Utilization of a Co-extruded Mixture of Flaxseed and Pulses (linPRO) in Broiler Breeders

by

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ABSTRACT

UTILIZATION OF A CO-EXTRUDED MIXTURE OF FLAXSEED AND PULSES (LINPRO) IN BROILER BREEDERS

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The objective of this thesis is to examine utilization of a co-extruded mixture of flaxseed and pulses (linPRO) in broiler breeders (BB). BB were fed a basal corn and soybean meal diet or the basal diet plus linPRO with or without fibre degrading enzyme (FDE). Ileal digesta and excreta samples were collected for determination of standardized ileal digestible amino acids (SID of AA) and apparent metabolizable energy (AME). Samples of egg and liver were collected to evaluate enrichment of egg yolk with long chain polyunsaturated fatty acids (PUFA), with specific focus on ω-3 fatty acid and crude fat content of liver. BB digested significantly more amino acids and energy in the control and linPRO diets than broiler chicks and there was no effect of FDE Feeding linPRO significantly increased levels of ALA and DHA in the egg without adverse effect on liver.

Dedication

To my parents, Thanabalan Naguleshapillai and Shanthini Thanabalan

To my advisor, Dr Elijah Kiarie

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Abbreviations

AA	Amino Acid
AAL	Amino Acid Loss
AGP	Antibiotic Growth Promoter
AID	Apparent Ileal Digestibility
ALA	Alpha-linolenic Acid
AME	Apparent Metabolizable Energy
AMEn	Nitrogen Corrected Apparent Metabolizable Energy
ANF	Antinutritional Factors
AR	Apparent Retention
BB	Broiler Breeders
BC	Broiler Chicks
CCAC	Canadian Council On Animal Care
CFC	Chicken Farmers Of Canada
CHEP	Canadian Hatching Egg Producers
CP	Crude Protein
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
EAAL	Endogenous AA Losses
EPA	Eicosapentaenoic Acid
FA	Fatty Acids
FDE	Fiber Degrading Enzyme
FID	Flame Ionization Detector
FLHS	Fatty Liver Haemorrhagic Syndrome
GE	Gross Energy
LA	Linoleic Acid

NDF	Neutral Detergent Fiber
PUFA	Polyunsaturated Fatty Acids
SID	Standardized Ileal Digestibility
TiO2	Titanium Dioxide
UPLC	Ultra Performance Liquid Chromatography
VLDL	Very Low-Density Lipoproteins

Chapter 1. Introduction

Genetic selection has dramatically increased the performance of poultry breeder flocks and their progeny. For example, growth performance and meat yield has improved linearly each year, along with greater feed utilization efficiency in broilers and turkeys (Havenstein et al., 2003; Havenstein et al., 2007). This trend will likely continue in the future as new technologies in genetics, biotechnology, and developmental biology are introduced and adopted by the poultry industry (Ferket, 2012). Between 2005 and 2010, the duration to produce a 2.5 kg broiler reduced by 0.74 days per year (Gous, 2010). One of the implications of decreasing time to slaughter weight is that the period of embryonic development will increasingly constitute a greater proportion of a bird's life. For example, a 21-day incubation period, which is a crucial window in gastrointestinal development, accounts for about 30% of the life of a 2.5 kg broiler at slaughter (Ferket, 2012). Therefore, the embryonic and post-hatch developmental period represents a significant phase in attaining quality broilers at slaughter. The nutrients deposited in the egg by the hen are the only sources of nutrients available to the embryo, and may be the last chemical means by which the hen may transfer an epigenetic message to its offspring. Therefore, investigations on the effect of maternal nutrition on during the incubation period could help improve embryonic health, hatchability and chick viability.

Egg fat is of considerable importance in the nutrition of the developing embryo as a source of energy and essential fatty acids, such as linoleic (18:2 ω-6) and α-linolenic (18:3 ω-3) acids. During incubation, yolk lipids provide fatty acids that are utilized for energy, synthesis of polyunsaturated fatty acids (PUFA), as well as synthesis of eicosanoids by the embryo. 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). The

incorporation of ω-3 PUFA into the egg through maternal diet enrichment could have beneficial effects on chick long-term health and performance post-hatch.

Surprisingly, little consideration is given to fat composition in broiler breeder diets and what effect it may have on reproduction or the immune and inflammatory response in offspring. Current broiler breeders diets are generally high in ω-6 fatty acids. For example, ω-6 fatty acids constitute over 50% of total fatty acids found in standard poultry rations, while ω-3 fatty acids only constitute about 3%. This is primarily due to the high reliance on corn, soybean oil and and terrestrial animal fat sources as the main lipid sources in these feeds. Several poultry studies have shown enriching breeder diets with ω-3 PUFA increased retention of ω-3 PUFA in tissues cell membranes, reduced plasma non-esterified fatty acids, altered expression of pro-inflammatory cyclooxygenase-2 protein and reduced production of pro-inflammatory eicosanoids, and suppression of cell-mediated immunity (Cherian, 2011).

Most common feed ingredients are low in ω-3 PUFA, however, marine oils, fish meal and some oilseeds are good sources of ω-3 fatty acids which can be used to enrich diets (Rymer and Givens, 2005). The cost of these ingredients are generally high due to limited supply, notably in the case of marine fish oils and fish meal (Kanakri et al., 2017). Flaxseeds are oilseeds rich in ω-3 fatty acids produced on a wide scale in Canada and may be considered as an alternative for dietary enrichment. Flaxseeds contain 40% crude fat and ALA consistutes about 58% of the total fatty acids (Caston et al., 1994). However, flaxseeds also contains high levels of non-starch polysaccharides (NSP), such as mucilages, and several antinutritional factors (e.g.phytic acid, antipyrodoxine, trypsin inhibitors and hydrocyanic acid) (Kiarie et al., 2009). Processing can be used to reduce the levels of certain ANFs and improve nutrient bioavailabilty. Processing

techniques, such as grinding and extrusion, can contribute to breaking the outer fibourous hull of the seed and which will allow for access to the fat located in the seed.

In this context, a Canadian company located in the heartland of flaxseed production (O & T Farms Ltd, Saskatoon) has been very successful in developing a flaxseed based product (linPRO) that is widely accepted and adopted by table eggs producers in Canada and elesewhere (O&T Farms, 2017). linPRO is a dry extruded product consisting of full-fat flaxseed and ground pulses, usually field peas, (1:1 wt/wt). The flaxseed in linPRO is used as a whole seed and is mixed with the ground field peas before dry co-extruding both ingredients. In addition to providing nutrients, field peas serve as a carrier of the flaxseed oils.

The effects of linPRO based diets on table egg enrichment with ω-3 fatty acids are well documented (Jia et al., 2008 and Nain et al., 2012). However, there is little research on the incorporation of linPRO into broiler breeder diets and the potential benefits in the breeder flocks and subsequently the progeny. Furthermore, current apparent metabolizable energy (AME) and amino acid values of linPRO have only been documented in broilers and layer hen (O&T Farms, 2010). As broiler breeders are restricted fed, they could have different digestion capabilities when compared to birds fed *ad libitum*. Therefore, AME and amino acid values of linPRO could be different in broiler breeders.

Chapter 2. Literature review

State of broiler industry and challenges thereof

Global human population is estimated to reach 9.6 billion in 2050 and during this period, broiler chicken production is expected to grow by 121% to satisfy the protein demand of the growing population (FAO, 2011). Chicken is the most widely consumed meat in Canada with 32 kg per capita consumption. To meet this demand, Chicken Farmers of Canada (CFC) raise more than 700 million broiler chickens with an overall annual contribution of $6.8 billion to the Canadian economy https://www.chickenfarmers.ca/good-for-canada/. In 2016, Canadian hatcheries set a total of 859 million eggs; 735 million of these eggs were supplied by 243 broiler hatching egg producers (93% of which are members of the Canadian Hatching Egg Producers (CHEP). In this context, CFC relies on CHEP for supply of resilient and robust chicks. The reliance of broiler farmers on successful hatcheries of healthy chicks is a globally accepted concept (Yassin et al., 2008). Therefore, optimizing performance in broilers starts with improved chick quality (Gous, 2010).

The concept of maternal nutrition to bolster progeny performance and well being

The influence of maternal nutrion on chick quality in terms of growth, carcass quality and immune development is well documented (Kidd, 2003). Nutrients deposited into the egg by the hen are the only source of nutrients available to the embryo, and this may be the last chemical means by which the hen may improve embryonic health, hatchability and chick viability. Amongst the possible constraints in broiler performance are maternal effects on egg size, deficiency of maternally derived immunity, rate of gut maturation and problems of supplying sufficient nutrients in the early phase of growth (Havenstein et al., 2003; Gous, 2010; Chang et al., 2016).

Breeder nutrition is a key contributor to better chick viability, growth and carcass yield, as well as bird welfare. Lowering protein levels in broiler breeder diets (16% to 10%, reduced in 2% increments) and relative protein intake (26 g/day to 16 g/day, reduced in 3 g/day increments) negatively affected egg sizes and subsequently chick weight at hatch (Lopez and Leeson, 1995). Conversely, when protein levels of broiler breeder diets were increased from 14.5% to 17.4% a 12% increase in breeder body weight was reported, which is known to negatively affect production and chick quality (Shivazad et al., 2003).

Energy intake of broiler breeders has also been reported to affect hatchability, chick quality and broiler performance. Spratt and Leeson (1987) investigated the effects of feeding broiler breeder diets containing 19 or 25 g protein and either 325, 385 or 450 kcal nitrogen-corrected metabolizable energy on progeny performance. While protein intake of broiler breeders had no effect on offspring body weight at day 0, the energy intake of broiler breeders was reflected in higher offspring body weights at day 0 relative to increasing levels of energy (Spratt and Leeson, 1987). Furthermore, male offspring body weights at day 20 were affected by maternal energy intake, where the highest intake had the heaviest chick. Broiler breeders fed increasing levels of energy also resulted in increased carcass protein and reduced fat in male offspring at slaughter, indicating that maternal nutrition plays a significant role in broiler performance.

Manipulation of fat in broiler breeder diets has also been reported to affect chick quality and broiler production. Effects of adding poultry fat, corn oil or lard to breeder diets on the growth, performance and slaughter yield of progeny were investigated in a series of experiemnts (Peeble et al., 1999a). The body weight gain of broilers from hens fed corn oil was significantly higher than broilers from hens fed poultry fat (Peebles et al., 1999a). Added dietary fat affected the yolk concentration of oleic, linoleic and arachidonic acids which was interpreted to have affected

integration of fats into the embryo and therefore the hatched chick quality (Peebles et al., 1999a). It has been reported that that adding corn oil, palmitic acid and linoleic acid increased hatchability and decreased embryonic mortality (Wilson, 1996). A higher incorporation of PUFAs in corn oil over poultry fat could have contributed to the higher growth rate in broiler between 0 and day 21 (Peebles, 1999a). Lastly, micronutrients such as vitamins in broiler breeder diets have also been examined for their effects on progeny performance. For example, broiler breeder diets supplemented with zinc-L-selenomethionine increased the hatch weight of chicks in 29 week old breeders (Urso et al., 2009). Therefore, research indicates an influence of maternal broiler breeder diets on progeny performance.

Composition of the egg and fat metabolism in the developing embryo

The direct influence of maternal diets is seen in the egg as the composition is mainly dependent on three factors; genetic traits, the age of the breeder flock and the maternal nutrition (Yadgary et al., 2001; Şahan et al., 2010; Speier et al., 2012). In general, the albumen represents approximately 65-75 % of an egg and is composed of 12% protein and 88% water (Yadgary et al., 2001). Comparatively, the yolk consists of 50% water, 15% protein, less than 1% carbohydrates and 33% fat (Yadgary et al., 2001). Due to the nutrient dense composition, fatty oxidation of the yolk accounts for 90% of avian embryonic caloric requirements acting as the main nutrient supply for the developing embryos and chicks in the immediate period post-hatch (Donaldson, 1981). The embryo relies on fatty acids for phospholipids for membrane formation and triglycerides for energy storage (Donaldson, 1981). Before the embryo can use the fatty acids for development, they must be transferred to the yolk by broiler breeders. Dietary lipid digestion involves the reduction of lipid fractions into its fatty acid components (Hurwitz et al., 1973). Micelles are formed during digestion through bile salts which emulsify fat along with the aid of co-lipase.

Pancreatic lipase then hydrolyses the emulsified triacylglycerols at the sn-1 and sn-3 positions to release 2-monoacylglycerols (MAG) and two free fatty acids (FFA) while cholesterol esterase hydrolyses cholesterol-fatty acids into cholesterol and FFA. These fractions are then absorbed through the intestinal lumen and then re-esterified within the endoplasmic reticulum of enterocytes before transport (Krogdahl, 1985). The FFA are transformed into TAG and form portomicrons that are transferred to the hepatic portal circulation (Bensadoun and Rothfeld, 1972; Krogdahl, 1985). Synthesized lipid components are transported to the ovary by yolk targeted very-low-density-lipoproteins from the liver, the main site of fat digestion (VLDL) in breeders.

Synthesis of LCPUFAs

Evolutionarily, lipids are a vital source of energy and essential fatty acids such as linoleic (**LA 18:2 n-6**) and α-linolenic (**ALA 18:3 ω-3**) during embryogenesis and early post-hatch development (Noble and Cocchi, 1990; Speake et al., 1998). From the 2nd week of incuation onwards, there is a rapid intake of lipid components by the embryo which continues until the residual yolk is completely absorbed post hatch (Cherian, 2015). In mammialian and avaian species, ALA 18:3 ω-3 and LA 18:2 n-6 cannot be synthesized *de novo* and must to be supplied in the diet (NRC, 1994). This essentiality is due to the inability of these animals to insert a double bond beyond δ-9 carbon due to the lack of Δ-12 and -15 desaturases (Brenner, 1971). However, once a double bond is inserted at the 3rd and 6th carbon (from CH3 end locations) more double bonds can be added to form longer chain FA such as eicosapentaenoic acid (**EPA, 20:5 ω-3**), docosapentaenoic acid (**DPA, 22:5 ω-3**) and docosahexaenoic acid (**DHA, 22:6 ω-3**) (Brenner, 1971).

The conversion of ALA to DHA involves seven sequential steps, involving three desaturases, three chain elongases and one chain-shortening reaction (**Figure 2.1)**. Alpha linolenic

acid is converted to stearidonic acid *via* Δ6 desaturase, followed by the elongation of stearidonic acid to eicosatetraenoic acid *via* elongase 5 . Eicosatetraenoic acid is subseuquntly converted to EPA *via* Δ5 desaturase. EPA is then further elongated *via* elongase 2, producing 13 tetracosapentaenoic acid which is then converted to tretracohxeaenoic acid *via* Δ6 desaturase. Tretracoheanoic acid is then converted to DHA through partial peroxisomal β-oxidation. n-6 PUFA metabolism for linoleic acid follows a similar pathwawy, with the resulting arachadonic acid as the major metabolite.

Currently, little consideration is given to the composition of the breeder hen dietary FA composition and what effect it may have on reproduction, hatchabiltiy, or progeny performance and immune response (Cherian, 2011, 2015). In a typical breeder ration, 50% of the total FA is repersented by LA with ~3-3.5% ALA (Cherian, 2008). This is attributed to predominant use of corn and soybean meal diets as these ingredients contribute high levels of n-6 fatty acids and are low in ALA concentration. **Table 1** highlights the differences of fatty acid profiles between commonly used feed ingredients in poultry diets. Corn and soybean meal are composed of > 40% 18:2 and < 10% 18:3 fatty acids, contributing to the wide gap in LA to ALA ratio. While the conversion pathway of ALA to DHA is biologically limited, enviormental factors such as diet also influence ALA to DHA conversion. Diets rich in LA competitively inhibit the metabolism of ALA to stearidonic acid by decreasing hepatic Δ6 desaturase expression. Therefore, decreasing dietary LA levels while increasing ALA levels, resulting in a closer ALA:LA ratio has been shown to promote DHA synthesis from ALA .

Flaxseed as a strategy for enriching poultry products with of ω-3 FA

Flax (*Linum usitatissimum*) have been cultivated for centuries and has found wide use as a food product (Singh et al., 2011). Full-fat flaxseed is composed of approximately 40% oil, 22%

crude protein, 28 % dietary fibre and 7.7% moisture (Canadian Grain Commission, 2017). One of the unique aspects of flaxseed is seen in its fatty acid composition; of the 40% oil up to 58% of the fatty acids are alpha-linolenic acid (**ALA, C18:3 ω-3**). Comparatively, other common oilseeds such as soybean and canola have approximately 9.6% C18:3 ω-3 according to Canadian Grains Commission (2017). Canada is the largest producer of flaxseeds, accounting for almost 80% of the global trade (Goyal et al., 2014).

In humans, dietary flaxseed intake has been reported to significantly increase EPA in the plasma and DHA in tissues, implying that a significant amount of ALA is elongated to the longer chain PUFA (Harper et al., 2006, Layne et al., 1996). The use of flaxseed to enrich poultry products such as eggs with ω-3 for human consumption is well documented (Caston et al., 1994; Konieczka et al., 2017). Laying hens have the ability, although not efficiently (< 6%), to elongate and desaturate ALA from flaxseed, to EPA and DHA (Neijat et al., 2016a; Neijat et al., 2016b). However, there appears to be a tolerable limit to how much flaxseed can be included in a ration, due to anti-nutrients or palatability. For example, in an experiment using 240 18-wk-old White Leghorn pullets, investigated the effects of various dietary levels of flaxseed (Leeson et al., 2000). Birds were fed ad libitum over twelve 28-day periods, and measurements of egg weight, egg production, eggshell deformation, and body weight taken. The overall effect of 20% flaxseed-based diet was negative and resulting in poor weight gain, reduced egg production and increased feed intake. High levels of anti-nutritional factors or lower apparent metabolizable energy (AMEn) of the diet due to flaxseed were suspected as the cause for the adjustment in laying hen feed intake (Leeson et al., 2000).

Anti-nutritional factors (ANF) in flaxseeds

As previously stated, flaxseed incorporation into diets is limited by the presence of anti-nutritional factors. The fibrous hulls account for approximately 30-39% of the seed weight (Wanasundara and Shahidi, 1997). This fiber is unique with a high content of crystalline cellulose with tightly bound galactans (Maijala et al., 2012). The inner hull, also known as the spermoderm is surrounded on the inside and outside by mucilage. Mucilage is made up of acidic and neutral polysaccharide components in a 2:1 ratio. The acidic portion is composed of 25.3% L-rhamnose, 11.7% L-galactose, 8.4% L-fructose, and 29.1% D-xylose and the neutral portion is composed of 20% L-arabinose and 76% D-xylose/D-galactose (Shim et al., 2014). Low pressure size exclusion chromatography revealed 1,4-linked β-D-xylose in the neutral fraction and 1,2 linked L-rhamnose om the acidic fractions (Warrand et al., 2005). Monogastric animals are unable to break β-linked sugars, affecting the digestibility of flaxseeds (Alzueta et al., 2002; Kiarie et al., 2007). Furthermore, mucilage leads to increased viscosity of the digesta due to its high water absorbing capacity, ultimately leading to poor growth performance in poultry (Rodríguez et al., 2001; Alzueta et al., 2003).

Flaxseeds also have relatively high content of phytic acid. *myo*-lnositol hexaphosphate, which, is a form of phosphorus storage in plant feedstuffs (Woyengo and Nyachoti, 2013). Majority of phosphorus (> 65%) is bound in phytate form in most plant feedstuffs and is unavailable to monogastric animals (Kiarie and Nyachoti, 2010). As a negatively charged molecule, phytate binds with positively charged dietary and endogenous molecules such as nutrients, digestive enzymes and mucins subsequently impacting gut function negatively (Woyengo and Nyachoti, 2013). Phytase, the required enzyme to hydrolyze phytate, is absent in avian and mammalian intestinal secretions. However, the use of phytase technology has led to

increased bioavailability of phytate bound phosphorous in feedstuffs for monogastric animals/ (Kiarie et al., 2015).

Another ANF present in flaxseed is cyanogenic glycosides. Cyanogenic glycosides are mainly present as linustatin and neolinustatin in flaxseed. When degraded by β–glucosidase in the hindgut, low levels of the toxic compound hydrogen cyanide is released, an inhibitor of cytochrome oxidase (Moghadam and Cherian, 2017). Hydrogen cyanide presence has been reported to cause negative effects on growth and nutrient utilization in broilers due to reduced palatability due to bitterness (Alzueta et al., 2002; Anjum et al., 2013). Flaxseeds also contain linatine, a dipeptide of glutamic acid and proline. linatine acts as a vitamin B6 antagonist, and poses the threat of vitamin B6 deficiency, ultimately resulting in impaired nutrient absorption (Moghadam and Cherian, 2017). Some of the effects of the ANFs present in flaxseed can be mitigated through processing of flaxseed.

Feed processing

Processing can be adapted to either individual ingredients or complete diets. Processing technologies such as grinding, heating, and extrusion are used to improve the availability of nutrients and potentially digestibility (Eggie, 2010). Grinding by either hammer or roller mills increases the surface area of the diet or ingredient as well as physically breaking up diets, increasing digestive enzyme accessibility (NRC, 2012). While smaller particle sizes are associated with greater feed utilization due to increased surface area, factors such as uniformity and homogeneity of particle sizes are challenges faced in grinding processes (Kiarie, E and Mills., A 2019).

Thermal processes such as heating, with or without pressure, also affect nutrient digestion (NRC, 2012). Heating is particularly beneficial in starch rich diets due to the gelatinization through

cell swelling and rupture, in moisture rich ingredients (de Vries et al., 2012). In flaxseed, thermal processes such as heating can destroy heat labile ANFs such as linatine and cyanogenic glycosides. Conversely, due to the potential for oxidation or heat-induced hydrolysis of exposed ALA, thermal processing of flaxseed requires the identification of temperatures which minimize oxidation (Choo et al., 2007).

Extrusion, a combination of heat and pressure processing, forces ingredients through a barrel and die to manipulate the shape of an ingredient. Traditional extrusion methods precondition ingredients with steam or hot water, allowing for starch gelatinization, protein denaturation and subsequent higher nutrient digestibility. Dry extrusion, uses friction as the sole source of heat to effectively rupture cells – improving efficiency of oil extraction compared to tradition extrusion while also reducing processing costs (Ellis et al., 2004). However, dry- extrusion can lead to significant undesirable losses of oil at the terminal end of the die. In order to combat this, previous studies recommend the use of absorbent binders as a means of preserving the exposed oil, while also increasing the friction produced by the shear flow (de Vries et al., 2012, Eggie, 2010).

Application of exogenous enzyme to improve flaxseed nutritive value

In poultry, it is recommended that flaxseed be processed to destroy or deactivate heat labile ANFs such as linatine and cyanogenic glyscosides . The extrusion technology is an efficient detoxification technique for flaxseed which operates under controlled conditions of pressure, temperature, screw speed and feeding rate (Wu et al., 2008). However, even with extrusion, the flaxseed still contains components of the seed's cellular matrix that may trap some of the lipid fraction as seen with other seed types (Cassady et al., 2009), which may be a factor in reducing digestibility or availability of ALA. For example, human studies comparing flax as whole, milled,

and oil observed 77% greater plasma ALA levels in flaxseed oil cohorts compared to milled flaxseed cohorts (Austria et al., 2008).

The fiber component in flaxseed has been associated with depression on growth performance in pigs and poultry (Rodriguez et al., 2001; Kiarie et al., 2007; Leung et al., 2018). Enzyme technology has been used in poultry diets since the 1980's to improve both nutritive values of ingredients and efficiency of use (Slominski, 2011). Commercially, poultry diet often include two types of exogenous enzymes: carbohydrases and phytases targeting non-starch polysaccharides (NSPs) and phytate respectively. Indeed most of the current enzyme market is represented by phytases (60%) and carbohydrases (30%) (Kiarie et al., 2013). Carbohydrases take three potential modes of actions which improve nutrient digestibility in poultry: (1) decrease digesta viscosity by depolymerization of soluble NSP, (2) hydrolysis of (1 → 4)-β- linkage sugar bonds present in insoluble NSP present in the cell wall of the seed coat, and (3) hydrolysis of (1 → 4)-β- linkage sugar bonds in one or both types of NSP and the formation of shorter oligosaccharides which are more readily available for gut bacteria utilization (Cowieson and Adeola, 2005; Jia and Slominski, 2010; Bao et al., 2013; Kiarie et al., 2014). Generally, xylanases and β-glucanases compose most of the fibre degrading enzymes which fall into the carbohydrase category. The addition of exogenous enzymes not only targets the ANFs found naturally in feedstuffs, but also any undesirable bonds created as a result of the Maillard reaction during dry extrusion.

As previously mentioned, mucilage accounts for 8% of the total flaxseed and presents many undesirable nutritive properties. Flaxseed mucilage is comprised of a neutral and acidic polysaccharide component, where the neutral fraction is composed of branched arabinoxylan with a β-D-xylan backbone with arabinose and galactose side-chains (Alzueta et al., 2002). Xylanase

works to liberate nutrients within the cell wall by cleaving the (1 → 4)-β- linkages on the xylan backbone (Alzueta et al., 2002). As well, mucilage presents issues due to its water soluble nature, creating a viscous environment wherein the digestion and absorption of nutrients is inhibited, with fat digestibility suffering the most (Jia and Slominski, 2010). The may be due to the size of chylomicrons relative to mucilage products, reducing their activity.

While xylanase is the most prominent enzyme needed for flaxseeds, multi-composite enzymes are more common for targeting a complete diet. Jia and Slominski (2010) reported a significant increase in both ileal and total tract fat digestibility and decrease in viscosity in broilers fed diets with an 15% inclusion of coarse ground flaxseed and an enzyme composite providing 1, 400 pectinase, 160 U cellulase, 2, 400 U xylanase, 1, 200 U glucanase, 1,500 U mannanase, and 50 U galactanase. Increased body weight gain in broiler chicks was also reported when full-fat flaxseed diets were supplemented with high levels of multi-carbohydrase inclusion (Slominski et al., 2006). With broilers fed coarse milled flaxseeds supplemented with a multi-composite carbohydrase consisting mainly of xylanase, glucanase, and mannanase, there was a 21% decrease in ileal digesta viscosity (de Vries et al., 2012). It is hypothesized that decreasing the viscosity of digesta increases ability to absorb nutrients, allowing for increased growth. Studies that did not see a decrease in digesta viscosity but rather an increase in body weight gain in broilers were associated with reduced nutrient encapsulating effect of the cell walls (Slominski et al., 2006). It is important to note that enzyme inclusion does not always result in a positive effect, but rather no effect at all. For example, in Alzueta et al., (2002) study, there were no effects of enzyme inclusion on linseed digestibility which was hypothesized to be directly related to there being an effect digesta viscosity. There were no significant differences in digesta viscosity in the control vs enzyme group, implying that the composite used may not have degraded the mucilage portions of

flaxseed diets. Therefore, the composition of enzymes must be fine tuned to meet the requirements of specific feedstuffs (Alzueta et al., 2002; Kiarie et al., 2016).

Methods for determining nutritive value of poultry feed ingredients

In recent years, the estimation of ingredient digestibility has been optimized to priovide accurate values specific to an animal. Adeola (2001) describes a direct method of determining digestibility, where the test feed ingredient is the sole component in the test diet. However, it is not always possible to formulate diets with one ingredient because of poor palatability and considerations of meeting animal physiological needs that may influence digestibility. In these cases, the difference or regression methods are used (Fan and Sauer, 1995). Briefly, the test ingredient is incorporated into the basal diets in such a manner that all energy and amino acids supplying ingredients are kept constant - ultimately allowing for the calculation of digestible energy and amino acids for the test ingredient. When the regression analysis method is used, multiple points are used and then the digestibility is extrapolated to the 100% replacement point as a means of determining the digestibility of the test diet (Fan and Sauer, 1995).

Before digestibility can be determined, ileal digesta and fecal matter must be collected from the animals. The total collection method requires a significantly longer adaptation period and is provided in conjunction with constant FI level and frequency (Kong and Adeola, 2014). This allows for the homeostatic state of digestive and metabolic processes. Excreta is collected from the beginning of feed intake recording until the beginning of the next meal. This method may pose issues of inaccurate sampling. An alternative is to use the marker technique which relies on the inclusion of a marker which much be indigestible, nonabsorbable, nontoxic while also travelling through the digestive tract at a relatively uniform rate (Kong and Adeola, 2014). The marker must also be easy to recover and analyze for; an example would be titanium dioxide.

Differences in physiology, metabolism and digestibility between layers, broilers and broiler breeders

Years of genetic selection improved reproductive traits in laying hens and production traits in broiler chicks. Broiler breeders, unlike broiler chickens or layers, have been selected to be successful both reproductively while also carrying genetics for their offspring's production capacity. Physiological and metabolic differences between BB and layers are seen as early as at the embryonic stage. To begin, broiler breeder eggs have heavier yolk masses compared to layer yolk masses, despite no difference in total mean masses of the egg (Ho et al.,2011). Furthermore, embryonic weights of broiler breeders at day 14, 16 and 19 were significantly higher than the weight of layer embryos (Ohta et al., 2004, Everaert et al., 2008). The rate at which the yolk sac, the main source of nutrients, is utilized can account for the differences in embryonic weight and the reflective of hatch weight. Broilers reflect the same patterns as their parental genetics, where the embryonic weights of boiler chickens are significantly higher than layers throughout incubation. The difference in growth rate is further reflected in hatching hours – layers begin to hatch at 491 h of incubation whereas broiler and broiler breeder chicks are 100% hatched by 501 or 502 h (Druyan, 2010). Once hatched, there is also a notable difference in nutrient utilization between layers, broilers and broiler breeders. Regardless of the type of the feed, broiler breeders metabolize about 2.5% less energy than layers (Buzala and Janicki., 2016). Pishnamazi et al., (2005) conducted experiments with feeding common poultry feedstuffs in order to evaluate the differences of AMEn in Cobb broiler breeders and White Leghorn laying birds. Despite no difference in feed intake, the AMEn of corn, soybean meal and wheat bran was significantly higher in the Leghorns (3065, 2185, 1440 kcal/kg respectively) compared to broiler breeders (2842, 2040 and 1333 kcal/kg respectively). Physiologically, layers have a more muscular gizzard as well as longer intestines relative to body weight which may increase the breakdown of feed as well as the

absorptive capacity (Shires et al., 1987). Shires et al., (1987) also reported a longer retention of digesta in the crop and gizzard of laying type bids than broiler breeders – increasing the potential of nutrient absorption due to retention time. Research suggests that broiler breeders are less efficient in using feedstuff for metabolizable energy. Broiler breeders are limit fed to avoid reproductive and skeletal issues associated with obesity, compared to their progeny (broiler chicks) who are fed *ad libitum*. Due to the restriction of feed in broiler breeders, digestibility of ingredients may differ based on diet transit time, age, rearing environment and metabolic needs (Sakomura, 2004). As the balance of energy intake is crucial for broiler breeder production, it is important for accurate quantification of ME value of feedstuffs.

Determination of nutritive value of flaxseed products in poultry

The nutrient profile of flaxseed has generally been reported based on the digestibility values calculated for broiler chicks' or laying hen assays (Evonik, 2010; Adeola et al., 2016). The apparent metabolizable energy (AMEn) value for flaxseed has been reported to be high in roosters (3750 kcal/kg (Lee et al., 1995); 3560, Gonzalez-Esquerra and Leeson, 2000) and laying hens (3894 kcal/kg, University of Saskatchewan, 2017). The AMEn values for broilers are generally lower than those of adult birds but they are also very variable. For example, values of 2055 and 2118 kcal/kg have been reported in broilers of 6 and 9 weeks, respectively (Gonzalez-Esquerra and Leeson, 2000) and other have reported higher values for broiler chickens (3260 kcal/kg), (Ortiz et al., 2001). Incorporation of flaxseed in practical poultry diets often results in variable responses and dependent on a multitude of factors such as processing, enzymes, and cultivation time. For example, values of flaxseed fed in mash versus steam crumble resulted in significantly different AMEn (kcal/kg) in broilers, roosters and laying hens with mash valued at 3560, 3650, 3330 kcal/kg respectively and steam crumble valued at 4580, 4280, 4140 (kcal/kg) respectively

(Leeson and Summers, 2009). Variable AMEn values have been reported for flaxseed in most strains of birds, however very little research has been done on the digestibility of flaxseed for broiler breeders.

It is also important to consider fluctuation reported in feed intake of birds fed diets containing flaxseed, especially in restricted fed broiler breeders (Leeson et al., 2000; Aymond and Elswyk, 1995). Previous studies reported variations in broiler breeder performance as a result of varying inclusions of fat supplying ingredients (Donaldson et al., 1957; Brake and Carolina, 1990). A deficiency of ALA has been reported to cause reduce egg production, increased incubation mortality and reduce growth (Herstad et al., 2000). As well, unlike broiler chicks, broiler breeders are able to ferment flaxseed fibre in the ceca and subsequently produce short chain fatty acids that can contribute energy which should be accounted for when formulating diets (Leung et al., 2018). Furthermore, it is important to characterize the impact of fiber degrading enzymes on the nutritive value of flaxseed and enrichment of egg yolk with ω-3 FA. Enzyme inclusion will potentially affect the nutritive value of flaxseeds as their main role is to increase nutrient utilization (Tahir et al., 2015).

Summary

Flaxseed meal is an economical and environmentally sustainable product for the enrichment of poultry products with ω-3 FA. However, the presence of ANF must be considered when incorporating this ingredient in practical poultry diets. Moreover, because of high oil content, processing flaxseed is challenging for the feed manufacturing processing due to potential for fires. In order to overcome these challenges, flaxseed meal can be ground and then dry extruded with an absorbent ingredient for example pulses to produce a co-extruded product such as linPRO. However, high fibrous content in flaxseed can result in undesirable viscous digesta, ultimately affecting nutrient utilization. In conjunction with mechanical processing, poultry diets can be

supplemented with exogenous fiber degrading enzymes to increase nutrients utilization. As proven with laying hens, feeding linPRO to broiler breeders may allow for the manipulation of fatty acids profile egg yolk of broiler breeders. This can subsequently enrich hatching eggs with ω-3 FA for the progeny. Nutritive value of flaxseed products has been determined in poultry though the data is variable. Most of the data has been determined in broiler chick and laying hen assays and may not be applicable to limit fed broiler breeders to prevent health and reproductive issues. Moreover, they have larger fibre fermentation capacity than broiler chicks. Therefore, for accurate feed formulation there is a need to determine the nutritive value of linPRO in broiler breeders. Moreover, the role of fibre degrading enzymes in improving nutritive value of linPRO and release of ALA for deposition in egg yolk is not known.

Figure 1 : Simplified metabolic pathway of α-linolenic acid to docosahexaenoic acid, adapted from Sprecher, 200

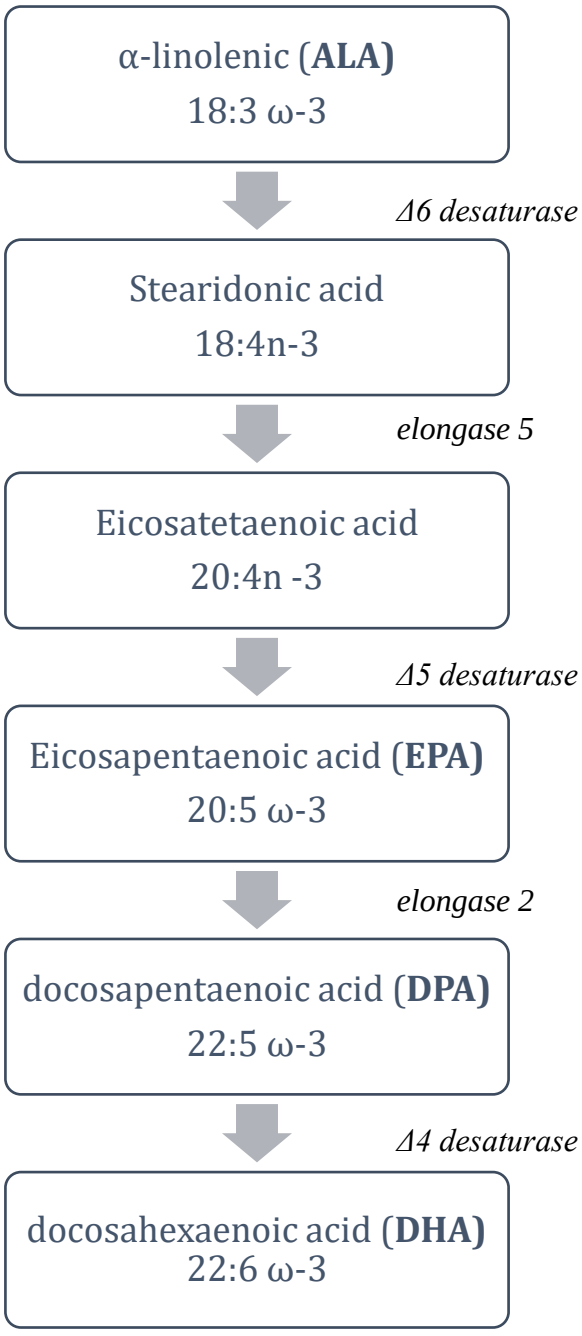

Table 2.1 Fatty Acid composition as a % of crude fat content of common feed ingredients used in poultry diets.

Fatty Acid (%)	Ingredient		
	Corn	**Soybean Meal**	**Flaxseed Meal**
18:2	44.24	39.83	11.03
18:3	1.37	5.55	40.65
PUFA	45.61	45.38	51.68

(NRC,2012)

Chapter 3. Hypotheses and Objectives

Feeding linPRO to broiler breeders has potential to enrich broiler breeder eggs with omega-3 fatty acids. However, for accurate feed formulation, it is necessary to determine the nutritive value of linPRO in broiler breeder hens because they are restricted fed and they could have different digestion capabilities compared to birds on *ad libitum* such as broilers and layers.

Hypotheses

1) Digestible amino acids and energy values of linPRO are different for broiler breeders compared to values derived from standard broiler chick assay
2) Feeding linPRO to broiler breeders will enrich egg yolk with ω-3 FA with no adverse influence on hepatic fat content
3) Supplemental fiber degrading enzymes (**FDE**) will enhance utilization of linPRO in broiler breeders

Objectives

Overall objective: To evaluate utilization of a co-extruded mixture of flaxseed and peas (linPRO) in broiler breeders

Specific objectives, were:

1) To determine standardized ileal digestibility of amino acids and apparent metabolizable energy of linPRO fed to broiler breeders with or without FDE
2) To evaluate effects of feeding linPRO to broiler breeders with or without FDE on egg yolk fatty acids composition and hepatic fat content

Chapter 4: Digestible amino acids and apparent metabolizable energy in co-extruded flaxseed and pulse (linPRO) fed to broiler breeders with or without fiber degrading enzymes

ABSTRACT : Standardized ileal digestibility (**SID**) of amino acids (AA) and AMEn of linPRO was determined in broiler breeder hens (**BB, Exp. 1**) and broiler chicks (**BC, Exp. 2)** with or without a fibre degrading enzymes (**FDE**) supplying 200 U of xylanase, 600 U of β-glucanase, 2,800 U of cellulase, 400 U of mannanase, and 2,500 U of amylase (Superzyme -OM, Canadian BioSystems) per kg of feed. Diets contained 0.5% TiO_2 as an indigestible marker and were fed in mash form. In Exp. 1, diets were a corn soybean meal basal diet and basal diet with energy- and AA- yielding ingredients replaced with 18% linPRO with or without FDE. Sixty, 26-week-old Cobb 500 BB were placed in cages, allocated to three diets and fed once daily a for 30 d. Excreta samples were collected from d 28-30 and all birds were sacrificed on d 30 for ileal digesta. In Exp. 2, a semi-purified corn-starch diet containing 90% linPRO was prepared with or without FDE. A N-free diet was also fed to estimate basal endogenous AA losses (**AAL**). A total of 240 d old Ross x Ross 708 male chicks were placed in cages and fed commercial starter diets until d 13. On d 13, chicks were weighed, allocated to 24 cages and fed experimental diets to d 21. Excreta samples were collected from d 18-21 and all birds were sacrificed on d 21 for ileal digesta. There was no (P>0.05) interaction between age and FDE or main effect of FDE on SID of AA and AMEn in linPRO, however, the main effect of age was such that SID of AA and AMEn in linPRO were higher (P<0.01) in BB than in BC. The AMEn was 4,012 and 2,105 kcal/kg for BB and BC, respectively. In conclusion, BB had higher SID of AA and AMEn than BC, suggesting differences in digestive tract capacity and the impact of feeding schedule on nutrient digestibility of linPRO. Supplemental FDE did not influence utilization of nutrients in linPRO.

Introduction

The first critical step in determining feeding value of a feedstuff is the characterization of their available energy and standardized ileal digestibility (**SID**) amino acids. Digestible AA and energy of ingredients for poultry diets have traditionally been determined using a broiler chick assay (NRC, 1994). However, due to the nature of broiler breeder feeding management, principally feed restriction regimen, it is important to quantify digestibility of feedstuffs in a broiler breeder assay. A dry extruded product consisting of full-fat flaxseed and ground pulses, predominately field peas (1:1 wt/wt, linPRO) was provided by O & T Farms Ltd, Saskatoon, Canada. The flaxseed in linPRO is mixed with the ground pulses before dry extruding both ingredients through a propriety process. In addition to providing nutrients, pulses serve as a carrier of the flaxseed oils through the formation of a protein-fat matrix. Furthermore, linPRO is mechanically processed in order to reduce anti nutritional factors (**ANFs**), residue fiber may inhibit digestibility, and thus inclusion of exogenous fiber degrading enzymes (FDE) may improve digestibility. Lastly, from industry perspectives quantifying differences in ingredient digestibility between broilers and breeders may help in refining values for certain feedstuffs particularly those rich in fiber. Recent research at the University of Guelph indicated broiler breeder hens had higher fermentation capacity of various fiber sources including flax meal (Leung et al., 2018). Application of fibrous feed ingredients in broiler breeder diets does not consider fermentability which could provide maintenance energy through absorbed volatile fatty acids (VFA) in the ceca. 11% derivation of maintenance energy from VFAs in the ceca has been reported for mature birds and approximately 3.5 % for young birds, however values have not been reported for broiler breeders (Annison et al. 1968, Jorgensen et al., 1996, Jamroz et al., 2001). Therefore, the objective of this chapter was to determine standardized ileal digestibility of amino acids and apparent metabolizable energy

content in linPRO fed to broiler breeder hens with or without FDE. A broiler chick assay was also incorporated to compare the amino acids and AMEn values.

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).

Experiment 1: Broiler breeder hens

Sixty, 26-week-old Cobbs 500 broiler breeder pullets were procured from Ontario Broiler Hatching Egg and Chick Commission (Guelph, ON, Canada). A standard corn-soy basal diet (Table 4.2) was formulated to meet broiler breeder nutrient specifications (Cobb, 2016). Experimental diets were made by adding 18% linPRO in the basal diet such that the ratio of energy and AA contributing ingredients were maintained at a constant ratio to allow determination of AA and AMEn in linPro using the substitution method (Fan and Sauer, 1995; Adeola et al., 2016; Kiarie et al., 2016). The linPRO diet was top dressed and fed with or without FDE supplying 1,200 U of xylanase, 600 U of β-glucanase, 2800 U of cellulase, 400 U of mannanase, and 2,500 U of amylase per kg of feed, inclusion level of 0.5kg/MT (Canadian Bio-Systems, Calgary, Alberta, Canada). All diets contained titanium dioxide (TiO_2) as an indigestible marker and were fed as mash. The birds were reared on a commercial pullet farm up to 26 weeks of age and were transported to campus animal holding units at the Department of Animal Biosciences for experimentation. Hens were placed in 30 cages (65 cm×30 cm×45cm). Cages were housed in environmentally controlled rooms kept at temperatures of 20℃ and received 16 hours of fluorescent illumination.

All birds were fed the basal diet for 1 week adaptation period. The 3 diets were randomly assigned to 10 replicate cages (n=10) based on equalized 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 day 27 and pooled per cage for the determination of AMEn. Birds were euthanized by cervical dislocation on day 28. Ileal contents were expressed by gentle flushing with distilled water and frozen at - 20˚C until analyzed.

Experiment 2: Broiler chick assay

A total of 240-day old Ross x Ross 708 broiler chicks were procured from a commercial hatchery (Maple Leaf Foods, New Hamburg, ON, Canada) and allocated to 24 identical cages (10 chicks per pen) based on body weight. A semi-purified corn-starch diet containing 90% linPRO (Table 2) was prepared with or without FDE. Additional N-free diet was fed to estimate basal endogenous AA losses (**EAAL**) (Adeola et al., 2016). Diets contained TiO_2 as an indigestible marker and fed as a mash. The cages were housed in environmentally controlled room. The room temperature was set to breeder recommendation of 32°C on day 0 and gradually decreased to 27°C by day 17. Birds were exposed to fluorescent lighting in a 23 hr of light (20+ lux) for the first 4 days and then a 16 light: 8 dark (10-15 lux) light cycle for the remainder of the experiment in accord with Arkell Poultry Research Station standard operating procedures. Broiler chicks were fed a commercial starter diets until d 13 for adaptation as standard procedures of broiler chick assay (Kiarie et al., 2014; Adeola et al., 2016). On day 13, chicks were weighed, allocated to 24 cages (10 birds/cage), and assigned experimental diets in a completely randomized design to give 8 replicates per diet. The birds had free access to feed and water. Excreta samples were collected from day 18-21 and all birds were sacrificed on day 21 for ileal digesta collection. Ileal digesta

and excreta from birds within a cage were be pooled, resulting in 8 samples per dietary treatment, and frozen at - 20˚C immediately after collection.

Sample processing and chemical analyses

Excreta and ileal digesta samples were thawed, pooled by cage and subsequently freeze dried. Samples of linPRO, experimental diets, freeze dried excreta samples and freeze-dried ileal contents were finely ground using a coffee grinder. All samples were analyzed for dry matter, crude fat, crude protein (**CP)**, neutral detergent fiber (**NDF)**, and gross energy **(GE)**. linPRO samples, diets and ileal digesta were further analyzed for amino acid 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). NDF concentrations were determined using ANKOM 200 Fibre Analyzer (ANKOM Technology, Fairport, NY) using methodology described by (Van Soest et al., 1991). Amino acid compositions (without arginine, cysteine, methionine and tryptophan) were determined after acid hydrolysis through Ultra performance liquid chromatography (UPLC, Waters corporation, Millford, CA, USA) according to AOAC 982.30 mod (AOAC 1995). The recovery of enzyme in feed was performed by CBS laboratories and only xylanase was analyzed using a modified method based on the Megazyme xylanase assay kit (Megazyme International Ireland Ltd., Bray, Ireland).

Calculations and statistical analysis

The apparent ileal digestibility (**AID**) of amino acids and apparent retention (**AR**) of components in experimental diets were calculated using digestible marker method using the following equation (Adeola et al., 2016).

Equation 1: AID or AR, % = [1 − (T in diet/T in ileal or excreta) × (N in ileal or excreta/N in diet)] × 100.

Where T is the concentrations of titanium dioxide in the diet, ileal digesta and excreta; N is concentration of any nutrient (amino acid, crude protein, gross energy, NDF) in diet, ileal digesta and excreta.

Because the experimental diets in Exp. 1 had other amino acids and energy yielding ingredients and experimental diets in Exp 2 had energy yielding ingredients, the AID of amino acids (AA) in Exp.1 and AR of energy yielding components in linPRO was calculated using a difference method (Fan and Sauer, 1995). The corn-soybean meal diet and N-free diet were used as basal diets in Exp.1 and Exp.2, respectively using the following equation:

Equation 2: $DA = D_B + (D_D - D_B)/P_A$

Where:

1) In Exp.1 (broiler breeder hens) D_A = digestibility of AA or retention of GE or N (%) in linPRO; D_B = digestibility (equation 1) of N or AA or retention of GE or N (%) in the basal diet (corn-soybean meal based diet); D_D = digestibility of N or AA or retention of GE or N (%) in assay diet (corn-soybean meal-linPRO); and P_A = proportion (decimal percentage) of linPRO in assay diet.
2) In Exp.2 (broiler chick assay) D_A = retention of GE or N (%) in linPRO; D_B = retention (equation 1) of GE or N (%) in the N-free diet (corn starch based); D_D = retention of GE

or N (%) in assay diet (cornstarch-linPRO); and P_A = proportion (decimal percentage) of linPRO in assay diet.

The SID values calculated by correcting AID of AA values for basal endogenous amino acid losses (**EAAL**) determined in Exp 2 in birds fed N-free diet.

Equation 3: EAAL, mg/kg = AA in ileal × (Tin diet/T in ileal).

Equation 4: SID of AA, % = AID + (EAAL/AA in diet) × 100.

The apparent metabolizable energy (**AME**) content in linPRO was calculated using the following equation:

Equation 5: AME, kcal/kg= [(AR of GE for linPRO, %) × (GE content in linPRO, kcal/kg)]/100.

Nitrogen-corrected AME (**AMEn**) was determined by correction for zero nitrogen retention by simple multiplication with 8.22 kcal per gram nitrogen retained in the body as described by (Hill and Anderson, 1958).

Equation 6: AMEn, kcal/kg = AME − (8.22 × ANR), where ANR = apparent N retained (g kg^{-1} of feed intake from equation 2).

The cage was the experimental unit. Comparative data of broiler breeder and broiler chick assays were analyzed using the GLMMIX procedures of SAS (SAS Inst., Inc., Cary, NC). The model included the main effects of assay method (broiler breeders and broiler chicks), FDE (- and +) and associated two-way interactions. For the apparent retention of components in experimental diets fed to broiler breeder hens (Exp. 1); a one-way ANOVA GLM model in which the diet was the fixed effect. Treatment differences were considered significant at $P < 0.05$.

Results and discussion

The analyzed chemical composition of experimental diets is shown in Table 4.3. Accurate determination of digestible nutrients in feed ingredients is the cornerstone for estimation of nutrient requirements and formulating diets for poultry. For these reasons, researchers have developed various digestibility protocols for estimating digestible nutrients in feed ingredients (Fan and Sauer, 1995; Woyengo et al., 2010; Adeola et al., 2016). Test diet designs range from a direct approach where the ingredient of interest is the sole source of nutrients in the test diet to indirect (substitution) where ingredient of interest is incorporated in the basal diet to create a test diet. In the later design, calculations are made to derive nutrient digestibility in the test ingredient. In these approaches, an assumption is made that digestibility values of a feed ingredient should be independent of the digestibility protocol. This approach was applied in this chapter to determine digestible AA and AMEn in linPRO in broiler breeder and broiler chick assays. It was hypothesized that use of nutrients, including amino acids and energy, in feed ingredients may vary according to the physiological status of the bird (Huang et al., 2006).

The analysed enzyme concentration in experimental diets were as follows: Exp. 1, xylanase levels in linPRO+ diet were higher than that in the control diet and linPRO- diet without enzyme i.e. 1,482, 185 and 170 xylanase units/kg, respectively. Xylanase was the only enzyme assayed as way of confirming accurate feed mixing as it is the most crucial of fibre degrading enzymes.

Standardized ileal digestibility of amino acid

There was no interaction ($P>0.05$) between assay method and FDE on AID (Table 4.4) and SID (Table 4.5) of amino acids (Table 4.4). The main effects were such that broiler breeders had higher ($P<0.05$) AID and SID of AA than broiler chicks (Tables 4.4 and 4.5). Among

indispensable AA, Thr (88.6%) was least digestible and Phe (97.3%) was most digestible in broiler breeders whereas Ile (63.1%) was the least digestible and His (76.5%) was most digestible in broiler chicks. It is generally assumed that amino acids digestibility does not change between the class of birds, and amino acid digestibility values generated with roosters are widely used in feed formulations for broilers and layers (NRC, 1994). However, differences in digestive tract capacity due to maturity and feeding schedules can impact nutrient utilization as demonstrated in the current study.

Apparent retention of components in broiler breeders

In comparison with basal corn-soybean meal-based diets broiler breeders fed linPRO diets retained significantly less dry matter, organic matter and crude protein (Table 4.6). Mucilage, a water-soluble polysaccharide component found in flaxseed increases the water holding capacity of digesta leading to a lower retention of dry matter. Although extrusion physically breakdown ANFs in flaxseed, mucilage presence may impede nutrient absorption by increasing the viscosity of intestinal contents. 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 (Rodríguez et al., 2001). 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 diet effects on AR of fat in the current study. Similarly, Leung et al. (2018) observed higher fat retention in broiler breeder hens fed flax meal compared with a control corn-soybean meal diet. Findings suggest that broiler breeders may have gastrointestinal capacity to overcome deleterious effects of flaxseed soluble fiber on dietary fat utilization. When considering diet composition, linPRO diets had twice as much fat, which could also explain the lack of difference in retention between treatments.

Birds fed the control diet also had a higher (P=0.006) retention of crude protein than birds fed linPRO diets (Table 4.6). Birds fed linPRO diets had a significantly higher (P=0.007) flow of NDF than the control diet translating to a lower retention of fibre. The results of this study agree with the well understood concept wherein high fibre 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. Despite the lower retention of fibre and protein in linPRO diets, the apparent retention of gross energy was not different (P=0.139) from the control (Table 4.6). As with fat retention, the AR of GE results are contradictory to what has been previously reported. Ortiz et al. (2001) reported a linear decrease in energy utilization in broilers as the inclusion rate of flaxseed increased. It is possible that broiler breeders due to the maturity of the digestive tract and relative length compared to broilers, can utilize most of energy yielding components in flaxseed and/or overcome deleterious effects of ANFs.

There were no (P>0.05) enzyme effect on AR of measured components in linPRO (Table 4.6). We can hypothesize that adequate amounts of enzyme may not have been ingested as the dosage used was recommended for broilers. As well, due to their restricted feeding regimen broiler breeders have become extremely effective at utilizing available nutrients and therefore enzyme inclusion may make no difference. Furthermore, the composite used may not have been the most suitable for reducing the antinutritional factors of flaxseeds. As previously mentioned, increased digesta viscosity due to mucilage is one of the main ANF inhibiting nutrient absorption and digestion. Previous research shows that the use of 300 U cellulase, 3950 U mannanase and 5000 U pectinase can significantly reduce digesta viscosity in mash flaxseed diets, however our composite did not contain any pectinase and inadequate amounts of mannanase (Jia and Slominski,

2010). Furthermore, the targeting of the pectic polysaccharides found in the cell wall was found to be most effective when the aforementioned enzymes were used with the addition of glucanase and mannanase and while these two enzymes were present in our composite it was at lower concentrations.

Apparent metabolizable energy

There was no interaction (P>0.05) between experiments and FDE on AR of GE, AME and AMEn in linPRO (Table 4.7)**.** The AMEn for broiler breeders and broiler chicks were 4012 and 2105 kcal/kg respectively, AME_n linPRO in broiler breeders was more than (P <0.001) for broiler chicks (Table 4.7). The AME_n for linPRO fed with or without FDE were 2975 and 3533 kcal/kg, respectively in broiler breeder experiment. These values agree with previously reported values for flaxseed products. The apparent metabolizable energy (AMEn) value for flaxseed has been reported to be high in roosters (3750 kcal/kg (Lee et al., 1995); 3560, Gonzalez-Esquerra and Leeson, 2000) and laying hens (3894 kcal/kg, University of Saskatchewan, 2017). The AMEn values for broiler chicks are generally lower than those of adult birds but they are also very variable. For example, values of 2055 and 2118 kcal/kg has been reported in broilers of 6 and 9 weeks, respectively (Gonzalez-Esquerra and Leeson, 2000). Other studies have reported higher AMEn flaxseed fed to broiler chickens, for example 3260 kcal/kg (Ortiz et al., 2001). High soluble fiber digestibility through cecal microbial fermentation has been widely reported in mature layer hens and broiler breeders and could be a factor in the increased AME_n (Mateos and Serrano, 2012). However, relatively the energy derived from VFAs do not represent a large portion of ME. Due to feed restriction regimen, larger digestive tract volume and microbial population, it is possible that broiler breeders can absorb more nutrients due to the increased period the digesta stays in the GIT tract. Supplemental FDE did not influence utilization of energy in linPRO.

Conclusions

The SID of AA and AME content in linPRO differed significantly between broiler breeders and broilers implying that the differences in feeding schedule and digestive capacities. However, it ought to be recognized that several factors such as rate of passage, physiological status as related to growth or maintenance, feed consumption, or nutritional adequacy of test diets may influence digestibility measurements, and such differences may be responsible, at least in part, for the differences observed between digestibility values of linPRO in broilers breeder hens and broiler chicks. Nonetheless, the data suggested that broiler breeder digestibility values should be used for incorporating linPRO in broiler breeder hen feeding programs. The fiber degrading enzyme supplement did not influence utilization of nutrients in linPRO.

Table 4.1. Analyzed nutrient composition of linPRO sample, as fed basis

Item	linPRO
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
(Ly/Crude protein) x 100	6.52

Table 4.2. Composition of basal and nitrogen free diets, as fed basis

Ingredients	Breeders assay		Broiler chick assay	
	Control	linPRO	Nitrogen free diet	linPRO
Corn	61.45	45.0	-	-
Soybean meal, 46 CP%	25.92	25.2	-	-
linPRO	-	18.0	-	90.1
Soy oil	1.67	1.15	2.50	0.15
Limestone	7.92	7.81	1.29	1.10
Cornstarch	-	-	76.8	4.55
Mono calcium phosphate	0.66	0.64	2.32	1.62
Sodium bicarbonate	0.11	0.12	0.04	0.04
Sucrose	-	-	8.25	0.49
Cellulose	-	-	5.00	-
Potassium carbonate	-	-	1.77	0.08
Salt	0.30	0.30	0.38	0.38
Titanium dioxide	0.50	0.50	0.50	0.50
Methionine	0.07	0.05	-	-
Choline chloride, 60%	0.41	0.01	-	-
Vitamin-trace mineral premix[1]	1.00	1.00	1.00	1.00
Calculated Provisions				
AME, kcal/kg	2800	2,846	2,872	3,471
Crude protein, %	16.78	20.0	0.00	20.0
Calcium, %	2.89	2.89	0.88	0.90
Available phosphorus, %	0.44	0.38	0.43	0.43

[1]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.

Table 4.3. Analyzed chemical composition of experimental diets, as fed basis

	Broiler breeder assay		Broiler chick assay	
	Control	linPRO[2]	NFD[1]	linPRO[2]
Dry matter, %	90.0	92.4	92.8	91.8
Organic matter, %	77.7	79.7	87.6	84.3
Crude protein, %	15.5	19.2	0.34	26.9
Gross energy, kcal/kg	3,644	4,997	3,529	4,685
Neutral detergent fiber, %	11.5	11.5	-	23.1
Crude fat, %	3.15	6.42	3.82	15.6
Ash, %	13.7	12.7	5.20	7.50
Starch, %	39.2	32.1	-	-
Indispensable amino acids, %				
His	0.75	0.41	0.01	0.51
Ile	1.18	0.64	0.04	0.91
Leu	2.11	1.23	0.10	1.42
Lys	1.29	0.81	0.08	1.25
Phe	1.58	0.85	0.07	1.06
Thr	0.96	0.55	0.01	0.84
Val	1.25	0.72	0.09	1.05
Dispensable amino acids, %				
Ala	1.27	0.75	0.02	0.96
Asp	2.65	0.16	0.02	2.15
Glu	6.19	3.80	0.84	5.01
Gly	1.43	0.74	0.02	1.21
Pro	1.78	0.11	0.02	0.88
Ser	1.38	0.79	0.02	1.10

[1]Nitrogen free diet

[2]linPRO diets with or without enzyme

Table 4.4. Apparent ileal amino acid digestibility (**AID**, %) in linPRO fed to broiler breeder hens (Exp. 1) and broiler chicks (Exp. 2) with or without fiber degrading enzyme[1]

	Assay			FDE			Assay*FDE					P Values		
							Broiler breeder assay		Broiler chick assay					
	Breeders	Broilers	SEM	-	+	SEM	-	+	-	+	SEM	Age	FDE	Assay*FDE
Indispensable amino acids														
His	88.3[a]	74.7[b]	1.44	81.8	80.9	1.37	88.3	88.2	75.3	73.6	2.04	<0.001	0.650	0.682
Ile	83.9[a]	55.4[b]	1.46	68.4	70.9	1.72	53.0	57.7	83.8	84.0	2.52	<0.001	0.309	3.840
Leu	85.6[a]	66.5[b]	1.46	77.0	75.1	1.55	85.7	85.5	68.3	64.6	2.31	<0.001	0.392	0.415
Lys	89.2[a]	70.0[b]	1.38	80.4	78.8	1.47	89.1	89.2	71.7	68.4	2.19	<0.001	0.441	0.405
Phe	86.3[a]	61.3[b]	1.67	73.0	72.3	1.58	86.5	86.1	64.0	58.5	2.35	<0.001	0.192	0.255
Thr	80.8[a]	67.4[b]	1.73	74.4	73.8	1.64	80.7	80.9	68.1	66.7	2.44	<0.001	0.797	0.720
Val	83.6[a]	59.9[b]	1.73	72.7	70.8	1.64	83.6	83.6	61.8	58.1	2.45	<0.001	0.429	0.442
Dispensable amino acids														
Ala	86.5[a]	74.3[b]	1.41	80.9	80.4	1.34	86.4	86.5	75.4	74.3	2.00	<0.001	0.809	0.766
Asp	84.5[a]	76.7[b]	1.44	81.4	79.8	1.37	84.4	84.5	78.5	75.1	2.05	<0.001	0.406	0.377
Glu	79.9[a]	50.2[b]	1.85	67.5[a]	62.5[b]	1.76	80.2	79.6	54.9	45.5	2.71	<0.001	0.049	0.081
Gly	88.6[a]	75.6[b]	2.37	82.9	81.2	2.25	88.6	88.5	77.3	73.9	3.35	<0.001	0.599	0.607
Pro	88.0[a]	67.8[b]	1.57	78.4	77.3	1.48	88.1	87.9	68.8	66.7	2.21	<0.001	0.594	0.669
Ser	88.0[a]	71.9[b]	1.53	80.2	79.7	1.45	87.8	88.2	72.5	71.2	2.16	<0.001	0.816	0.689
Tyr	87.2[a]	56.5[b]	1.96	72.5	71.2	1.86	87.3	87.1	57.6	55.3	2.77	<0.001	0.629	0.702

[1] Values considered signficantly different were letters are different at $P<0.05$

Table 4.5. Standardized ileal amino acid digestibility (**SID**, %)[1] in linPRO fed to broiler breeder hens (Exp. 1) and broiler chicks (Exp. 2) with or without fiber degrading enzymes

Item	Assay			FDE			Assay*FDE					Probabilities		
							Broiler breeder assay		Broiler chick assay					
	Breeders	Broilers	SEM	-	+	SEM	-	+	-	+	SEM	Assay	FDE	Assay*FDE
Indispensable amino acids														
His	91.9a	76.5b	1.44	84.7	83.8	1.94	91.9a	91.9a	77.4b	75.7b	2.04	0.028	0.507	0.989
Ile	95.2a	63.1a	1.81	80.3	78.0	1.72	95.1a	95.3a	65.5b	60.8b	2.29	<.001	0.355	0.316
Leu	91.8a	70.7b	1.64	82.2	80.3	1.55	91.8a	91.8a	72.5b	68.8b	2.32	<.001	0.392	0.409
Lys	96.4a	73.4b	1.64	85.7	84.1	1.64	96.2a	96.6a	75.1b	71.7b	2.31	<.001	0.515	0.419
Phe	97.3a	67.9b	1.69	84.1	81.1	1.60	97.5a	97.0a	70.7b	65.2b	2.39	<.001	0.198	0.263
Thr	88.6a	72.8b	2.33	80.9	80.5	1.65	88.3a	89.0a	73.5b	72.1b	2.46	<.001	0.873	0.652
Val	91.6a	64.9b	1.74	79.1	77.4	1.65	91.5a	91.7a	66.7b	63.1b	2.46	<.001	0.465	0.412
Dispensable amino acids														
Ala	91.6a	77.7b	1.42	84.9	84.5	1.35	91.5a	91.7a	78.3b	77.2b	2.00	<.001	0.84	0.739
Asp	87.6a	78.8b	1.45	83.9	82.4	1.38	87.4a	87.7a	80.5b	77.1b	2.05	<.001	0.462	0.36
Glu	90.3a	59.3b	1.88	77.3	72.3	1.79	90.5a	90.0a	64.0b	54.6b	2.74	<.001	0.054	0.081
Gly	92.2a	77.6b	2.44	87.7	84.1	2.38	92.2a	92.2a	79.3b	5.9b	3.46	<.001	0.623	0.622
Pro	90.3a	69.8b	1.57	80.6	79.5	1.49	90.4a	90.2a	70.8b	68.7b	2.22	<.001	0.607	0.657
Ser	93.7a	75.5b	1.56	84.8	84.4	1.51	93.5a	94.0a	76.1b	74.8b	2.20	<.001	0.844	0.677
Tyr	92.9a	61.0b	1.97	77.6	76.3	1.87	93.0a	92.8a	62.1b	59.8b	2.78	<.001	0.649	0.683

[1]Calculated by correcting values for apparent digestibility (AID, Table 3.4) for basal endogenous losses from birds fed N-free diets in Exp.2 : 10.5, 66.9, 57.1, 40.3, 67.6, 44.5, 50.2, 27.1, 41.4, 40.8, 23.7, 17.0, 38.9, and 27.7 mg/kg DM intake for His, Ile, Leu, Lys, Phe, Thr, Val, Ala, Asp, Glu, Gly, Pro, Ser and Tyr, respectively.

Table 4.6: Apparent retention of components in broiler breeder hens fed corn-soybean meal basal or basal diet containing linPRO with or without fiber degrading enzymes[2]

		linPRO[2]			
Item	Control	-	+	SEM	P-Value
Retention, %					
Dry matter	67.9a	56.5b	54.1b	2.83	<0.01
Organic matter	77.7a	66.6b	64.1b	2.13	<0.01
Crude protein	41.6a	18.4b	15.8b	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
Flow g/kg					
NDF	91.07b	117.7a	129.8a	8.04	0.007

[2] linPRO diets fed with or without fibre degrading enzyme composite. Supplied 1,200 U of xylanase, 150 U of β-glucanase, 500 U of cellulase and 5,000 U of protease per kg of feed.

Table 4.7. Apparent metabolizable energy (AME) and AMEn in linPRO fed to broiler breeder hens (Exp. 1) and broiler chicks (Exp. 2) with or without fiber degrading enzymes[1]

	Gross energy	Apparent metabolizable energy, AME	Apparent metabolizable energy corrected for N, AMEn
Assay			
Breeders	79.1a	4343.0a	4011.6a
Broilers	45.5b	2494.3b	2105.4b
SEM	1.13	62.05	53.2
FDE			
-	62.7	3438.3	3076.0
+	61.9	3399.0	3041.1
SE	1.65	59.09	50.69
Assay*FDE			
Breeder -	79.2a	4346.1a	4015.5a
Breeder +	79.1a	4339.8a	4007.7a
Broiler -	46.1b	2530.4b	2136.4b
Broiler +	44.8b	2458.2b	2074.4b
SE	1.65	90.63	77.76
P values			
Assay	<.0001	<.0001	<.0001
FDE	0.636	0.636	0.623
Assay*Enzyme	0.690	0.690	0.703

[1]supplied 1,200 U of xylanase, 150 U of β-glucanase, 500 U of cellulase and 5,000 U of protease per kg of feed.

Chapter 5: Effects of feeding linPRO with or without fiber degrading enzymes on egg yolk fatty acids composition and liver fat content in broiler breeders

ABSTRACT: The objective of this study was to determine egg yolk FA composition and liver weight in BB fed linPRO. Diets and feeding procedures are described in chapter 4. Eggs were collected from d 28-30 consecutively, and yolks were separated, weighed, pooled by cage, and frozen until analysis. All birds were killed on d 30, and livers removed, weighed, and freeze dried. Frozen yolks were lyophilized to determine dry yolk weight. Dried yolk samples were hydrolyzed with methanol, and FA concentrations were analyzed using gas chromatography. Freeze dried livers were analyzed for total crude fat content. Dried liver and yolk weights as well as liver fat and total yolk FA were not influenced by treatments ($P > 0.05$). Birds fed the control diet had lower concentration of α-linolenic acid (C18:3 ω-3) compared with birds fed linPRO- and + ($P < 0.001$). The concentration of C18:3 ω-3 was 7.5, 36.8 and 37.3 mg/g dried yolk in the control, linPRO- and linPRO+, respectively. Furthermore, birds fed control diet displayed lower concentrations of docosahexaenoic acid (C22:6 ω-3) than birds fed linPRO diets ($P < 0.001$). The concentration of C22:6 ω-3 was 4.1, 9.1 and 8.5 mg/g of dried yolk in control, linPRO- and linPRO+, respectively, indicating higher conversion of C18:3 ω-3 to C22:6 ω-3 in birds fed linPRO. The omega 6:3 ratio in the control yolks was higher than birds fed linPRO ($P < 0.001$). The study indicated that feed-restricted BB enrich egg yolks with omega-3 FA from flaxseed products without adverse effects on the liver. Supplementation with FDE did not affect the deposition of fatty acids in hens fed linPRO.

Introduction

Flaxseed products have been proposed as a potential strategy to enrich broiler breeder (**BB**) eggs with omega-3 fatty acid to benefit progeny. By feeding laying hens diets with flaxseed,

producers can manipulate the fatty acid profile of eggs. (Aymond and Van Elswyk (1995) reported ALA levels of 212 mg/egg *vs.* 13 mg/mg in diets containing 15% flaxseed compared to control diets, respectively. Lewis et al. (2000) reported similar numbers in laying hens fed diets containing flaxseeds *vs* control diets with ALA levels of 277 mg/egg and 17 mg/egg respectively. While the ability to deposit high levels of *ω-3 FA* in laying hens fed flaxseed products has been extensively reported, there is minimal research in broiler breeders.

Layers have been intensively selected for egg production, broiler breeders have been selected for meat yield and growth. Selection programs for broiler chicken parent stock gives a lot of consideration on increased weight gain. However, the interaction between nutritional and reproductive traits is complex and continually changing with the introduction of new genetics (Hocking et al., 2002; van Krimpen and de Jong, 2014). It is necessary to provide enough, but not excessive nutrient intake for maximum egg production (Zuidhof et al., 2017). In this context, broiler breeders are restricted fed in order to maintain chick quality and prevent reproductive issues which may stem from overweight birds. This feeding management practice may affect the quantity of fatty acids that are deposited into the egg yolk and subsequently available for the embryo. Therefore, it is essential to quantify whether broiler breeders can deposit sufficient fatty acids (FA) into the egg yolk despite limited access to feed.

More than 90% of the fatty acids synthesized in birds occurs in the liver, in contrast to mammals whose adipose tissue is responsible for most of the fatty acid synthesis (Squires and Leeson, 1988). Indeed, the proportion of fat in livers can exceed 40% in laying hens due to their inability to export lipoprotein to adipose and other tissues (Squires and Leeson, 1988). For this reason, chickens, particularly laying birds are at high risk for developing fatty liver disease (Bain et al., 2016; Rozenboim et al., 2016; Robinson and Kiarie, 2019). Therefore, proper feed

management is critical for liver health maintenance in laying hens (Bain et al., 2016). The lipid components of egg yolks are synthesized in the liver and then transported into the ovaries by lipoproteins, specifically very low-density lipoproteins (**VLDL**) (Squires and Leeson, 1988). At the onset of egg production, hepatic lipoproteins shift from generic VLDL to yolk targeted VLDL and act as the sole method of lipid transfer into the yolk. As the only lipogenic tissue in the breeder, the effects of a fat rich linPRO

The objective of this study was to determine egg yolk FA composition and liver weight and crude fat in BB fed a dry-extruded flaxseed product linPRO, a mixture of full-fat flaxseed and ground pulses (1:1 wt/wt). A secondary objective was to investigate the efficacy of fiber degrading enzymes (FDE) on utilization of fatty acids in linPRO.

Materials and methods

Details on birds, diets and experimental procedures are described in chapter 4, n=10. Eggs were collected from d 28-30 consecutively, and yolks were separated, weighed, pooled by cage, and frozen (- 20°C) until analysis. All birds were weighed and killed on d 30, and livers removed, weighed, and freeze dried.

Laboratory analyses

Frozen yolks were lyophilized to determine dry yolk weight. Dried yolk samples (0.5g) were extracted for fatty acid determination using methods described by O'Fallon et al. (2007) and Nelson et al. (2008). Freeze dried yolk samples were aliquoted in 16 x 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 afterwards, 3.0 ml of hexane were added prior to 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℃ 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 x 0.25 mm x 0.25 um 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℃ while flame ionization detector (FID) temperature was 280℃. Initial oven temperature was 150℃ which was held for 1 min, then increased to 180℃ at a rate of 10℃/min, from 180˚C to 200˚C at 2℃/min and from 200℃ to 240℃ at 1℃/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 (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).

Calculation and statistical analyses

Liver weight was expressed on live broiler breeder weight prior to sacrifice whereas fatty acid values in egg yolk were expressed as a function of egg yolk weight. The cage was the experimental unit. The data was subjected to one-way ANOVA using GLIMMX procedures of SAS with the diet was the fixed effect. Treatment differences were considered significant at $P < 0.05$.

Results and discussion

Yolk weight, liver weight and crude fat content

The dry yolk weight was not (P=0.52) affected by diets, the values were 33.7, 30.0 and 27.1 g for the control, linPRO- and linPRO+ diets, respectively (Table 5.1). There was no (P=0.

51) treatment differences in liver weight (Table 5.1). Fat content in livers of birds fed the control diet was higher (P <0.001) than in birds fed linPRO and the values were 29.50, 16.49, 17.40% for control, linPRO- and linPRO+, respectively. 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., 2019). Although none of the treatments surpassed the threshold (>35% liver crude fat) required to be classified as fatty liver haemorrhagic syndrome (**FLHS**), the potential risk with feeding high fat ingredients is still a concern (Squires and Leeson, 1988). FLHS in both laying hens and broiler breeders leads to excessive lipid peroxidation in the liver causing the weakening and breakdown of lipid membranes by overwhelming antioxidant defence mechanisms, ultimately resulting in liver haemorrhaging (Wu and Squires, 1997; Gonzalez-Esquerra and Leeson, 2000). As noted in Table 4.3, the control diet had higher starch level than the linPRO based diets i.e. 39.2 and 32.1%, respectively. High levels of starch content in corn-soy-based diets have been shown to induce occurrences of FLHS in laying hens (Rozenboim et al., 2016). Furthermore, despite the higher fat content, linPRO diets also had higher level of crude protein compared with the control diet (Table 4.3). Studies have shown that the balance between energy, protein and fat is essential for reducing the occurrence of FLHS (Rozenboim et al., 2016). Results of the current study agree with the findings of Caston et al. (1994) and Schumann et al. (2000) who reported a reduction of hepatic fat content in birds fed ground flaxseed compared to a conventional corn-soy diet. Although the mechanism of reduction in FLHS is not understood Schumann et al., (2000) hypothesized that high intakes of ω-3 fatty acids may reduce the concentration of arachidonic acid by down regulating Δ6-desaturase activity. As arachidonic acid is positively correlated with thrombocyte aggregation, reduction may be beneficial. Dietary fat

influences the lipids found in the body, and therefore it is important to consider the fat content in the liver in relationship to the fat content of the yolk.

Egg yolk fatty acid enrichment

There was no treatment effect ($P>0.05$) in total fatty acid content in egg yolk (Table 5.2). 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 fatty acid profile of the yolk itself. 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 fatty acid composition were achieved through the manipulation of FA deposition. As expected, feeding linPRO significantly ($P<0.01$) increased the levels of ALA (38.8 mg/g) in the egg yolk compared with the control diet (7.5 mg/g) (Table 5.2). Due to the widely understood competition between ALA and LA for the same desaturase enzymes, FA deposition is directly related to the relative ratios of omega-6/omega-3 in the diet (Brenner, 1971; Neijat et al., 2016a). The reduced ratio between omega-6/omega-3 has been reported to enhance the deposition of ALA as well as reduce the efficiency of endogenous conversion of ALA to EPA (Neijat et al., 2016a; Neijat et al., 2016b) The omega-6/omega-3 ratio in the control diet was significantly higher than the ratio present in the linPRO diets (Table 4.3). In addition, omega-6/omega-3 ratios in the FA composition of the egg yolk was reflective of the diet composition, where the control egg yolks had a significantly higher omega-6/omega-3 ratio (Table 5.3). Furthermore, due to this reduced ratio and subsequent competition for enzymes, the conversion of ALA to DHA was significantly higher in birds fed the linPRO diets. 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).

Despite the presence of the enzyme, there was no significant effects of enzyme inclusion on fatty acid enrichment in egg yolk or liver responses (Tables 5.1 and 5.2).

Conclusion

Despite being restricted fed, broiler breeders are in fact able to manipulate and enrich the fatty acid profile of egg yolks from flaxseed-based diet. Increased levels of ALA and DHA were observed in birds fed diets containing linPRO. There was no effect of feeding linPRO on the total fat of the yolk and liver. Lastly, FDE had no effect on the enrichment of egg yolk fatty acid profile.

Table 5.1. Yolk and liver weights in broiler breeders fed control corn soybean meal diet or control plus linPRO with or without multi-carbohydrase supplement

		linPRO			
	Control	-	+	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
Weight (DM basis) (g)/g BW	16.9	17.9	17.2	0.57	0.510
Crude fat content, %	29.5a	16.5b	17.4b	0.52	<0.001

Table 5.2. Fatty acid concentration in egg yolk (mg/g of dry yolk) of broiler breeders fed control corn soybean meal diet or control plus linPRO with or without fiber degrading enzymes.

		linPRO			
	Control	-	+	SEM	*P* value
Fatty acid, mg/g					
Myristate (C14)	2.1a	1.6b	1.6b	0.05	<.001
Palmitate (C16)	155.7a	135.1b	134.4b	1.69	<.001
Palmitoleate (C16:1)	20.0a	14.1b	12.0b	1.94	<.001
Heptadecanoate (C17)	0.9a	1.1a	2.3a	1.02	0.342
Stearate (C18)	47.0a	44.6a	46.0a	1.22	0.176
Oleate (C18:1)	214.8a	203.6b	202.0b	4.16	0.001
Linoleic Acid (C18:2n-6)	84.0a	89.7a	89.5a	2.36	0.040
Alpha-Linolenic Acid (C18:3n-3)	7.5b	36.8a	37.3a	3.34	<.001
11-14 Eicosadienoate (C20:2)	0.06a	0.04a	0.03a	0.01	0.080
Homogamma Linolenate (C20:3)	0.1a	0.07b	0.06b	0.02	<.001
Arachidonic Acid (C20:4n-6)	9.4a	6.9b	6.5b	0.40	<.001
Docosatetraenoic (C22:4)	0.9a	0.6b	0.5b	0.08	<.001
Docosapentadenoic (C22:5)	1.1b	2.1a	1.7ab	0.25	0.002
Docosahexaenoic Acid (C22:6n-3)	4.1b	9.1a	8.5a	0.49	<.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	11.6b	45.9a	45.8a	2.62	<.001
Ratio Omega 6:3	14.1a	2.1b	2.1b	1.10	<.001
Omega 6 to Total %	0.2	0.2	0.2	0.003	0.240
Omega 3 to Total %	0.02b	0.08a	0.08a	0.005	<.001

Chapter 6. General discussion and conclusions

The poultry industry has made tremendous advances in genetic selection resulting in reduction of days to market. However, the decrease to market time translates into greater reliance on maternal nutrients and factors deposited in eggs in early stages of chick's life. One proposed nutritional strategy is ω-3 FA inclusion into maternal diets due to the role of yolk lipids and fatty acids during embryogenesis for energy, synthesis of polyunsaturated fatty acids (PUFA)-rich membrane phospholipids, and eicosanoids by the embryo (Cherian., 2011, Gonzalez., 2011). Flaxseed product inclusion into maternal diets may be a means to successfully supply ω-3 FA in the egg. Flaxseed is abundant in ω-3 FA - in fact they contain the highest content of PUFA (57.7%) relative to available oil (40%). However, availability of oil flaxseed may be impeded is by a fibrous matrix containing high levels of crystalline cellulose with tightly bound galactans – one of many ANF present in flaxseed (Wanasundara and Shahidi, 1997). Flaxseeds also have mucilage, an antinutritional factor known for increasing water retention of digesta leading to decrease nutrient digestibility (Rodríguez et al., 2001; Alzueta et al., 2003). Furthermore, the relatively high level of phytic acid, cyanogenic glycosides and linatine affects gut function negatively (Kiarie et al., 2015). Mechanical processing such as dry extrusion can force seed coat breakage, reduce fibre length – subsequently increasing nutrient digestibility. In order to maximize nutrient digestibility, dry extruded flaxseed can be paired with enzymes and incorporated into diets. Fiber degrading enzymes (FDE) accounts for the bulk of the poultry enzyme market and could to increase nutrient availability in flaxseed. Before these ingredients can be incorporated into breeder diets, accurate nutritive values must be determined. The differences in feeding schedules and maturity of the GIT could present differences in nutritive value of feedstuffs between broiler breeders and traditional

broiler chick assay. Lastly, as breeders are restricted fed it is important to considered whether ω-3 FA enrichment of the egg yolk is possible.

Therefore, the overall objective of this thesis was to determine utilization of a co-extruded mixture of flaxseed and pulses in broiler breeders. Study presented in chapter 4 determined the SID of AA and energy values (AMEn) in broiler breeders compared with values generated from a broiler chick assay. The product evaluated was supplied by O&T Farms in Saskatoon and is a dry co-extruded contained full fat flaxseed and ground pulses, linPRO (1:1 wt/wt). Fiber degrading enzymes were included in order to evaluate effect on increasing nutrient digestibility. In the broiler breeder assay, birds fed linPRO based diets retained significantly less dry matter, organic matter, crude fibre and crude protein than their control diet fed counterparts. There was no difference in AR of fat between birds fed the control diets and those fed linPRO diets. As previously stated, there are ANFs such as mucilage and high fibre present in flaxseed which is known to impede nutrient absorption and utilization.

There was no interaction ($P>0.05$) between assay method and FDE on AID (Table 4.4) and SID (Table 4.5) of linPRO amino acids. However, broiler breeders had higher ($P<0.05$) AID and SID of AA of linPRO than broiler chicks. Among indispensable AA, Thr (88.6%) was least digestible and Phe (97.3%) was most digestible in broiler breeders whereas Ile (63.1%) was the least digestible and His (76.5%) was most digestible in broiler chicks. The significant differences in SID and AID of AA implies that nutrient utilization is affected by both feeding schedules and digestive capacity. AME_n of linPRO for broiler breeders was significantly higher (4012 vs 2105 kcal/kg) than value determined for broiler chicks. As breeders have a longer and mature digestive tract it is possible that they can digest fibre and increase nutrient absorption. When compared to the reported values for layer hens, the determined AMEn values for broiler breeders was higher

(4012 vs 3664 kcal/kg) than reported values in laying hens. This is contrary to what has been previously reported in broiler breeders vs layers, generally it is expected that broiler breeders are approximately 2.5% less efficient in the absorption of nutrients (Buzala and Janicki., 2016). The current study used an 18% inclusion versus a 20% inclusion level in the reported layer study – the 2% difference flax could potentially influence the viscosity of the digesta enough to see a difference in energy utilization (O&T Farms, 2017).

There was no enzyme effect on nutrient digestibility which may be attributed to a multitude of factors. To begin, restricted fed birds have been reported to exhibit acute adaptive behaviours and increase endogenous activities of enzymes such as sucrase, amylase, lipase and trypsin (Pinheiro et al., 2004). This onset is then followed by morphological changes in intestine once restricted for an extended period of time as well as a selective modulation of enzymes dependent on the available substrates in the digesta. Therefore, broiler breeders may have already been digesting feedstuffs at their maximum potential. In broiler breeders, the restriction of feed may also limit the quantity of enzyme ingested in diets due to the limited amount of feed. Lastly, it is possible that the enzymes were not effective in reducing digesta viscosity, a major role of enzymes, resulting in impaired transport of digestive enzymes and nutrients.

Chapter 5 presents the effects of feeding linPRO on egg yolk fatty acid profile and the liver health. There was no significant difference in dry egg yolk weight or total fatty acids ($P>0.05$), implying that the differences in fatty acid profile was influenced by dietary treatment. linPRO significantly increased the levels of ALA in the egg yolk compared with birds fed the control diet. Furthermore, the conversion of ALA to DHA was significantly higher in birds fed linPRO diets. These finding imply that not only is it possible to enrich the eggs of restricted fed broiler breeders, but that the bird is also able to convert ALA to the biologically usable DHA. Lastly, liver weight

and fat content were evaluated as an indices of liver health; the liver of birds fed linPRO diets had lower crude fat levels ($P<0.001$) than birds fed control diets. As fatty liver is a concern in laying hen, these results were considered positive. Enzymes had no effect on egg yolk enrichments.

The SID of AA and AMEn values can be used to formulate accurate diets for broiler breeders. These diets can then be fed to broiler breeders in order to enrich eggs, providing FA for the developing embryo and subsequent effects on progeny growth performance and resilience to production environment stresses. Indeed, the use of ω-3 enriched maternal diets may result in more robust progeny and provide a supplementary solution to the antibiotic free movement.

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