
C22:5 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:6	< 0.02	0.03	< 0.02	0.24	< 0.02	< 0.02	< 0.02
C24:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C24:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total n3	0.12	1.20	0.26	0.82	0.13	0.11	0.21
Total n5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total n6	1.99	1.74	1.86	1.56	1.48	1.59	1.76
Total n7	0.27	0.09	0.13	0.30	0.06	0.13	0.13
Total n9	1.77	1.23	2.26	1.14	0.93	2.41	1.54
Total fatty acids	5.67	5.18	5.35	5.24	5.24	5.23	5.11
MUFAs	2.07	1.35	2.44	1.49	1.00	2.56	1.70
PUFAs	2.13	2.97	2.13	2.51	1.61	1.71	1.98
SFAs	1.44	0.85	0.77	1.22	2.62	0.96	1.42
Metabolizable Energy (Kcal/kg)	1,433	1,429	1,438	1,423	1,432	1,412	1,411
Crude Protein, %	23.06	23.19	21.88	22.63	22.75	24.38	23.25
Crude Fat, %	5.42	5.12	5.46	5.27	5.25	5.18	5.05
Crude Fiber, %	2.1	2.3	2.3	2.3	2.5	2.3	2.3
Ash, %	5.64	5.55	5.66	5.57	5.30	5.73	5.77

¹Experimental diets were analyzed for fatty acid composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

²SFA =Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Expression Levels of Ileal DAT

According to the Western blot results, FLAX and OLIV treatments exhibited a significant increase ($P < 0.05$) in the relative DAT levels at 20 d compared to CON, similar to CANO, COCO, FISH, and LARD (Figure 1A). DAT levels at 41 d were not influenced by dietary fat types among treatments but were lower ($P <$

0.05; 0.101–0.438) than the CON treatment (1.00; Figure 1B). The expression levels of DAT at 55 d showed significant differences between the dietary fat types ($P < 0.05$), with FLAX (1.601) and CANO (1.711) treatments having a higher expression level than the CON (1.00) treatment. However, the relative DAT expression levels of COCO and LARD treatments were the least expressed with FISH and OLIV similar to CON (Figure 1C).

Table 5. Fatty acid composition of experimental diets.¹

Fatty acids ²	Grower diets						
	CON (%)	FLAX (%)	CANO (%)	FISH (%)	COCO (%)	OLIV (%)	LARD (%)
C4:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C6:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C8:0	< 0.02	< 0.02	< 0.02	< 0.02	0.18	0.02	< 0.02
C10:0	< 0.02	< 0.02	< 0.02	< 0.02	0.13	< 0.02	< 0.02
C11:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C12:0	< 0.02	< 0.02	< 0.02	< 0.02	1.00	0.14	< 0.02
C14:0	0.03	0.03	< 0.02	0.15	0.39	< 0.06	0.04
C14:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C16:0	1.02	0.62	0.63	0.81	0.72	0.76	1.02
C16:1	0.16	< 0.04	< 0.04	0.19	< 0.04	0.04	0.07
C16:2	< 0.02	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02
C16:3	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	< 0.02
C16:4	< 0.02	< 0.02	< 0.02	0.05	< 0.02	< 0.02	< 0.02
C17:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C17:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:0	0.22	0.18	0.14	0.16	0.19	0.17	0.38
C18:1	1.58	1.22	2.12	1.17	0.99	2.27	1.68
C18:2	1.85	1.74	1.93	1.53	1.53	1.59	1.89
C18:3 n-3	0.11	1.05	0.24	0.12	0.12	0.12	0.29
C18:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:4	< 0.02	< 0.02	< 0.02	< 0.05	< 0.02	< 0.02	< 0.02
C20:0	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:1	< 0.02	< 0.02	0.03	0.02	< 0.02	< 0.02	0.02
C20:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:5 n-3	< 0.02	0.04	< 0.02	0.30	< 0.02	< 0.02	< 0.02
C21:5	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:4	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:5 n-3	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	< 0.02
C22:5 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:6	< 0.02	0.03	< 0.02	0.21	< 0.02	< 0.02	< 0.02
C24:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C24:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total n3	0.11	1.13	0.24	0.74	0.13	0.12	0.32
Total n5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total n6	1.88	1.76	1.95	1.58	1.55	1.61	1.93
Total n7	0.24	0.09	0.14	0.28	0.07	0.12	0.16
Total n9	1.60	1.24	2.17	1.11	1.01	2.29	1.71
Total fatty acids	5.24	5.15	5.42	5.08	5.44	5.39	5.69
MUFAs	1.87	1.35	2.36	1.43	1.09	2.42	1.89
PUFAs	2.01	2.91	2.21	2.44	1.69	1.74	2.27
SFAs	1.34	0.88	0.84	1.18	2.66	1.22	1.51
Metabolizable Energy (Kcal/kg)	1,449	1,447	1,450	1,467	1,445	1,430	1,487
Crude Protein, %	21.31	21.13	21.38	21.44	20.13	21.13	18.81
Crude Fat, %	5.27	5.49	5.32	5.21	5.30	5.29	5.68
Crude Fiber, %	2.1	2.0	2.1	1.9	2.0	2.0	1.9
Ash, %	4.60	4.64	4.39	4.49	4.21	4.48	3.97

¹Experimental diets were analyzed for fatty acid composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321.

²SFA =Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Expression Levels of Ileal 5-HTT

At 20 d, the FLAX, CANO, and LARD treatments exhibited higher expression levels of 5-HTT than the CON treatment (2.728, 2.814, and 2.614 respectively vs. 1.00, $P < 0.05$; but CON treatment showed similar levels to COCO, FISH, and OLIV treatments (Figure 2A). At 41 d, the expression levels of 5-HTT were similar in CON and COCO treatments but were lower ($P < 0.05$) in FLAX, CANO, FISH, OLIV, and LARD treatments (Figure 2B). 5-HTT

expression levels at 55 d was greatest in FISH treatment with treatments FLAX, CANO, and LARD expression levels higher than the CON treatment ($P < 0.05$; Figure 2C).

Pro-Oxidant Capacity

The effect of dietary fat types on oxidative status in plasma is shown in Figure 3. Dietary fat differences significantly influenced ROMs (reactive oxygen

Table 6. Fatty acid composition of experimental diets.¹

Fatty acids	Finisher diets						
	CON (%)	FLAX (%)	CANO (%)	FISH (%)	COCO (%)	OLIV (%)	LARD (%)
C4:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C6:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C8:0	< 0.02	< 0.02	< 0.02	< 0.02	0.17	< 0.02	< 0.02
C10:0	< 0.02	< 0.02	< 0.02	< 0.02	0.12	< 0.02	< 0.02
C11:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C12:0	< 0.02	< 0.02	< 0.02	< 0.02	0.94	< 0.02	< 0.02
C14:0	0.02	0.03	< 0.02	0.16	0.37	< 0.02	0.04
C14:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C15:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C16:0	1.08	0.70	0.68	0.92	0.78	0.93	1.21
C16:1	0.19	0.06	0.05	0.21	< 0.05	0.09	0.12
C16:2	< 0.02	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02
C16:3	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	< 0.02
C16:4	< 0.02	< 0.02	< 0.02	0.05	< 0.02	< 0.02	< 0.02
C17:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C17:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:0	0.23	0.20	0.15	0.18	0.20	0.20	0.41
C18:1	1.59	1.36	2.19	1.10	1.03	2.27	1.97
C18:2	1.83	1.84	1.84	1.58	1.54	1.66	1.92
C18:3 n-3	0.09	1.16	0.22	0.12	0.10	0.09	0.15
C18:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C18:4	< 0.02	< 0.02	< 0.02	< 0.05	< 0.02	< 0.02	< 0.02
C20:0	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:1	< 0.02	< 0.02	0.03	0.03	< 0.02	< 0.02	0.02
C20:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:3 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C20:4 n-6	< 0.02	< 0.02	< 0.02	0.02	< 0.02	< 0.02	< 0.02
C20:5 n-3	< 0.02	0.05	< 0.02	0.31	< 0.02	< 0.02	< 0.02
C21:5	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:3	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:4	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:5 n-3	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	< 0.02
C22:5 n-6	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C22:6	< 0.02	0.03	< 0.02	0.22	< 0.02	< 0.02	< 0.02
C24:0	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
C24:1	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Total n3	0.10	1.26	0.22	0.77	0.11	0.10	0.18
Total n5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total n6	1.88	1.86	1.87	1.63	1.56	1.70	1.96
Total n7	0.26	0.12	0.15	0.31	0.10	0.18	0.22
Total n9	1.61	1.39	2.23	1.29	1.05	2.29	1.88
Total fatty acids	5.30	5.68	5.43	5.60	5.52	5.54	6.06
MUFAs	1.91	1.54	2.43	1.66	1.17	2.50	2.14
PUFAs	1.99	3.15	2.11	2.57	1.71	1.81	2.17
SFAs	1.38	0.98	0.89	1.34	2.64	1.21	1.73
Metabolizable Energy (Kcal/kg)	1,443	1,447	1,457	1,446	1,458	1,455	1,436
Crude Protein, %	23.31	22.81	21.81	23.63	22.13	22.81	22.25
Crude Fat, %	5.04	5.37	5.40	5.29	5.44	5.40	5.22
Crude Fiber, %	2.0	2.0	2.0	2.2	2.1	2.1	2.0
Ash	4.85	4.95	5.03	4.98	4.66	5.17	4.99

¹Experimental diets were analyzed for fatty acid composition by Eurofins Scientific Inc. Nutrient Analysis Center, 2200 Rittenhouse Street, Suite 150, Des Moines, IA 50321. ²SFA = Saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

metabolites) ($P < 0.05$). FISH exhibited the highest pro-oxidant capacity with a value of 36.5 mg Carr U, which is >27.20 Carr U, this therefore indicates the presence of oxidative stress (Morucci et al., 2022). Similarly, oxidative stress was assessed in CANO and COCO; however, birds in the CON, FLAX, OLIV, and FLAX treatments did not exhibit oxidative stress, as their ROMs values were within the normal range (18.25 ± 1.25 to 25.5 ± 0.87 mg Carr U, respectively).

DISCUSSION

Dietary fats vary greatly in fatty acid composition. For example, pork lard and beef fat contain high amounts of saturated fatty acids (SFA), while fish oil is composed of higher levels of n-3 polyunsaturated fatty acids (PUFA). Fat is an important dietary nutrient, which can be derived from diet or transformed from carbohydrates and proteins (Hu et al., 2018). Dietary fats

Table 7. Effect of dietary fat types on growth performance of broiler chicks (d 1–21).

Treatments ¹	D 1 to 7 (Parameters measured) ²				D 8 to 21 (Parameters measured) ²			
	Body weight (BW , kg/bird) ³	Body weight gain (BWG , kg/bird)	Feed intake (FI , kg/bird)	FCR ⁴ (kg:kg)	BW (kg/bird) ³	BWG (kg/bird)	FI (kg/bird)	FCR ⁴ (kg:kg)
CON	0.161	0.119	0.115	0.962	0.860	0.692 ^b	0.846	1.222 ^a
FLAX	0.158	0.120	0.132	1.098	0.912	0.752 ^a	0.875	1.163 ^b
CANO	0.163	0.122	0.127	1.054	0.891	0.728 ^{ab}	0.868	1.193 ^{ab}
FISH	0.162	0.124	0.118	0.952	0.899	0.737 ^a	0.865	1.175 ^{ab}
COCO	0.156	0.116	0.119	1.027	0.886	0.724 ^{ab}	0.875	1.208 ^{ab}
OLIV	0.156	0.118	0.119	1.014	0.880	0.719 ^{ab}	0.870	1.209 ^{ab}
LARD	0.153	0.112	0.117	1.041	0.886	0.724 ^{ab}	0.881	1.216 ^a
SEM	0.004	0.003	0.006	0.055	0.012	0.011	0.017	0.013
P-value	0.4797	0.2277	0.4084	0.5376	0.1058	0.0450	0.8516	0.0088

^{a-c}Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

¹Treatment **CON** consisted of chicks fed corn-soybean meal (SBM) basal with poultry fat; Treatments **FLAX**, **CANO**, **FISH**, **COCO**, **OLIV**, and **LARD** consisted of chicks given similar diet to **CON**, but with flaxseed oil, canola oil, fish oil, coconut oil, olive oil, and lard, respectively, replacing poultry fat as the fat type.

²Values represent the mean of 5 replicate pens per treatment.

³Values are based only on weight of live birds

⁴FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

Table 8. Effect of dietary fat types on growth performance of broiler chicks (d 22–56).

Treatments ¹	D 22 to 42 (Parameters measured) ²				D 43 to 56 (Parameters measured) ²			
	Body weight (BW , kg/bird) ³	Body weight gain (BWG , kg/bird)	Feed intake (FI , kg/bird)	FCR ⁴ (kg:kg)	BW (kg/bird) ³	BWG (kg/bird)	FI (kg/bird)	FCR ⁴ (kg:kg)
CON	3.116	2.227 ^{bc}	3.304 ^{ab}	1.483 ^b	4.919 ^c	1.672	3.238	1.904
FLAX	3.283	2.340 ^{abc}	3.505 ^{abc}	1.498 ^{ab}	5.126 ^{abc}	1.724	3.309	1.933
CANO	3.151	2.223 ^c	3.357 ^{bc}	1.510 ^{ab}	5.095 ^{abc}	1.862	3.477	1.781
FISH	3.281	2.383 ^{ab}	3.697 ^a	1.552 ^a	5.219 ^{ab}	1.833	3.633	2.002
COCO	3.178	2.252 ^{bc}	3.434 ^{abc}	1.525 ^{ab}	4.943 ^{bc}	1.698	3.307	1.970
OLIV	3.307	2.410 ^a	3.395 ^{bc}	1.407 ^c	5.325 ^a	1.927	3.600	1.830
LARD	3.286	2.371 ^{abc}	3.587 ^{ab}	1.513 ^{ab}	5.054 ^{abc}	1.706	3.343	2.003
SEM	0.056	0.049	0.084	0.015	0.088	0.064	0.139	0.056
P-value	0.1041	0.0386	0.0359	0.0012	0.0358	0.0572	0.3085	0.0616

^{a-c}Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

¹Treatment **CON** consisted of chicks fed corn-soybean meal (SBM) basal with poultry fat; Treatments **FLAX**, **CANO**, **FISH**, **COCO**, **OLIV**, and **LARD** consisted of chicks given similar diet to **CON**, but with flaxseed oil, canola oil, fish oil, coconut oil, olive oil, and lard, respectively, replacing poultry fat as the fat type.

²Values represent the mean of 6 replicate pens per treatment.

³Values are based only on weight of live birds.

⁴FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

Table 9. Effect of dietary fat types on growth performance of broiler chicks (d 1–56).⁵

Treatments ¹	Cumulative: D 1 to 56 (Parameters measured) ²			
	Body weight (BW , kg/bird) ³	Body weight gain (BWG , kg/bird)	Feed intake (FI , kg/bird)	FCR ⁴ (kg:kg)
CON	4.919 ^c	4.793 ^c	9.299	1.517 ^{abc}
FLAX	5.126 ^{abc}	4.964 ^{abc}	9.536	1.519 ^{abc}
CANO	5.095 ^{abc}	4.937 ^{abc}	9.220	1.507 ^{bc}
FISH	5.219 ^{ab}	5.076 ^{ab}	10.075	1.568 ^a
COCO	4.943 ^{bc}	4.808 ^{bc}	9.354	1.559 ^{ab}
OLIV	5.325 ^a	5.172 ^a	9.660	1.468 ^c
LARD	5.054 ^{abc}	4.921 ^{abc}	9.642	1.555 ^{ab}
SEM	0.088	0.086	0.276	0.018
P-value	0.0358	0.0446	0.3813	0.0084

^{a-c}Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

¹Treatment **CON** consisted of chicks fed corn-soybean meal (SBM) basal with poultry fat; Treatments **FLAX**, **CANO**, **FISH**, **COCO**, **OLIV**, and **LARD** consisted of chicks given similar diet to **CON**, but with flaxseed oil, canola oil, fish oil, coconut oil, olive oil, and lard, respectively, replacing poultry fat as the fat type.

²Values represent the mean of 6 replicate pens per treatment.

³Values are based only on weight of live birds.

⁴FCR = feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

⁵Cummulative = growth performance data from d 1 to 56

vary differently in fatty acid composition and have been implicated in affecting the digestion and fat bioavailability in the small intestine under the emulsifying functions of bile acids (McKimmie et al., 2013). In the present

study, we evaluated how the dietary fat types in the gut influences the expression levels of intestinal ileal neurotransmitter transporters, DAT and 5-HTT. Our results demonstrated that dietary fat types differentially

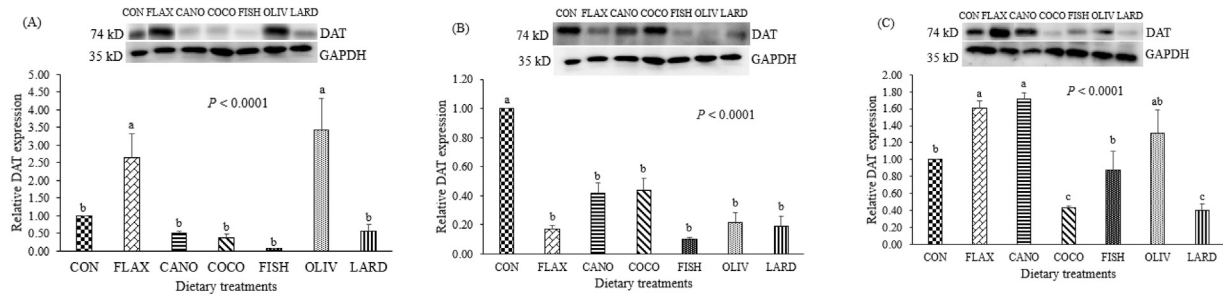


Figure 1. Effect of dietary fat types on the expression levels of ileal DAT at 20 d (A), 41 d (B), and 55 d (C). The data are expressed as means $\pm$ SEM, with $n = 5$ per treatment. ^{a, b, c} means with a different superscript are significantly different ($P < 0.05$).

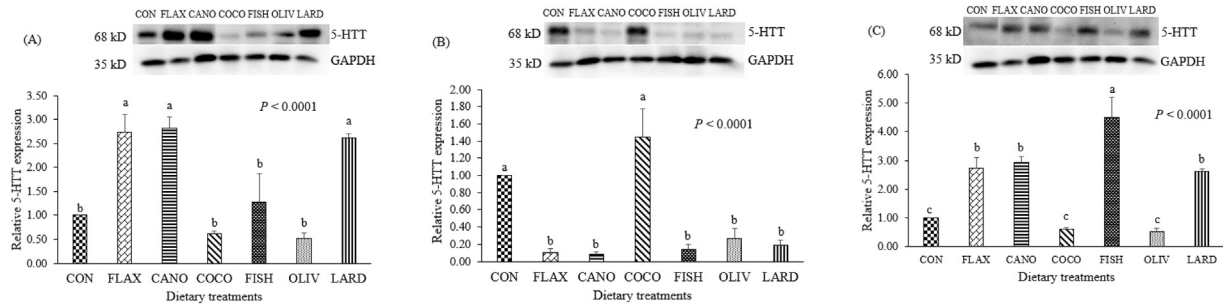


Figure 2. Effect of dietary fat type on the expression levels of ileal 5-HTT at 20 d (A), 41 d (B), and 55 d (C). The data are expressed as means $\pm$ SEM, with $n = 5$ per treatment. ^{a, b, c} means with a different superscript are significantly different ($P < 0.05$).

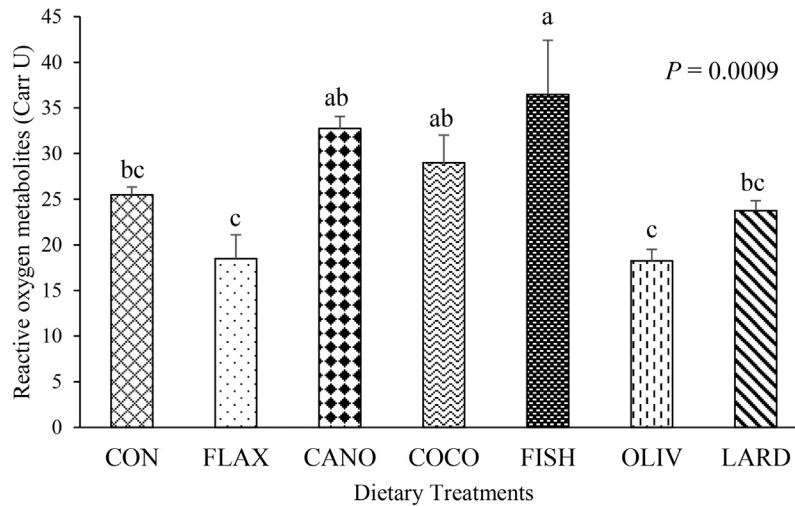


Figure 3. Effect of dietary fat type on plasma oxidative status. The data are expressed as means $\pm$ SEM, with $n = 5$ per treatment. ^{a, b, c} means with a different superscript are significantly different ($P < 0.05$).

influenced the expression levels of the DAT and 5-HTT over the 3 different time points.

The growth performance parameters measured demonstrates that the fat types used in the dietary treatments were well utilized by the chickens and are comparable to the CON treatment. Our findings correspond to studies that involved the inclusion of dietary fat types in chicken diets (Baéza et al., 2013; Konieczka et al., 2017; Turcu et al., 2020; Geng et al., 2022), who reported that dietary

inclusion of saturated, monounsaturated or polyunsaturated fat types, did not adversely affect broiler performance. Varying intake of saturated fatty acids often results in increased intestinal permeability, impaired gut barrier function, and increased adipocyte (Dewulf et al., 2011; Matsunaga et al., 2009; Cani et al., 2008). There has been some connection between gut health and the metabolism of neurotransmitters, and enteric challenges that affect intestinal permeability might impact the

production and regulation of neurotransmitters. Changes in gut permeability could potentially influence the composition of the gut microbiota, which in turn might influence the expression levels of neurotransmitters. However, it is important to note there are limited studies in understanding these mechanisms and connections between the enteric nervous system, neurotransmitters, and gut health. Although there is fascinating evidence that may link the enteric nervous system and gut health, more research needs to be done to determine how the metabolism of dietary fats influences the production of neurotransmitters and its transporters.

DA and 5-HT neurotransmitters play important roles in modulating the gut. The 5-HTT aids the reuptake of 5-HT, which promotes gut motility and influences peristaltic movement coordination (Shine et al., 2022). Similarly, DAT regulates the DA transmission required for motivation and reward. Notably, DA functions primarily as a modulator, inhibiting and balancing excitatory signals while regulating the gut functions and splanchnic circulation (Serio and Zizzo, 2023). The ENS consists of neurons that regulate varying gastrointestinal functions including gut motility and secretion, in addition to the myenteric plexus, which controls peristalsis and vascular tone influenced by these neurotransmitters (Popowycz et al., 2022).

The fatty acid composition of each dietary fat type is the main factor influencing neurotransmitter production, signaling and transport. Studies by Delion et al. (1996) and Zimmer et al. (2000) have shown that PUFAs differentially affect the binding of DAT receptors. However, there are limited studies regarding the effects of dietary fat type on 5-HTT binding and receptor and binding. Across all ages of the broiler chickens in this study, the expression levels of DAT in the saturated dietary fat types (LARD and COCO) were similar (Figure 1) and this could be attributed to their comparable fatty acid composition. At 55 d, the unsaturated dietary fat types (OLIV, CANO, FISH, and FLAX) produced higher expression levels of DAT than the saturated dietary fat types, which suggests that unsaturated fats can enhance the intestinal DAT levels. This difference could be linked to the easy breakdown involving the digestion of unsaturated fat and formation of micelles. As the fatty acid metabolites interact and modulate ion channels, it aids the signaling by the G-protein coupled receptors, which possibly explains the increased expression levels of DAT (Antollini and Barrantes, 2016). In contrast to what was observed at 55 d, there were no differences in DAT levels observed at 41 d amongst the unsaturated and saturated dietary fats, suggesting age could influence intestinal DAT. The unsaturated dietary fat types influenced the expression of neurotransmitter transporters which is evident in our findings. The breakdown of the dietary fat types and fibers results in the production of SCFAs by the gut bacteria, which can adhere to free fatty acid receptors on the ileal epithelial cells (Kim et al., 2003; Lenartowski and Goc, 2011; Dicks, 2022). These SCFAs interact with neurons in the gut tissues thereby influencing synthesis of neurotransmitters (Lyte et al., 2024).

The polyunsaturated dietary fat types, including the CANO, FLAX, FISH has been suggested to play a role in supporting healthy serotonin function and have shown potential towards serotonin receptors and its transporter binding (du Bois et al., 2006). Our relative 5-HTT expression levels was similar for treatments FLAX and CANO with FISH treatment showing similar or better 5-HTT levels. Interestingly, LARD a saturated fat type showed similar 5-HTT expression levels as the polyunsaturated, which indicates that the metabolites present in the LARD treatment are somewhat easily attached to the receptors and transporters (Mawe and Hoffman, 2013). In contrast to the 55 d observation, differences at 41 d were not evident in the relative 5-HTT levels amongst the saturated and unsaturated dietary fats FLAX, CANO, FISH, OLIV, and LARD except for COCO, which showed relatively higher 5-HTT levels. Similar to DAT, age might be a limiting factor, however COCO could play a role in supporting its metabolites to 5-HT receptors.

The d-ROM test serves as a standard method used for assessing the pro-oxidant capacity by measuring the concentration of hydroperoxides concentrations in plasma, which are part of the reactive oxygen metabolites group. The production of these hydroperoxides results from the oxidation of the peptides, proteins present in the dietary fat types (Hidalgo and Alaiz, 2001). Our pro-oxidant test results showed lower oxidative stress levels in FLAX and OLIV treatments but were comparable to CON and LARD treatments (Figure 3), however, they were still within the normal range. Unsaturated fats OLIV and FLAX are known for their anti-inflammatory properties and could be attributed to the decreased oxidative stress response (Barceló-Coblijn and Murphy 2009). However, LARD contains a blend of saturated and unsaturated fats and could have influenced its metabolism thereby reducing the oxidative stress in the system (Xu et al., 2013). Saturated fats are known to influence oxidative stress which is linked to increased ROS production (Famurewa et al., 2019) and was evident in COCO. However, oxidative stress level in COCO was reduced, and could be attributed to its antioxidant and anti-inflammatory effect as reported by Nevin and Rajamohan (2004) and Vysakh et al. (2014). Interestingly, unsaturated fats FISH and CANO recorded similar levels of stress, and this could be attributed to the improper balance of omega-3s present in both fats. The buildup of free radicals within the broiler chicken's cells could give rise to oxidative stress posing a threat to the health of the animal and lead to reduced performance and susceptibility to diseases (Lykkesfeldt and Svendsen 2007).

In conclusion, a diet supplemented with 3% of flaxseed and olive oil showed considerable potential to increase the expression of intestinal DAT without inducing oxidative stress. Moreover, flaxseed oil increased the expression levels of 5-HTT without inducing oxidative stress. Further study needs to be conducted to delineate the mechanism by which the fatty acid profile composition of each dietary fat type influences the ileal neurotransmitter synthesis.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.104114.

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