Influence of Dietary Fat Inclusion on the Ileal Digestibility of Amino Acids in Broilers

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List of Contents

List of Abbreviations:

AA: Amino acid(s)

CAD: Coefficient of apparent digestibility

DM: Dry matter

EL: Endogenous Loss(es)

GE: Gross energy

NSP: Non-starch polysaccharide(s)

List of Tables

Chapter 1: General introduction

The inclusion of fats in broiler diets has long been an important aspect of proper poultry diet formulation. Fat is included largely as a source of metabolizable energy and to increase palatability (Baião and Lara, 2005). Previous studies, have indicated other benefits may be realized through the inclusion of dietary fat in poultry diets, such as decreases in endogenous amino acid losses and increases (Li et al., 1994; Allahyari-Bake and Jahanian, 2017).In broiler diets, ingredient sources of fat are often chosen based off of economic and availability factors. Compositionally, fats from differing sources will differ, and have a variety of effects and these fats may have differing impacts on these possible effects on endogenous amino acid losses and amino acid absorption. Dietary fats can often be classified into animal and plant fats, both of which often show significant compositional differences.

Understanding these effects could have major impact in industry production. Decreased protein utilization in broilers represents decreased growth rates and less efficient feed utilization. Decreased efficiency can lead to increased feed costs, increased growth times and increased costs due to increased feed requirements. By possibly increasing protein utilization through fat inclusion, decreased industry costs and decreased food waste could be realized, having positive economic and environmental impacts.

The present study was divided into two parts. The first part looked to establish a connection between increased dietary fat and endogenous amino acid losses in broilers, as well as a elucidate a difference between soybean oil (plant fat) and beef tallow(animal fat) and how these different fats impact the rate of endogenous amino acid losses.

The second part involves studying the effect that soybean oil and beef tallow have on nutrient digestibility in the intestine, specifically amino acid absorption at the ileal level. The possible interaction effects of dietary grain sources (maize and wheat) was also taken into account.

Chapter 2: Literature review

2.1 Introduction

While fats and oils have been included in broiler diet formulations for years, their primary purpose for inclusion has been as a source of energy and to increase palatability of the feed. However, the influence of dietary fats on aspects of digestion has not been studied in sufficient depth.

There have been studies illustrating a link between dietary fat and nutrient digestibility in general (Allahyari-Bake and Jahanian, 2017; Merriman et al., 2016) and on amino acid absorption specifically (Imbeah and Sauer, 1991). At the current time, fat inclusion level is mainly governed by economic availability of specific fat sources and the nutrient density of the feed. Evidence does, however, seem to point to specific characteristics of the added fat being an important determinant of this effect (Thacker et al., 1994; Zollitsch, et al., 1997).

The objectives of this literature review are to:

i) Present an overview of important characteristics of different dietary fat sources

ii) Examine the factors affecting endogenous losses of amino acids, and

iii) Evaluate the link between dietary fat and amino acid digestibility

2.2 Fats in broiler diets

Table 2.1: Fatty acid composition of common dietary fats

	Soybean oil	Canola oil	Palm oil	Coconut oil	Fish (tuna) oil	Poultry oil	Beef tallow
Mono-Unsaturated FA %	28.5	59.5	22.5	.5	23.3	45.5	40.5
Poly-Unsaturated FA %	57.5	30.7	1.25	8.8	49.4	23.5	3
Saturated FA %	13.5	9.6	76	90.5	27.2	30.5	56
Type	Vegetable	Vegetable	Vegetable	Vegetable	Animal	Animal	Animal

Sources: Kostik et al., (2013); Suseno et al., (2014); Woolgate and van der Veen (2014)

The term “fat” is generally used to describe any dietary lipid and can be described in the most common categories of triglycerides, sterols and phospholipids (Wiseman, 1984). Lipids are non-polar organic molecules that are notably water insoluble (Baião and Lara, 2005). Triglycerides are the most relevant fat in animal nutrition, as they make up the majority of the composition of dietary fats (Baião and Lara, 2005). Triglycerides consist of three fatty acids esterified with glycerol. These triglycerides are broken down into free fatty acids to be absorbed and utilized by the bird (Wiseman, 1984). Individual fatty acids are identified based on a number of factors: saturation, the number of hydrogen ions bound to the fatty acid chain, chain length, which also determines the number of double bonds and shape of the fatty acid, and point of attachment in the triglyceride. All of these characteristics play a role in how a triglyceride is digested and absorbed by the bird.

Dietary fats are an important component of poultry diets, used as a source of metabolizable energy and to increase feed palatability (Baião and Lara, 2005). Compositionally, a number of different fat sources can be used. When deciding which source to use, nutritional considerations must be balanced with economic ones. Common sources of fat include soybean oil, tallow, fish oil, and canola oil (Baião and Lara, 2005). These fats are often described in terms of whether they are sourced from animal or plant sources (Baião and Lara, 2005). Compositionally, plant fats tend to contain higher amounts of unsaturated fatty acids and longer chain fatty acids (Orsavova et al., 2015). Animal fats tend to contain more saturated fatty acids and short-chain fatty acids (Orsavova et al., 2015). These observations are not universally true. As Table 2.1 illustrates, fatty acid composition can still vary between different plant and animal fats.

Dietary fats are included in broiler diets predominantly as a source of dietary energy, and for palatability (Baião and Lara, 2005). Lipids are also an important nutrient involved in the maintenance of cellular structures (Baião and Lara, 2005). In practice, dietary fats are primarily chosen as a feed ingredient based on cost and availability. Geography, economics and availability play a huge role in ingredient selection (Baião and Lara, 2005). However, specific characteristics of the fat added can affect how the fat is utilized, such as degree of saturation of constituent fatty acids, chain length and molecular positioning (Renner and Hill, 1961; Baião and Lara, 2005)

Digestion of fats is governed by pancreatic lipase (Wiseman, 1984) which hydrolyze triglycerides into free fatty acids. Other pancreatic secretions also aid in the fat digestion process (Wiseman, 1984). It has been observed that younger birds produce less of these pancreatic secretions, lowering their efficiency of fat absorption (Jimenez-Moreno et al., 2009). The duodenum is the primary site of excretion from the pancreas (Wiseman, 1984, Baião and Lara, 2005; Erdaw et al., 2017). The resultant monoglycerides, triglycerides and free fatty acids conjugate with released bile salts, forming micelles (Wiseman, 1984; Baião and Lara, 2005), which are able to be absorbed by intestinal villi. The majority of fat absorption in chickens takes place in the proximal small intestine, specifically the lower jejunum and to a lesser extent the upper ileum (Renner, 1965). Absorption does also occur in the upper jejunum and lower ileum, but at even lower rates (Renner, 1965; Rodríguez-Sánchez et al., 2017).

Fatty acid saturation is one characteristic of dietary fats that is important in poultry nutrition. In terms of absorbability, broilers have been shown to more efficiently absorb and digest unsaturated fatty acids over saturated fatty acids (Ward and Marquardt, 1983; Allahyari-Bake and Jahanian, 2017). The disparity does decrease with age, with older birds better able to digest saturated fats than younger birds (Tancharoenrat et al., 2015). This superior digestibility of unsaturated fatty acids is due to the fact that that unsaturated fatty acids have a greater ability to form micelles with bile salts, which is the primary mechanism for fat digestion (Baião and Lara, 2005). Previous studies have also shown that the presence of unsaturated fatty acids in a diet of mixed unsaturated and saturated fatty acids can actually improve the digestibility of saturated fatty acids by encouraging micelle formation (Renner and Hill, 1961; Li et al., 1990). Li et al. (1990) demonstrated that, in pigs, a combination of coconut oil and soybean oil improved feed conversion and increased absorption when compared to soybean oil or coconut oil alone. .

Fatty acid chain length also factors into the digestibility of fatty acids. It has been shown that short-chain fatty acids are more easily absorbed than long-chain fatty acids (Renner and Hill, 1960; Hurwitz et al., 1979; War, et al., 1983). Li et al. (1990) found that both coconut oil (short-chain, saturated fatty acids) and soybean oil (long-chain, unsaturated fatty acids) both had superior digestibility than grease (long-chain, saturated fatty acids). The decreased digestibility of long-chain fatty acids is thought to be due to their lower micelle-forming activity (Tancharoenrat et al., 2014).

Finally, the structural-positioning of the constituent fatty acids also affects digestibility. Renner and Hill (1961) found that palmitic acid with its point of attachment in -position 2 was more readily absorbed than monopalmitin. The same results did not, however, hold true for stearic acid, which was also tested (Renner and Hill, 1961). This observation may be related to the fact that pancreatic lipase hydrolyses triglycerides at positions 1 and 3, meaning that having a point of attachment at the position 1 would inhibit hydrolysis (Tancharoenrat et al., 2014; Ravindran et al., 2016). Fatty acids attached at position 2 are overall more readily absorbed (Vilà and Esteve-Garcia, 1996). Linoleic and oleic acids have been shown to be more readily digested than palmitic and stearic acid in their natural forms, suggesting more favorable composition and positioning for hydrolysis (Ward and Marquardt, 1983). Thus, fats with fatty acid configurations at position 2 are the most optimally digested fatty acids, as compared with those with fatty acids in positions 1 and 3.

2.3 Endogenous amino acid losses

Maintaining the proper amount and balance of amino acids is an important consideration in broiler feed formulation. Along with amino acids ingested in feed, endogenous proteins secreted into the digestive tract are also present in the digesta (Ravindran, 2016). These proteins, like the ones present in the feed, do cycle through the digestive system. Like feed proteins, some of these are re-absorbed, and some are not. This unabsorbed portion is lost to the animal and is known as endogenous losses. There are two types of endogenous losses inherent to the biology of the animal, namely basal losses, or non-specific losses, and specific losses. The basal losses are independent of the diet (Ravindran, 2016). Specific losses refer to endogenous losses caused by certain components present in the diet (Ravindran, 2016). These losses are governed by the presence and specific composition of anti-nutritional factors and dietary fiber, especially non-starch polysaccharides (NSPs). Understanding the relationship between NSPs and endogenous losses is an important part of maximizing dietary protein efficiency. This is because NSPs have been shown to have a major effect on the specific AA losses (Angkanaporn et al., 1994)

2.3.1 Measurement of basal endogenous losses

Measuring endogenous amino acid losses is important in order to understand the true digestive impact of certain diets and to calculate the true digestible amino acid content of an ingredient, rather than the apparent content (Zuber et al., 2016). At this point in time, extensive research has concluded that the ileum is the preferred segment of the digestive tract to measure these losses, due to the extensive bacterial fermentation that occurs in the hindgut

(Ravindran, 2016). A number of techniques have been developed to assess the basal endogenous losses after obtaining ileal digesta samples.

2.3.1.1 Protein-free diets

The most common and simple method is the use of a protein/nitrogen-free diet (Adeola et al., 2016). This technique involves feeding the animals a diet containing no protein and then taking an ileal digesta sample. Any amino acid detected in the digesta is assumed to be endogenous losses (Brestensky et al., 2017). This method is attractive because of its low cost and experimental investment (Adeola et al., 2016). Studies, however, have shown a number of flaws in this technique (Adeola et al., 2016; Brestensky et al., 2017). It is known that protein-starved animals will begin to break down muscular proteins in order to meet amino acid requirements (Brestensky et al., 2017). As a result, the endogenous amino acid losses observed are not truly reflective of those in normally-fed birds, which is the goal of the procedure in the first place (Brestensky et al., 2017). There have been various methods proposed to address this issue, while still maintaining accuracy when it comes to measuring endogenous losses. Feeding of highly-digestible proteins, as opposed to none at all, may be the best way of utilizing this method (Adeola et al., 2016; Brestensky et al., 2017). Casein is a common choice (Adeola et al., 2016). Brestensky, et al. (2017) concluded that the use of casein in the diet seemed to provide more accurate results in terms of endogenous loss measurement. It was, however, observed that casein per se may have increased EL of some amino acids due to its slower breakdown in the gut. These researchers also evaluated adding synthetic amino acids in the diet and still concluded that the casein diet resulted in more accurate results (Brestensky et al., 2017). The simplicity and convenience, as well as it being of lower costs than the previously-mentioned methods, makes this method a popular choice for estimating EL.

2.3.1.2 Regression method

The regression method is yet another way to estimate EL. In this method, multiple diets of increasing levels of dietary protein are fed, with none of the diets reaching zero protein, and ileal digesta samples are collected (Adeola, et al., 2016). These different treatments are then used to construct a regression equation, where the ileal amino acid contents can then be determined at zero dietary protein level (Ravindran, 2016). This method is thus able to overcome the aforementioned issues that occur when an animal is fed a protein-free diet. This diet has been demonstrated to have accuracy on par with a casein diet and more accurate results than a protein-free diet (Adeola et al., 2016). The protein-free diet method has also shown an increased secretion of proline and glycine that is not observed with the regression method (Adeola et al., 2016). The main issue with this method is the cost and time investments. Since multiple treatments are necessary to construct the regression equation (with more treatments leading to a more accurate equation), there are increased costs, increased labor and longer time necessary to obtain the results. These have limited the use of this method (Ravindran, 2016). Occasionally, these studies also report negative estimations for EL, which is not physiologically realistic. The fact that such obviously false results could occur, even occasionally, has led many to question its use (Ravindran, 2016). As a result of these, this method is much less popular than the protein-free method.

2.3.1.3 Fasting method

This method is the simplest, but also the most inaccurate. It involves fasting the birds for a period of 48 hours before taking an excreta or ileal sample. Aside from similar problems as with the protein-free diet method, this method has also been shown to underestimate EL (Adeola et al., 2016). This is because EL levels are related to dry matter intake. This method has the lowest cost and resource investment, but is not commonly used in modern experiments due to high inaccuracy and welfare issues, though this method was more common in past decades.

2.3.2 Sources of endogenous protein

Endogenous losses in the gastrointestinal tract originate from a number of sources. These sources include intestinal mucus secretions (Adedokun et al., 2011), digestive enzymes (Adeola et al., 2016), bile and epithelial cells (Tamminga et al., 1995). It is important to note that losses of some of these sources are greater than the others (Adedokun et al., 2011) and are relevant in understanding the process. The amino acids that appear most prominently in endogenous proteins are glutamic acid, aspartic acid, serine and threonine (Adedokun et al., 2011).

Mucin is a glycoprotein that is the main component of mucus, which coats the epithelial layer of the digestive tract (Adedokun et al., 2011). Since mucus is constantly secreted by the epithelial cells, a large amount of mucus will inevitably make its way into the digesta. Threonine in particular has been found to appear in high levels in ileal digesta, attributable to secreted mucin (Adedokun et al., 2011). Other amino acids attributable to mucin or epithelial cell secretions are serine, proline and cysteine (Tamminga et al., 1995).

Mucins are resistant to degradation, meaning that it is difficult for digestive enzymes to break it down and reabsorb it (Tamminga et al., 1995).

Along with mucus secretions, the secreting epithelial cells slough off into mucosal secretions, resulting in another avenue of loss. It is believed that the protein in these epithelial cells is more easily digested, resulting in less loss than from mucus secretion (Tamminga et al., 1995).

Within the pre-ileal digestive tract, a number of enzymes and secretions are released at different segments. Pre-pancreatic secretions that add to EL include saliva (in mammals), gastric juices and bile (Tamminga et al., 1995).

There are also a number of enzymes secreted by the pancreas that enter the small intestine. These enzymes include proteases, lipases and amylases (Tamminga et al., 1995). The amount of lipase secreted by the pancreas is dependent on the dietary fat content (Tamminga et al., 1995), while amylase secretion is largely governed by the starch content (Tamminga et al., 1995). In general, the composition of the diet has a large impact on the composition and relative amount of secreted digestive enzymes (Adedokun et al., 2011). Conceivably, all enzymes secreted in order to facilitate digestion have the potential to be lost as EL, as there is not 100% efficiency when it comes to reabsorption of these proteins (Tamminga et al., 1995). Despite this, enzymatic and digestive secretions are generally more easily reabsorbed than mucosal and epithelial cell secretions (Ravindran, 2016)

2.3.3 Effects of feed components

As previously mentioned, the nature of digestive and enzymatic secretions is heavily related to the nutritional composition of the diet. Beyond this, however, there are also a number of substances present in feed that can have inhibitory effects or cause negative digestive reactions that increase EL.

2.3.3.1 Non-starch polysaccharides

Indigestible dietary fiber, also known as non-starch polysaccharides (NSPs), are the main substances related to this. It has been observed that increased cellulose content (cellulose is one example of a NSP) increases EL (Adedokun et al., 2011). It should be noted that, while the amount of losses increase, the amino acid profile of endogenous protein is relatively unaffected (Adedokun et al., 2011; Ravindran, 2016). NSPs are a wide range of substances, with a wide range of effects. The exact composition of a grain in regards to NSPs, as well as the concentration of NSPs, can influence EL. These substances either affect digestive secretions or mucin secretion (Cowieson and Ravindran, 2007). NSPs have also been observed to reduce lipid digestion (Smits and Annison, 1996), which is believed to be related to digestive viscosity changes brought on by increased soluble NSPs in the feed.

Despite general trends, not all NSPs have identical effects (Zuber et al., 2016). The individual structure of each NSP is still the ultimate determinant of how it will affect digestion, and in some cases NSP presence was not correlated with decreased growth or increased EL (Angkanaporn et al., 1994; Zuber et al., 2016). Another factor that can address these discrepancies is interactions between NSPs themselves (Zuber et al., 2016; Maurer et al. 2017).

Arabinoxylan is a polymer of arabinose and xylose classified as a hemicellulose. It is also a type of pentosan, with arabinose and xylose being two pentose sugars. These NSPs are found in wheat, as well as related grains like barley and rye (Angkanaporn et al., 1994). Like other NSPs, the presence of pentosans in the diet depress growth rate The mechanisms of action for these effects seem to be due to a combination of an increase in EL and a reduction in protein utilization (Angkanaporn et al., 1994). A study by Angkanaporn, et al. (1994) found an across-the-board increase in endogenous ileal amino acid secretions as pentosans obtained from wheat were supplemented into broiler diets (Angkanaporn et al., 1994). However, the same effects were not observed with the supplementation of cellulose or polyethylene, indicating that not all NSPs cause an increase in EL (Angkanaporn et al., 1994).

ß-glucans are another NSP common in wheat, barley, oats and rye (Angkanaporn et al., 1994). ß-glucans have been shown to decreases daily weight gain in chickens, as well as altering digestive morphology and microbiome composition (Kazempour et al., 2017). These changes were speculated to increases losses associated with mucin secretion and epithelial cell shedding (Kazempour et al., 2017). In order to evaluate the specific effects of different NSPs and indigestible starches, Morel et al. (2005) conducted a study on weaner pigs. The pigs were fed diets either containing two levels of ß-glucan or two levels of indigestible starch, with all diets also containing cellulose. The results indicated that, while the resistant starch had no measurable effect on EL, ß-glucan increased endogenous losses, with EL increasing as the concentration of ß-glucan increased. The ß-glucan also seemed to increase losses associated with both mucin secretion and endogenous enzyme production. Taken together, ß-glucans have a negative impact on amino acid absorption, and increase endogenous losses when present in the diet.

2.3.3.2 Phytate

Phytate, also known as phytic acid, is widely distributed in the plants and is the major storage form of phosphorus in seeds. Phytate has the capacity to form complexes with proteins, including digestive enzymes, and it is thought that the main mechanism for its anti-nutritive effects is this tendency (Adedokun et al., 2011). Phytate is also observed to increase the flow of digesta (Cowieson and Ravindran, 2007); however, this increase in flow rate was only observed in specific amino acids. Phytate has a particular tendency to form protein complexes (Cowieson and Ravindran, 2007). Cowieson and Ravindran (2007) found that endogenous proteins seemed to lack methionine and that methionine is largely unaffected by phytate. It is thought that this complex-forming behavior causes the pancreas to respond by increasing the secretion of pepsin and other digestive enzymes (Cowieson and Ravindran, 2007). This is because these complexes trap amino acids, and make them resistant to digestion (Smits and Annison, 1996). The fact that it thus takes larger volumes of digestive enzymes to complete digestion in the presence of phytate means a large amount of EL in the form of over-secreted enzymes.

NSPs should also be noted to have complex interactions with dietary fat (Smits and Annison, 1996). In the context of the current review, these interactions are important to consider along with the normal effect of phytate on endogenous amino acid losses. Phytate is known to entrap bile salts and reduce fat solubility (Smits and Annison, 1996). This effect can increase the digesta viscosity. How these observations relate to dietary fat sources or levels in feed is unknown, but it is possible that there is some sort of interaction.

2.3.3.3 Fats

Dietary fat, while feed component in its own right, can also affect the digestion and absorption of other feed components. According to Jiménez-Moreno et al. (2009), inclusion of fat in poultry diets increases overall dry matter absorption and energy utilization in the gut. Jiménez-Moreno et al. (2009) found that unsaturated fatty acids in the diet lead to better overall increases in dry matter digestion than saturated acids, speculated to be due to the fact that chickens could more readily digest unsaturated fats.

Merriman, et al. (2016) conducted a study to elucidate the relationship between different sources of dietary fat and EL in pigs. The results indicated that the dietary fat increased the digestibility of a number of minerals, noting different results with fat of differing composition, with animal fats showing less increases in mineral digestibility. It was speculated that this effect may be due to increased gut retention. The observed effects would also allow for increased re-uptake of proteins and amino acids of endogenous origin in the small intestine.

The presence of dietary fats is overall thought to improve dry matter digestibility of a number of nutrients, but this effect is modulated by the source of the fat (Jiménez-Moreno et al., 2009) and presence of NSPs (Smits and Annison, 1996).

2.4 Measurement of amino acid digestibility

Measurements of digestibility can be either made at the ileum or over the total tract. . While historically excreta were used to measure digestive efficiency, ileal sampling is now regarded as more accurate in pigs (Sauer and Ozimek, 1986) and has also become the standard method of measurement in poultry (Adedokun et al., 2011). In pigs, studies have shown that the majority of protein digestion occurs in the small intestine (Sauer and Ozimek, 1986) and that the large intestine is largely the site of microbial fermentation, which does not significantly contribute to nutrient digestion and feed efficiency (Sauer and Ozimek, 1986). As a result of this, analyzing undigested protein in excreta does not present a clear picture of the exact nutrients that the animal has utilized, rather it also includes by products after microbial fermentation in the hindgut (Sauer and Ozimek, 1986). These considerations make ileal sampling the most effective way to determine amino acid digestibility.

2.5 Influence of fats on amino acid digestibility

While a large aspect of amino acid digestibility is related to digestive physiology, dietary composition also plays a role. Measuring the amount of amino acid utilized is a necessary process for maximizing dietary efficiency and maximizing profits in a production setting. The presence, or lack, of certain ingredients can have a measurable effect on the digestion and retention of other feed ingredients. Diet formulation is not conducted in a vacuum, but rather by balancing the effects some ingredients might have on the digestion of others. In the context of dietary fat, the level and composition can affect the digestibility of other nutrients, including proteins.

A number of studies have been performed in monogastric animals that have shown a link between the addition of dietary fat and amino acid digestibility.

Imbeah and Sauer (1991) fed pigs a maize-soy diet with diets supplemented at three levels of canola oil at 20, 60 and 100 g/kg diet as compared to a maize-canola meal diet with the same levels of canola oil additions. The results showed an increase in amino acid digestibilities for each level of canola oil. The effect was much more pronounced in the 20 g/kg canola oil diet, compared to the 100 g/kg diet (Table 2.2).

Table 2.2: Effect of dietary fat sources of amino acid digestibility

Amino Acid	Soybean meal diet			Canola meal diet		
	2% canola oil	6% canola oil	10% canola oil	2% canola oil	6% canola oil	10% canola oil
Arginine	90.2^{d}	90.4^{d}	91.7^{c}	84.5^{c}	82.3^{d}	85.8^{c}
Histidine	85.9^{d}	87.4^{cd}	87.9^{c}	80.0^{d}	79.8^{d}	83.4^{c}
Isoleucine	83.7	87.2	85.2	74.1^{c}	70.2^{d}	75.8^{c}
Leucine	83.2	83.9	85.5	76.8^{c}	73.5^{d}	78.7^{c}
Lysine	83.6^{d}	84.7^{cd}	85.9^{c}	74.9^{e}	71.0^{d}	76.9^{c}

Phenylalanine	84.2^{c}	84.8^{cd}	86.4^{c}	76.6^{cd}	74.2^{d}	78.5^{c}
Threonine	69.7^{d}	71.8^{d}	74.6^{c}	64.4^{c}	60.1^{d}	66.6^{c}
Valine	79.6^{d}	80.2^{d}	82.7^{c}	71.6^{c}	68.8^{d}	73.6^{c}

Source: Imbeah and Sauer (1991)

This study also measured the influence of fats on the rate of passage. It was observed, contrary to the speculation, that increased fat content do not affect the rate of passage of digesta. It was noted that the canola meal diets passed slower than soybean meal diets and this was attributed to the higher NSP content of the canola diets. These results indicate that if fat does increases viscosity, the viscous digesta does not behave in the same way as digesta that is viscous due to high NSP content (Imbeah and Sauer, 1991).

In a follow up study, Li and Sauer (1994) conducted a trial similar to the previous trial, but with the addition of four levels of 3.2, 6.2, 9.2 and 12.2 g/kg canola oil in a maize-soy diet. Results showed a linear increase in the digestibility of all amino acids, except methionine, cysteine and serine, with increasing canola oil inclusions. These results largely confirmed those of Imbeah and Sauer (1991), with the authors speculating a delay in gastric emptying may be the cause of this effect (Table 2.3). It is unknown if effects on a chicken gizzard would be analogous to those on a pig's stomach.

Table 2.3: Effect of differing dietary fat levels of amino acid digestibility in pigs

Amino Acid	Level of canola oil in diet (%)			
	3.2	6.2	9.2	12.2
Argenine[a]	90.4	90.8	92.1	91.8

Histidine[a]	86.9	88.2	88.7	89.2
Isoleucine[a]	85.6	85.1	86.8	87.0
Leucine[a]	83.6	84.1	85.2	85.5
Lysine[a]	85.4	86.8	88.6	88.6
Methionine	91.1	91.9	91.7	90.8
Phenylalanine[a]	84.7	84.4	86.3	86.2
Threonine[a]	77.8	79.1	80.4	81.4
Valine[a]	82.6	82.5	84.2	84.9
Alanine[a]	82.0	82.1	83.9	85.0
Aspartic acid[a]	83.7	83.5	86.1	85.9
Cysteine	78.3	79.6	80.1	78.9
Glutamic acid[a]	85.9	87.7	90.5	90.2
Glycine[a]	77.9	78.3	82.4	83.5
Serine	87.5	87.0	88.6	88.5
Tyrosine[a]	85.1	86.1	87.1	87.6

Source: Li and Sauer (1994)

In an attempt to investigate the impact different sources of dietary fat on AA digestibility, Li et al. (1990) conducted a study with weaner pigs supplementing the diets with one of the following sources: soybean oil, coconut oil, white grease, 50:50 soybean and coconut oil or 50:50 coconut oil and white grease. In all cases, pigs fed diets with supplemented fats, regardless of fat type, showed higher daily gains and amino acid digestibility (Table 2.4). The soybean oil diet seemed to show the greatest improvement in growth rate, followed by the white grease and then the coconut oil. The mixture of 50% soybean oil and 50% coconut oil showed the greatest gains and allowed for the highest nutrient digestibility. Morphological changes in the microvilli of small intestine were also observed, with diets that lead to increased digestibility also leading to longer microvilli and increased crypt depth. The authors attributed these differences to differences in the chain length or degree of saturation, which were believed to allow for increased cellular growth of the microvilli. Unsaturated and shorter chain fatty acids were believed to be better utilized and absorbed, with some unexplained benefit attributed to the mixed diet.

Jimenez-Moreno et al. (2009) conducted a study to examine the interaction between fiber content and dietary fat sources on performance, total tract nutrient retention and ileal digestibility of amino acids. Broilers were fed one of six diets containing three fiber sources (oat hulls, sugar beet pulp or no source), each with two fat sources (soybean oil or yellow grease). Results indicated that fat type had no effect on growth performance, but influenced ileal digestibility of AA and total tract retention, with the soybean oil showing higher digestibility and retention over the yellow grease. The sugar beet pulp diet had higher AA digestibility and total tract retention, while the oat hull diet had a negative impact.

Table 2.4: Effect of differing dietary fat sources on fat and dry matter digestibility in pigs

Ileal digestibility	No added fat	Soybean oil	Coconut Oil	Soybean/Coconut oil blend
Total fatty acids	78.0	85.5	86.1	87.5
Dry matter	85.2	78.3	80.3	79.7

Source: Li et al. (1990)

2.6 Mode of action

The available published data illustrates a positive link between dietary fat content and amino acid digestibility. Several authors postulate that the main cause of this observation might be longer retention time of digesta in the digestive tract (Turlington et al., 1989; Li et al., 1990; Imbeah and Sauer, 1991; Adedokun et al., 2011). However, this mechanism was not confirmed in some studies (Imbeah and Sauer, 1991). Other proposed mechanisms, which may work in tandem, include changes in intestinal morphology and microbial profile.

2.6.1 Increase in gut retention time

Gut retention/transit time refers to the overall time digesta spends traveling through the animal's digestive system. An increase in gut retention time involves the digesta taking a longer amount of time to completely pass through the bird's digestive system. It should be noted that, when compared to mammals, chickens have a lower overall gut retention time (Weurding et al., 2011), meaning contents pass through at a faster rate. Overall gut retention time is governed by the rate of gastric emptying as well as overall intestinal transit time. It is commonly accepted that different rates of digesta transit time will lead to differing rates of nutrient digestibility (Turlington et al., 1989). The exact mechanisms involved in this process, however, are clearly complex. Imbeah and Sauer (1991) reported that the concentration of fat in the diet had no effect on the protein digestibility in pigs. This study found significant differences in digesta passage rate of diets containing canola oil and soybean oil (Imbeah and Sauer, 1991). The authors also speculated that there may have been differences in gastric emptying rate. The data suggest that there are benefits in terms of digestibility and improved growth of including dietary fats, but the concentration of the fat was found to be less important than the composition of fat in this study. The study by Li and Sauer (1994) contradict the above finding claiming a linear relationship between dietary fat content and amino acid digestibility. This study did also reiterate the mechanism of a slower digestive flow rate with fat addition. It is thought that slower passage rate allows nutrients to spend longer time in the small intestine, giving more time for digestive enzymes to act and allowing more efficient digestion. In humans, dietary fats slow gastric emptying by interacting with intestinal receptors (Hunt and Knox, 1967). It is unknown if similar receptors exist in chickens, but an interaction with such receptors might also explain how dietary fats might delay gastric emptying.

2.6.2 Effects of fatty acid composition

There is ample evidence that indicates that the composition of dietary fat has an effect on nutrient digestibility. Particularly, chain length and saturation seem to be correlated with increased dry matter digestibility. Li et al. (1990) demonstrated that pigs fed a soybean oil diet had superior fat digestibility than those fed a diets supplemented with coconut oil (Table 6). It is unknown if these results are applicable to protein and amino acid digestibility. A study by Jimenez-Moreno et al. (2009) reached similar conclusions; it was observed that soybean oil, largely composed of unsaturated, long-chain fatty acids, led to superior nitrogen digestibility as compared to yellow grease, largely short-chain and unsaturated (Table 2.5). There is some question as to which of these traits, chain length or saturation, is more relevant to the effects on amino acid digestibility (Jimenez-Moreno, et al., 2009). In terms of diet formulation, this question might not be particularly relevant. Most common economically-viable dietary fat options will tend to be either long-chain and unsaturated or short-chain and saturated. In many cases, the decision is between a vegetable oil source, such as soybean oil, or an animal by product, like tallow. In this binary, the evidence suggests that vegetable oils are generally more favorable for inclusion. There are some exceptions to this that could be relevant depending on local economic conditions and cost-benefit analysis.

Table 2.5: Effect of soybean oil and yellow grease on Dry Matte and Nitrogen digestibility in pigs

	Dry Matter			Nitrogen		
	5 days	15 days	average	5 days	15 days	average
Soybean oil	75.0	74.6	74.8	75.6	72.8	74.2
Yellow grease	72.0	73.6	72.9	75.1	73.7	74.4

Source: Jimenez-Moreno et al. (2009)

The mechanism for this observation is likely related to the digestibility of fat, and is not exclusive to amino acid digestion. Long-chain and unsaturated fats in the diet have been shown to lead increased calcium utilization (Allahyari-Bake and Jahanian, 2017) as well as increased digestibility of other dietary nutrients, including starch and minerals (Jimenez-Moreno et al., 2009) when compared to short-chain and saturated fats. It is known that long-chain and unsaturated fatty acids are more easily digested in broilers, and more easily broken down by bile salts (Zollitsch et al., 1997). Since fat is known to form micelles in the intestine, it is possible that less digestible fats leave amino acids (and other nutrients) more likely to be trapped in micelles that remain undigested. Having a diet high in long-chain and unsaturated fatty acid reduces the likelihood that amino acids will pass through the small intestine still trapped in micelles, thus allowing for more efficient utilization.

2.6.3 Changes in digestive morphology

Evidence indicates that dietary fats can cause morphological changes in the small intestine. These changes, triggered by fat inclusion, could possibly lead to increased digestive capability and thus an increase in nutrient absorption. Li et al. (1990) observed that pigs with higher supplementary fat in their diets developed longer and rounder microvilli in small intestine compared to those fed diets with no added fat. It is possible that this observation may be tied to fatty acid binding proteins located on the epithelial lining. Increased contact with fatty acids would cause an increase in enterocyte proliferation, increasing microvilli length and roundness (Smits and Annison, 1996). The increased microvilli length would allow for increased digestive enzyme production, as well as increased contact with amino acids, leading to increased digestion efficiency and absorptive capacity (Li et al., 1990). Li et al. (1990) speculated that these changes in pigs may have been due to the increased fat allowing for more fatty acids to be used to regenerate more enterocytes. In the presence of increased and more digestible fat, the enterocytes of the microvilli were able to increase cellular production, due to their reliance on structural fats. This increased cell count would allow for increased villus height. There is also speculation that morphology changes in chickens may be related to increased bacterial colonization of the epithelial lining (Pan and Zu, 2014). These bacteria would colonize the intestinal lining, and by metabolizing some free fatty acids, increase the available nutrients for enterocytes, allowing for increased cell proliferation and increased villus height.

The composition of the fat might also have an influence on intestinal histology. Li et al. (1990) observed that a mixture of soybean and coconut oils resulted in longer microvilli in pigs compared to coconut oil or soybean oil individually. Similar observations were also made in rats, with a general observation that diet composition has an effect on intestinal microvilli (Goda and Takase, 1993). Rats fed a high fat diet showed increased mucosal and microvilli weight, as opposed to rats fed the equivalent calories in carbohydrates. The high fat diet also led to thicker microvilli caused by an increase in the number of villus cells present in the microvilli. The researchers noted that it was likely that long-chain triglycerides were triggering these morphological changes. This seems to make sense, as once again increased digestible fat availability would allow the fat to be incorporated into the enterocytes. The addition of dietary fat, specifically of long-chain fatty acids, seem to cause an adaptive change in the microvilli, increasing the number of villi cells produced, leading to increase in absorptive area. This might increase absorption, leading to in increased amino acid utilization, as the longer microvilli would be more efficient at AA absorption.

2.6.4 Bird Age

Bird age plays a huge role in how broilers utilize dietary nutrients. It has been found that the increases in amino acid digestibility afforded by increased fat content are markedly lower in chickens between 1 and 21 days of age, as opposed to older birds (Jimenez-Moreno et al., 2009). It has also been noted that younger birds have a distinctively harder time digesting saturated and long-chain fatty acids (Zollitsch et al., 1997). This impaired ability to digest fats explain the fact that dietary fats have a lower impact on amino acid digestibility in younger birds. Adedokun, et al. (2008) found that decreased digestibility of a number of nutrients (including fat) could be attributed to a difference in digestive secretion levels, with younger birds having lower levels of digestive enzymes.

2.6.5 Other factors

The majority of microbial activity in monogastric animals occurs in the large intestine. However, there is also a significant amount of microbial activity that occurs in the small intestine. It is speculated that this activity, and the nature of microbiota, could be modulated by the diet. There is evidence suggesting that increased digestive viscosity allows for a more favorable substrate for the proliferation of certain pathogenic bacteria (Smits and Annison, 1996). It should be noted though that this mechanism is normally applied largely to NSPs, fats are also reported to increase viscosity. A study by Harkins et al. (1964) found that the presence of increased dietary fats decreased digesta transit time and increased retention rate in rats. The study found that higher proportions of long-chain fatty acids and saturated fatty acids decreased flow rate even more so than unsaturated and short-chain fatty acids. It is not known if the viscous environment caused by the fat would allow for the same bacterial proliferation as a viscous environment induced by NSPs. It is also unknown what effect increased bacterial presence in the small intestine might affect AA digestibility and is almost certainly species-dependent, as the microbiome of poultry does differ significantly from that of pigs and rats (Pan and Yu, 2014).

There is also evidence that different fat sources may affect bacterial growth differently. A study by Knarreborg et al. (2002) found that supplemental tallow and soybean oil added to the diets did cause compositional changes in ileal bacteria microbiome (Table 2.6). It was also observed that these microbiome differences increased with bird age. Taking into account that fat digestibility differences between soybean oil and tallow increase with bird age, this may be related to these observed microbiome differences. While it is generally thought that increased bacterial activity would likely decrease nutrient utilization by the bird, this is not necessarily universal. What is known though is that NSP and fat contents in the diet both exert effects on the intestinal microbiota, and are likely to complement and interact with each other. These interactions may or may not exert a significant effect on the microbial ecosystem of the gut.

Table 2.6: Effect of dietary fat source on bacterial growth

	Bacterial counts (least squares mean log CFU)	
	Tallow+Lard	Soy oil
Bacteria/day		
Day 7		
Lactobacilli	8.52	8.62
Fermentative enterobacteria	5.40	5.06
Nonfermentative enterobacteria	4.29	4.39
C. perfringens	5.04	4.50
Day 14		
Lactobacilli	8.12	7.99
Fermentative enterobacteria	5.49	5.82
Nonfermentative enterobacteria	4.18	4.04
C. perfringens	6.35	5.50
Day 21		
Lactobacilli	7.76	7.41
Fermentative enterobacteria	6.09	6.35
Nonfermentative enterobacteria	4.18	4.53
C. perfringens	6.62	3.13

Day 35		
Lactobacilli	8.88	8.68
Fermentative enterobacteria	7.25	7.60
Nonfermentative enterobacteria	5.96	6.36
C. perfringens	8.04	5.44

2.7 Conclusions

The topic of how added dietary fat may affect nutrient digestibility and EL is one that has been yet to be fully explored. Experimental evidence seems to indicate a relationship between the presence of dietary fat and increased digestibility of amino acids. The primary mechanism causing this is speculated to be an increase in digesta retention time. The effect of increased dietary fat on endogenous losses is also unknown.

Chapter 3: Effect of dietary fat on endogenous amino acid losses

3.1 Abstract

The effect of dietary fat source on ileal endogenous amino acid losses in 21-day old broiler chickens was investigated. Three protein-free diets, namely a control diet with no added fat and test diets with 60 g/kg soybean oil or tallow additions, were formulated. Titanium dioxide was added as an indigestible marker in each of these diets. Each diet was assigned to six replicate cages (8 birds/ cage). Ileal samples were collected after slaughter and analyzed for dry matter, titanium oxide, nitrogen (N) and amino acids, including sulfur-containing amino acids. The endogenous losses of all amino acids, except cysteine,

were higher ($P < 0.05$) in broilers fed diets with no added fat, compared to those fed diets with fat. There was no significant ($P > 0.05$) differences in endogenous amino acid losses between soybean oil and tallow diets for any amino acid.

3.2 Introduction

Diet composition is known to influence the endogenous amino acid losses in poultry (Allahyari-Bake and Jahanian, 2017). These dietary components can include dietary fats (Baião and Lara, 2005). Fats of varying composition are a common element in many broiler diets, and their presence can have a particular effect on the amount of mucin and digestive enzymes secreted in the intestine. There have been extensive studies on the effects of added dietary fiber and its effects on endogenous amino acid losses in pigs and chickens (Morel et al., 2005; Cowieson and Ravindran, 2007), however there have been relatively fewer studies on how the amount and composition of added dietary fats affects endogenous amino acid losses in broilers.

The present study was designed to determine the effect fat has on the ileal endogenous amino acid losses in broilers. A secondary goal is to determine if there is a difference in endogenous losses between an animal-sourced fat (tallow) and a plant-sourced fat (soybean oil).

Previous studies in pigs (Imbeach and Sauer, 1991) and ducks (Akinde, 2017) indicate that dietary fat may not have an effect on endogenous AA losses when compared to diets without added fats. These studies compared low-fat diets with add-fat diets. Other studies in pigs (Li and Sauer, 1994) and rats (Harkins, 1964) indicate that increasing levels of dietary fat increase rut retention of digesta and intestinal amino acid absorption. It should be noted that these studies showing an effect included diets of increasingly higher levels of dietary fats without a no-fat diet to compare to.

The composition of the dietary fats may also have effect, especially when comparing fat sources with high level of saturated fatty acids as compared to fat sources with high levels of unsaturated fatty acids. The composition of vegetable fats, such as soybean and canola oils, is more heavily composed of unsaturated fatty acids, while animal fats are more heavily composed of saturated fatty acids (Blanch et al., 1996, Baião and Lara, 2005, Orsavova et al., 2015). Chickens show a generally lower digestibility of saturated fatty acids over unsaturated ones (Blanch et al., 1996, Renner and Hill, 1961), however this disparity does decrease with age (Wiseman, 1984). Kussaibati et al. (1982) found that young birds were less able to digest saturated fats than older birds. While this disparity decreases with age, overall fat digestibility also increases with age (Vieira et al., 1997). Fatty acid chain length is also an important compositional factor in dietary fats, with longer chain saturated fatty acids showing decreased digestibility when compared to shorter chain saturated fatty acids (Kussaibati et al., 1982). Vegetable fats, like soybean oil, usually contain a higher proportion of long-chain fatty acids (Orsavova et al., 2015), with some exceptions such as coconut (Orsavova et al., 2015) and palm oil (Rodríguez-Sánchez et al., 2017, Allahyari-Bake and Jahanian, 2017) which contain higher proportions of short-chain fatty acids. These characteristics affect how fats from specific sources might be utilized by the broiler. These characteristics may also be relevant as to how these fats affect endogenous secretions and losses.

3.3 Materials and Methods

3.3.1 Birds and housing

Day-old male broilers (Ross 308) were obtained from a local hatchery. The birds were raised in floor pens and fed a commercial starter diet for 14 days. On day 14, 144 broilers of uniform weight were selected and moved to 18 cages, with 8 birds per cage. The birds continued to receive the commercial starter diet until day 18. On day 18, test diets were introduced with each of the three diets being allocated to six cages. The diets were offered ad libitum and water available at all times.

3.3.2 Diets

Three nitrogen-free diets, namely with no added fat sources, with 60 g/kg soybean oil or with 60 g/kg tallow (Table 2.1) were developed. Titanium dioxide (5 g/kg) was included in all diets as an indigestible marker.

3.3.3 Digesta collection

On day 21, all birds were euthanized by an intracardial injection of sodium pentobarbitone solution and the contents of the lower half of the ileum were collected. Two birds from each cage were chosen at random for digesta collection. The ileum is defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. The ileum was divided into halves, and the digesta was collected from the lower half towards the ileo-caecal junction. Digesta within a cage were pooled, frozen immediately and freeze-dried. Diet and dried ileal digesta samples were ground to pass through a 0·5-mm sieve and stored in sealed containers at – 20ºC until chemical analyses.

3.3.4 Chemical analysis

The diets and digesta samples were analyzed for dry matter, titanium oxide, nitrogen (N) and amino acids, including sulfur-containing amino acids.

Nitrogen was determined by using a FP-428 nitrogen determinator (LECO® Corporation, St Joseph, MI) as described by Sweeney (1989). Amino acids were determined using high-performance liquid chromatography as outlined by Ravindran et al. (1999a). The samples were hydrolyzed under nitrogen with 8 mol/l HCl containing phenol (3 g/l) for 16 h at 120ºC. DL-norleucine was added to the hydrolysate as an internal standard. Each hydrolysate was then diluted and adjusted to pH 2•2 to 2•3, as per the amino acid standard (Standard H, Pierce Chemicals Co., Rockford, IL). The hydrolysates were passed through a 0•22-µm nylon 66 membrane filter (Alltech, Baulkham Hills, New South Wales, Australia). Aliquots of the hydrolysates were subjected to ion-exchange column chromatography, using a Shimadzu amino acid analysis system (Shimadzu Corp., Kyoto, Japan). Amino acids were eluted by a gradient of pH 3•20 sodium citrate eluent to pH 10•00 sodium citrate eluent at a flow rate of 0•4 ml/min and a column temperature of 60ºC. O-phthaldialdehyde (Sigma Chemicals Co., St Louis, MO) was used for post-column derivatization of amino acids. Chicken egg-white lysozyme (Seikagaku Co., Chuo-ku, Tokyo, Japan) was used as a control to confirm the reproducibility and accuracy of the amino acid determinations.

3.3.5 Calculations

The basal endogenous amino acid flow at the terminal ileum was calculated as milligrams lost per kilogram of dry matter intake (DMI) using the following formula (Moughan et al., 1992).

Basal endogenous amino acid flow (mg/g DMI)

$$= \frac{\text{Amino acid concentration in ileal digesta (mg/kg) x Titanium}_{\text{Diet}}\text{ (mg/kg)}}{\text{Titanium}_{\text{Digesta}}\text{ (mg/kg)}}$$

3.3.6 Statistical analysis

The data was analyzed using SAS 9.4 software, using a general linear model. Differences where $P < 0.05$ were considered significant.

Table 3.1. Ingredient composition (g/kg) of the nitrogen-free diets

	Control	Soybean oil	Tallow
Dextrose	907	847	847
Soybean oil	0	60	0
Tallow	0	0	60
Solkafloc (Cellulose)	35	35	35
Sodium bicarbonate	3.0	3.0	3.0
Sodium chloride	3.0	3.0	3.0
Dicalcium phosphate	19.5	19.5	19.5
Limestone	13.2	13.2	13.2
Dipotassium phosphate	12.0	12.0	12.0

Vitamin premix[1]	0.8	0.8	0.8
Trace mineral premix[1]	1.5	1.5	1.5
Titanium dioxide	5.0	5.0	5.0

[1] Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

3.4 Results

3.4.1 Fatty acid composition

Table 3.2 displays the analyzed fatty acid composition of soybean oil and tallow used in the experimental diets

Table 3.2. Fatty acid composition of soybean oil and tallow (g/kg diet)

	Tallow	Soybean oil
Saturated Fatty Acids		
C8:0 Caprylic	0.17	*
C10:0 Capric	0.75	*
C12:0 Lauric	1.23	0.10
C13:0 Tridecanoic	0.16	*
C14:0 Myristic	23.92	0.76
C16:0 Palmitic	197.81	103.43
C17:0 Margaric	9.68	1.28
C18:0 Stearic	156.83	40.81
C20:0 Arachidic	1.62	3.12
C21:0 Heneicosanoic	0.24	0.36
C22:0 Behenic	0.26	3.55
C24:0 Lignoceric	0.24	1.19
C23:0 Tricosanoic	0.11	0.46

Unsaturated Fatty Acids		
C14:1n5 - cis-9-Myristoleic	2.83	*
C16:1n9t Palmitelaidic	0.50	*
C16:1n7 - cis-9-Palmitoleic	26.84	0.82
C18:1n9t Elaidic	2.34	0.14
C18:1n7t Vaccenic	19.48	0.11
C18:1n9c Oleic	308.90	205.25
C18:1n7c Vaccenic	12.37	10.90
C18:2n6c Linoleic	46.84	573.99
C18:3n6 - cis-6,9,12-Gamma linolenic	0.50	*
C20:1n9 - cis-11-Eicosenoic	4.70	4.19
C18:3n3 - cis-9,12,15-Alpha linolenic	12.88	81.55
C20:2n6 - cis-11,14-Eicosadienoic	1.76	0.47
C20:3n6 - cis-8,11,14-Eicosatrienoic	1.13	*
C20:3n3 - cis-11,14,17-Eicosatrienoic	0.59	*
C20:4n6 - cis-5,8,11,14-Arachidonic	2.52	*
C20:5n3 - cis-5,8,11,14,17-Epa	3.79	0.42
C22:5n3 - cis-7,10,13,16,19-DPA	2.52	*
C22:6n3 - cis-4,7,10,13,16,19-DHA	6.42	*
Total Fat		
Saturated	393.0	155.1
Unsaturated	457.9	877.4
Unsaturated/Saturated ratio	1.17:1	5.66:1
Moisture %	0.1	*
Gross Energy (kJ/g)	39.3	39
Iodine value /kg	1265	576

* Represents trace amounts.

3.4.2 Ileal endogenous losses

Table 3.3 illustrates the influence of dietary treatments on endogenous AA losses. Results for all amino acids were significant ($P<0.05$), with the exception of cysteine. For all amino acids, the diet with no added fat showed higher ($P>0.05$) endogenous losses than tallow and soybean oil diets. For all amino acids, there was no significant difference ($P > 0.05$) between the endogenous losses in the tallow-supplemented and soybean oil supplemented diets.

Table 3.3: Influence of fat sources on the ileal endogenous amino acids in broilers (mg/kg dry matter intake)[1]

	No Fat	Soybean Oil	Tallow	P-value	SEM[2]
Essential AA					
Arginine	0.647^{a}	0.337^{b}	0.262^{b}	0.009	0.0799
Isoleucine	0.627^{a}	0.301^{b}	0.241^{b}	0.009	0.0815
Leucine	1.017^{a}	0.482^{b}	0.381^{b}	0.010	0.1352
Lysine	0.639^{a}	0.274^{b}	0.211^{b}	0.003	0.0763
Methionine	0.201^{a}	0.116^{b}	0.097^{b}	0.014	0.0232
Phenylalanine	0.559^{a}	0.278^{b}	0.232^{b}	0.009	0.0688
Threonine	0.836^{a}	0.491^{b}	0.446^{b}	0.009	0.0837
Valine	0.780^{a}	0.399^{b}	0.332^{b}	0.007	0.0916
Histidine	0.216^{a}	0.102^{b}	0.073^{b}	0.001	0.0221
Non-Essential AA					
Aspartic Acid	1.072^{a}	0.582^{b}	0.476^{b}	0.006	0.1174
Serine	0.738^{a}	0.430^{b}	0.404^{b}	0.010	0.0738
Glutamic Acid	1.574^{a}	0.836^{b}	0.658^{b}	0.011	0.1951
Alanine	0.617^{a}	0.330^{b}	0.256^{b}	0.005	0.0690
Tyrosine	0.574^{a}	0.278^{b}	0.226^{b}	0.011	0.0757
Cysteine	0.215	0.152	0.168	0.078	0.0188
Average	0.683^{a}	0.360^{b}	0.301^{b}	0.007	0.0775
Total	1.096^{a}	0.624^{b}	0.531^{b}	0.006	0.1123
Nitrogen[3]	2.046^{a}	1.265^{b}	1.067^{b}	0.009	0.2134

[a, b] Means in a column not sharing a common letter are significantly different ($P<0.05$).

[1] Each value represents the mean of six replicates (eight birds per replicate).

[2] Pooled standard error of means

[3] Total nitrogen losses

3.5 Discussion

The results indicate that, for all amino acids, the presence of dietary fat decreased endogenous losses and there w ere no significant differences between tallow and soybean oil.

These results contradict what was expected. Speculation in previous literature indicates that the inclusion of dietary fat would increase the gut retention time of the digesta. This would lead to increased production of digestive enzymes and mucus, which would lead to increased endogenous losses in the diets containing fat. Morel et al. (2005) found that the presence of certain NSPs in pig diets decreased digestive flow rate as well as increasing mucin production as a result.

A number of causes may explain this discrepancy and include changes in bacterial substrate, changes in the structure of the microvilli, and improved ileal absorption of amino acids due to increased gut transit time.

This observation could be related to morphological changes in the microvilli. A study conducted on rats found that a high-fat diet increased the length of intestinal microvilli and increased crypt depth, thus also increasing the proliferation of microvilli in the intestinal wall (Goda and Takase, 1993). Another study in pigs found similar results, but also indicated that pigs fed a combination of soybean and coconut oils had longer and rounder villi and increased crypt depth when compared to pigs fed diets with a singular oil or no fat (Li et al. 1990). An increase in villus area could explain the decrease in endogenous losses. The increased length would increase the efficiency of absorption, allowing better re-absorption of endogenous proteins in the chickens fed dietary fat. A study by Jenkins and Thompson (1989) in rats lends support to this suggestion. This study found that the replacement of dietary energy from glucose to fat increased mucin production, but also increased crypt depth of microvilli and proliferation of microvilli cells (Jenkins and Thompson 1989). While the study by Li et al. (1990) indicates that the composition of the fat may also have an effect, it does not entirely explain the observed differences between tallow and soybean oil. It is possible that these observations may be related, partly, to bacterial proliferation. Pan and Yi (2014) speculate that increased colonization of the intestinal walls may lead to a stimulation of microvilli length growth, crypt depth and the proliferation of microvilli cells. It is thus possible that these two mechanisms work in concert to reduce endogenous losses. Lam et al. (2012) observed that high fat, especially saturated fat, increase the permeability of the colonic walls in mice (Lam et al 2012). In their study, this was translated into an increase in general energy absorption leading to increased obesity.

Changes in the composition of microbiota may be another possible cause of this unexpected finding. While the primary area of bacterial activity in the gut is the large intestine, the small intestine still contains a microbiome. The bacteria in the small intestine are particularly related to mucus production (Tomas et al., 2016). Many of these species will, in some cases, colonize the intestinal wall. This colonization can often have the effect of disrupting and limiting mucus production (Tomas et al., 2016). This is likely due to the fact that many of these species use mucin as an energy and amino acid source (Pan and Yi, 2014) It has been speculated that increased retention time of undigested substrates can allow for increased proliferation of bacteria in the small intestine by acting as a substrate (Smits and Annison 1996). A study in mice by Onishi et al. (2017) found that increasing dietary fat resulted in blooms of bacteria in the small intestine and a shift in the relative proportion of different bacterial species. The composition of bacterial colonies also favors shorter retention times and strains found within the small intestine of the chicken are often selected to adhere to intestinal walls at a fast rate and proliferate quickly (Pan and Yu, 2014). As a result, it is possible that the increased retention time can have a much more drastic effect on the bacterial population in chickens than it might in pigs, mice or other mammals. There is also evidence indicating that the adhesion of these bacterial colonies to the intestinal wall can disrupt the release of digestive enzymes along with mucus (Pan and Yu, 2014). This would have the effect of inhibiting a source of endogenous losses. In mice, it was found that a high-fat diet substantially changed the microbiotic composition of the colon of mice (Hildebrandt et al., 2009). This was confirmed in another study with mice by Lam et al. (2012), who speculated that the high-fat diet allowed for greater bacterial proliferation, as dietary fat caused enrichment of certain genetic sequences encoding survivability mechanisms in the bacteria. Jensen (1998) found that the small intestine of young pigs featured a significant amount of protein fermentation by gut microbiota. Evidence has shown the colonial mucin bacteria do utilize amino acids present in small intestine digesta as well as amino acids of endogenous origin (Dai et al., 2015). Since many of these species do utilize mucins and other proteins of endogenous origin metabolically, this increased proliferation could have the effect of increasing the bacterial utilization of endogenous amino acids, increasing apparent digestibility.

Another possible cause for these observations is due to a shift in the area of amino acid absorption due to increased viscosity. Increased fat content in digesta has been shown to increase viscosity and increase transit time and gastric emptying of gut contents in humans (Hunt and Knox, 1968). It has also shown that increased fat content might increase the amino acid absorption of proximal segments of the small intestine. A study by Jenkins and Thompson (1992) in rats found that, replacing energy content from glucose to fat did shift the site of absorption of energy from the proximal segments of the intestine to the middle segments, and caused a shift in mucus production to these areas. It is, however, unknown if this was due to a chemical difference in glucose and fat absorption, or rather increased gut retention time (Jenkins and Thompson 1992). Booth et al. (1961) also observed an increased participation of the jejunum in nutrient digestion when fat was added to the diet. Previous results in pigs (Li and Sauer, 1994) indicate that increased dietary fats may decreases endogenous amino acid losses. This is contradicted in a study by Akinde (2017) in ducks that found no effect of increased concentrations of soybean oil on endogenous AA losses. It should be noted that there was no fat-free diet used as a control in this study. Previous studies do indicate that increases in fat content can shift the primary site of fat digestion to the jejunum and increase the absorption of dietary fat. These effects are likely due to delayed gastric emptying and increased retention time of digestive contents. A study by Merriman et al. (2016) found that supplemented fat improved absorption of dietary microminerals in pigs as compared to a no-fat diet, implying that these increases in absorption can affect the absorption of nutrients other than fat. It is possible that this delayed passage rate would also allow for increased opportunity of absorption of amino acids as well as fat. Thus, endogenously-secreted proteins are more readily re-absorbed before reaching the terminal ileum when there is increased fat in the diet. While it has been documented that increased viscosity by NSPs does increase gut retention time of digesta, NSPs have been documented to actually increase endogenous losses and inhibit re-uptake (Smits and Annison, 1996). While these effects are partially attributed to the increased viscosity, some have suggested that this is a simplistic and inaccurate assessment (Smits and Annison, 1996). Digesta transit effects attributed to increased fat content and increased NSP content likely cannot be compared.

None of these possible causes are mutually exclusive, and any and all of them might be occurring and causing the observed effects. Indeed, the assumption that the increased gut retention time would increase production of mucus and digestive enzymes could still be correct, and simply be counteracted by any or all of these observed effects.

As there was no significant difference between them, fat type may not be important in affecting endogenous amino acid losses in broilers, although some studies in pigs have indicated fat composition is relevant (Li et al., 1990; Merriman et al., 2016). Li et al. (1990) found improved amino acid digestibility imparted by added fat sources higher in unsaturated amino acids. Previous experiments have shown that degree of saturation (Rodríguez-Sánchez et al., 2017; Li et al., 1990) and chain length (Booth et al., 1961) are important in governing rate of fat absorption. The observed result of this experiment, that there is no effect, would mean that the level of digestibility of the dietary fat does not have any impact on the digestibility of other nutrients, such as end amino acids. One other explanation that may explain this result is the age of the birds. It has been observed that younger birds at less able to digest saturated fats (Adedokun et al., 2008, Erdaw et al., 2017). At 21-days old, the birds used in this experiment may have been old enough to see the discrepancy between saturated and unsaturated fatty acid digestibility lessen, leading to similar fat digestibilities from both treatments. This would then mean both fat treatments would have an equal impact on amino acid re-absorption.

3.6 Conclusion

The present data indicates that the presence of fat in broiler diets decreases endogenous amino acid losses and, implies that dietary fat may have beneficial effects beyond increased dietary energy and palatability.

As the mechanism for these results is still speculative, future studies would be best served by looking at three possible causes. This would include bacterial sampling of the small intestine, to discover if dietary fat results in a change in the microbiota composition. There are currently no published studies on the effect dietary fat has on microbiota in the small intestine in chickens. Studies in mice (Onishi et al., 2017) indicate that there could be compositional changes brought upon by changes in dietary fat. Intestinal segments could be examined to determine any morphological changes in the microvilli. Experiments in pigs (Li et al., 1990) and rats (Jenkins and Thompson, 1989; Goda and Takase, 1993) indicate that there may be an effect, but there are no published studies in chickens. Finally, isotope markers could be used to determine the rate of passage of gut contents, as well as the site of absorption. The results of this study could be used to optimize fat ratios in broiler diets and, allow more efficient and cost effective utilization of fats in diet formulation, balancing cost of dietary fat sources and the financial benefits of increased protein utilization.

Chapter 4: The interaction between grain type and dietary fat source on nutrient digestibility in broilers

4.1 Abstract

The present experiment was conducted to determine the effect of grain type and dietary fat source on coefficient of apparent digestibility (CAD) of nutrients, including amino acids, in broiler starters. Two cereals (maize and wheat) and three inclusions of fat sources (no fat, soybean oil and tallow) were evaluated in a 2 × 3 factorial arrangement of treatment diets with each being based on one grain and having one of three added fat sources. In general, where a significant main effect of grain type existed, maize-based diets resulted in higher ($P < 0.05$) nutrient digestibility than wheat-based diets in all the segments of digestive tract. Dietary fat inclusion, regardless of fat source, increased ($P < 0.05$) CAD of dry matter (DM) in upper ileum, CAD of fat in duodenum, upper jejunum, upper and lower ileum, and CAD of gross energy in upper ileum. Whilst both fat sources increased ($P < 0.05$) fat digestibility in upper and lower ileum compared with the diets with no fat, diet with soybean oil showed the highest ($P < 0.05$) CAD of fat. Of the two fat sources, only tallow resulted in higher CAD of DM in upper jejunum, and nitrogen in upper jejunum and upper ileum. There was no effect ($P > 0.05$) of fat source on starch digestibility in any segments of the digestive tract. Birds fed maize-based diets, regardless of fat source, had higher ($P < 0.05$) digestibility for all the amino acids except proline. Fat source had no effect ($P > 0.05$) on ileal CAD of amino acids. Overall, the present data showed that added dietary fat did increase DM and nitrogen digestibility in the lower intestinal segments.

4.2 Introduction

Dietary fat content likely has an impact on the digestion of a number of different nutrients in broiler diets other than fat itself. The type of cereal grains in poultry diets has been shown to influence the extent of nutrient digestion in poultry (Liu et al., 2014). Wheat-based diets have been reported to lower amino acid (AA) digestibility in broilers

(Angkanaporn et al., 1994) and that a grain, such as wheat, with higher concentration of non-starch polysaccharides (NSP) shows overall lower nutrient digestibility in poultry diets (Zuber et al., 2016).

Nutrient digestibility values in different segments of the digestive tract represent the proportion of the nutrients in the digesta that is digested and absorbed by the bird. The digestibility values in the upper gut, gizzard and duodenum, are usually negative values, indicating net excretion of endogenous nutrients (Hurwitz et al., 1979), and the fact that upper digestive tract segments are primarily sites of digestion rather than absorption; although some small amounts of absorption may still occur (Sklan et al., 1973; Hurwitz et al., 1979). Since the jejunum and ileum segments are where the majority of digestion and absorption takes place, these segments are of higher focus and digestibility values are routinely determined at the ileum level. Renner (1964) found that the majority of fat absorption occurs in the lower jejunum, followed by the upper ileum and then smaller amounts in the upper jejunum and lower ileum.

Previous studies have demonstrated a link between dietary fat inclusion and increased protein and dry matter (DM) absorption in poultry (Zollitsch et al., 1997; Mirghelen et al., 2013; Allahyari-Bake and Jahanian, 2017) and pigs (Sauer and Ozimek, 1986; Li et al., 1990; Li and Sauer, 1994; Merriman et al., 2016). In most of these studies, fats with long-chain fatty acids have improved nutrient absorption more efficiently than those encompassing short-chain fatty acids.

Although the influence of grain type and fat source on growth performance and nutrient digestibility in broilers has been investigated in a number of studies, information on the possible interaction of these factors on nutrient digestibility in different segments of the digestive tract and, more specifically, on ileal AA digestibility is limited, and merit further investigation. The present experiment was designed to investigate the influence of grain type (maize and wheat) and fat source (no fat, soybean oil and tallow) on the CAD of DM, nitrogen (N), starch, fat, gross energy (GE) in different segments of digestive tract and A digestibility in distal ileum in broiler starters.

4.3 Materials and methods

4.3.1 Diets

The experiment utilized a completely randomised deign with 2 × 3 factorial arrangement of treatments evaluating two grain types (maize and wheat) and three fat sources (no fat, soybean oil and tallow). Maize, wheat and soybean meal were obtained from a commercial supplier. Two basal maize- and wheat-soybean-based diets were formulated to contain 60 g/kg of dextrose. Six diets were then developed by adding 60 g/kg of either soybean oil or tallow to each basal diet at the expense of dextrose (Table 4.1). The diets contained 5.0 g/kg of titanium dioxide (TiO2, Merck KGaA, Darmstadt, Germany) as an indigestible marker the determination of nutrient digestibility.

Table 4.1 Experimental diet composition (g/Kg)

	Maize-soybean diet			Wheat-soybean diet		
Item	No fat	Soybean oil	Tallow	No fat	Soybean oil	Tallow
Maize	557.7	557.7	557.7	-	-	-
Wheat	-	-	-	605.1	605.1	605.1
Soybean Meal	338.7	338.7	338.7	292.5	292.5	292.5
Dextrose	60	0	0	60	0	0
Soybean oil	0	60	0	0	60	0

Tallow	0	0	60	0	0	60
DCP	20.4	20.4	20.4	20.1	20.1	20.1
Limestone (Omyacal)	11.8	11.8	11.8	11.8	11.8	11.8
Sodium chloride	2.5	2.5	2.5	2.4	2.4	2.4
Sodium bicarbonate	1.6	1.6	1.6	0.8	0.8	0.8
Vit Premix	0.8	0.8	0.8	0.8	0.8	0.8
Min Premix[1]	1.5	1.5	1.5	1.5	1.5	1.5
Titanium dioxide	5	5	5	5	5	5
Provision						
AME (MJ/kg)	12.21	13.44	12.89	11.82	13.05	12.5
Crude protein	210	210	210	210	210	210
Methionine	3.3	3.3	3.3	2.9	2.9	2.9
M+C	6.7	6.7	6.7	6.3	6.3	6.3
Lysine	11.5	11.5	11.5	10.5	10.5	10.5
Threonine	8	8	8	7.4	7.4	7.4
Crude fat	24.6	79.8	69.6	18.1	73.2	63
Crude fiber	25.5	25.5	25.5	29.6	29.6	29.6
Calcium	10	10	10	10	10	10
Available Phosphorus	5	5	5	5	5	5
Sodium	1.6	1.6	1.6	1.6	1.6	1.6
Chloride	1.9	1.9	1.9	1.9	1.9	1.9

[1] Supplied per kilogram of diet: ethoxyquin, 100 mg; biotin, 0.2 mg; Ca pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1.0 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

4.3.2 Birds and housing

Experimental procedures were conducted in accordance with the Massey University Animal Ethics Committee guidelines. A total of 360, day-old male broilers (Ross 308) were obtained from a local hatchery. These birds were raised in floor pens and fed a commercial starter diet for 14 days. On day 14, 288 birds of uniform body weight were selected and randomly assigned to 36 grower cages in an environmentally controlled room so that the average bird weight per cage was similar. Each of the six dietary treatments was then randomly assigned to six cages, each housing eight birds. Birds Received 20 h of fluorescent illumination per d. The temperature was maintained at 31 ºC on d 1, and was gradually reduced to 22 ºC by 21 d of age. The experimental diets, in mash form, were offered ad libitum from 14 to 21 d and water was freely available.

4.3.3 Digesta collection

On day 21, all birds per cage were euthanized by intracardial injection (1 mL per 2 kg live weight) of sodium pentobarbitone solution (Provet NZ Pty Ltd., Auckland, New Zealand) and the contents of the gizzard, duodenum, upper and lower jejunum, upper and lower ileum were collected as described by Ravindran et al. (2005). The ileum is defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. Digesta from birds within a cage were pooled, lyophilised, ground to pass through a 0.5 mm sieve and stored at 4 ºC until laboratory analysis. The diets and digesta samples were analyzed for DM, titanium (Ti), N, starch, fat and gross energy (GE). The diets and digesta samples were also analyzed for indispensable and dispensable AA.

4.3.4 Chemical analysis

Dry matter content was determined using standard procedures (methods 930.15 and 925.10; AOAC, 2005). Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. For titanium determination, the samples were first ignited at 500 oC to burn all organic material and the remaining minerals were digested (using 66% sulphuric acid) to release Ti, which was then determined using a colorimetric assay (Short et al., 1996). Total starch content was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Fat content was determined using Soxhlet extraction procedure (method 991.36; AOAC, 2005).

Nitrogen content was determined by combustion (method 968.06; AOAC, 2005) using a CNS-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). Amino acids were determined as described by Ravindran et al. (2005). Briefly, the samples were hydrolyzed with 6N HCl (containing phenol) for 24 h at 110 ± 2 ºC in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion-exchange HPLC system, and the chromatograms were integrated using dedicated software (Millennium, Version 3.05.01, Waters, Millipore, Milford, MA) with the AA identified and quantified using a standard AA mixture (Product no. A2908, Sigma, St. Louis, MO). The HPLC system consisted of an ion-exchange column, two 510 pumps, Waters 715 ultra WISP sample processor, a column heater, a post column reaction coil heater, a ninhydrin pump and a dual wavelength detector. Amino acids were eluted by a gradient of pH 3.3 sodium citrate eluent to pH 9.8 sodium borate eluent at a flow rate of 0.4-ml/ min and a column temperature of 60 ºC. Cysteine and methionine were analyzed as cysteic acid and methionine sulphone, respectively, by oxidation with performic acid for 16 h at 0 ºC and neutralization with hydrobromic acid prior to hydrolysis.

4.3.5 Calculations

The apparent digestibility coefficients were calculated from the dietary ratio of nutrients to titanium relative to the corresponding ratio in the digesta from different segments of the digestive tract.

$$\text{Nutrient digestibility coefficient} = \frac{(N / Ti)_f - (N / Ti)_d}{(N / Ti)_f}$$

where, $(N / Ti)_f$ = ratio of nutrient to titanium in feed

and $(N / Ti)_d$ = ratio of nutrient to titanium in digesta.

4.3.6 Statistical Analysis

The data was analyzed by two-way ANOVA to determine the main effects (grain type and fat source) and their interaction using the General Linear Models procedure of SAS (2004). Cage means served as the experimental unit for all data. Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test.

4.4 Results

4.4.1 Dry matter digestibility

Influence of dietary treatments on CAD of DM in gizzard and different intestinal segments is summarized in Table 4.2. A significant ($P < 0.05$) interaction between the grain type and fat source was observed for CAD of DM in gizzard. In wheat-based diets, gizzard DM digestibility was not influenced ($P > 0.05$) by fat source, whereas, in maize-based diets, soybean oil increased ($P < 0.05$) DM digestibility compared to the diets with no fat inclusion.

The grain type had a significant effect on CAD of DM in lower jejunum ($P < 0.05$), upper and lower ileum ($P < 0.001$), with higher ($P < 0.05$) DM digestibility in maize- than wheat-based diets. Fat source had a significant effect on DM digestion in upper jejunum ($P < 0.01$) and upper ileum ($P < 0.05$). Whilst both fat sources increased ($P < 0.05$) CAD of DM in upper ileum, only tallow resulted in higher CAD of DM in upper jejunum.

Table 4.2:

Influence of grain type and fat source on coefficient of apparent digestibility of dry matter in different segments of the digestive tract in male broilers at 21 days post-hatch[a]

Grain type	Fat source	Gizzard	Duodenum	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum
Maize	No fat	-1.401c	-0.142	0.360	0.586	0.657	0.726
	Soybean oil	-1.158b	-0.284	0.347	0.553	0.668	0.731
	Tallow	-1.309bc	-0.173	0.443	0.568	0.690	0.730
Wheat	No fat	0.516a-	-0.268	0.270	0.518	0.581	0.648
	Soybean oil	0.647a-	-0.037	0.318	0.537	0.624	0.666
	Tallow	-0.655a	-0.435	0.420	0.571	0.610	0.646

SEM[b]	0.0720	0.1204	0.0375	0.0153	0.0115	0.010
Main effects						
Grain type						
Maize	1.289-	-0.200	0.383	0.569a	0.671a	0.729a
Wheat	0.606-	-0.247	0.336	0.542b	0.605b	0.653b
Fat source						
No fat	0.958-	-0.205	0.315b	0.552	0.619b	0.687
Soybean oil	0.902-	-0.160	0.332b	0.545	0.646a	0.699
Tallow	0.982-	-0.304	0.432a	0.569	0.650a	0.68
Probabilities, P						
Grain type	0.001	0.636	0.135	0.038	0.001	0.001
Fat source	0.531	0.482	0.008	0.273	0.022	0.437
Grain type x fat source	0.045	0.108	0.620	0.070	0.232	0.639

.(Means in a column not sharing a common letter (a-c) are significantly different ($P < 0.05$

.[a] Each value represents the mean of six replicates of eight birds per replicate

.[b] Pooled standard error of mean

4.4.2 Nitrogen digestibility

Maize-based diets resulted in higher ($P < 0.05$) N digestibility than wheat-based diets in upper and lower ileum (Table 4.3). Tallow inclusion increased ($P < 0.05$) CAD of N in upper jejunum and upper ileum compared with no fat diets and higher ($P < 0.05$) N digestibility than the diets with soybean oil in lower jejunum.

Table 4.3:

Influence of grain type and fat source on coefficient of apparent digestibility of nitrogen in different segments of the digestive tract in male broilers at 21 days post-hatch[a]

Grain type	Fat source	Gizzard	Duodenum	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum
Maize	No fat	-0.154	-1.734	0.217	0.597	0.719	0.803
	Soybean oil	-0.082	-1.968	0.230	0.541	0.729	0.815
	Tallow	-0.201	-1.812	0.443	0.635	0.770	0.829
Wheat	No fat	0.024-	-1.623	0.205	0.534	0.669	0.761
	Soybean oil	0.131-	-1.027	0.201	0.561	0.703	0.776
	Tallow	-0.154	-1.792	0.392	0.626	0.718	0.785

SEM[b]	0.0520	0.258	0.0559	0.0328	0.0180	0.0117
Main effects						
Grain type						
Maize	0.146-	-1.838	0.297	0.591	0.740a	0.816a
Wheat	0.103-	-1.480	0.266	0.574	0.697b	0.774b
Fat source						
No fat	0.089-	-1.678	0.211b	0.566ab	0.694b	0.782
Soybean oil	0.106-	-1.497	0.215b	0.551b	0.716ab	0.796
Tallow	0.177-	-1.802	0.418a	0.630a	0.744a	0.807
Probabilities, P						
Grain type	0.323	0.100	0.508	0.524	0.007	0.001
Fat source	0.214	0.502	0.001	0.049	0.032	0.118
Grain type x fat source	0.248	0.162	0.943	0.453	0.726	0.979

.(Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$

.[a] Each value represents the mean of six replicates of eight birds per replicate

.[b] Pooled standard error of mean

4.4.3 Starch digestibility

A significant ($P < 0.05$) interaction between grain type and fat source was observed for CAD of starch in gizzard (Table 4.4). In wheat-based diets, starch digestibility was not influenced ($P > 0.05$) by fat source, but increased ($P < 0.05$) by soybean oil inclusion in maize-based diets. Birds fed maize-based diets, regardless of fat source, showed higher ($P < 0.05$) starch digestibility in lower jejunum, upper and lower ileum.

Table 4.4:

Influence of grain type and fat source on coefficient of apparent digestibility of starch[a] in different segments of the digestive tract in male broilers at 21 days post-hatch[b]

Grain type	Fat source	Gizzard	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum
Maize	No fat	-1.252c	0.800	0.926	0.956	0.974
	Soybean oil	-1.007b	0.768	0.905	0.948	0.963
	Tallow	-1.221c	0.781	0.897	0.951	0.969
Wheat	No fat	0.274a-	0.784	0.880	0.892	0.906
	Soybean oil	0.402a-	0.759	0.855	0.896	0.900
	Tallow	-0.417a	0.775	0.846	0.871	0.882
SEM[c]		0.0698	0.0316	0.0139	0.0097	0.0082

Main effects					
Grain type					
Maize	1.160-	0.783	0.909a	0.952a	0.969a
Wheat	0.364-	0.773	0.860b	0.886b	0.896b
Fat source					
No fat	0.763-	0.792	0.903	0.924	0.940
Soybean oil	0.705-	0.764	0.880	0.922	0.931
Tallow	0.819-	0.778	0.871	0.911	0.926
Probabilities, P					
Grain type	0.001	0.692	0.001	0.001	0.001
Fat source	0.280	0.665	0.082	0.389	0.232
Grain type x fat source	0.041	0.987	0.976	0.370	0.310

.(Means in a column not sharing a common letter (a-c) are significantly different (P < 0.05

.[a] Starch content could not be analyzed in duodenum due to low quantity of digesta samples

.[b] Each value represents the mean of six replicates of eight birds per replicate

.[c] Pooled standard error of mean

4.4.4 Fat digestibility

There was a significant ($P < 0.05$) interaction between grain type and fat source for CAD of fat in gizzard ($P < 0.001$) and lower jejunum ($P < 0.05$; Table 4.5). Fat digestibility was higher in the maize-based diets over the wheat-based diets when no additional fat was added. The fat in the wheat diets were significantly more digestible as soybean oil over tallow. There was no significant difference between the fat digestibility of tallow and soybean oil in the maize diets. The tallow and soybean oil were not significantly different in digestibility in the maize diets when compared to the wheat diets.

Grain type had a significant ($P < 0.05$ to 0.001) effect on fat digestibility in upper jejunum, upper and lower ileum; diets based on maize resulted in higher ($P < 0.05$) CAD of fat than those based on wheat. Dietary fat inclusion, regardless of fat source, increased ($P < 0.05$) CAD of fat in duodenum, upper jejunum, upper and lower ileum. Whilst both fat sources increased ($P < 0.05$) fat digestibility in upper and lower ileum compared with the diets with no fat, diet with soybean oil showed the highest ($P < 0.05$) fat digestibility.

Table 4.5:

Influence of grain type and fat source on coefficient of apparent digestibility of fat in different segments of the digestive tract in male broilers at 21 days post-hatch^{a}

Grain type	Fat source	Gizzard	Duodenum	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum
Maize	No fat	-2.605e	-2.479	-0.024	0.474c	0.682	0.731
	Soybean oil	-0.299bc	-0.458	0.471	0.784ab	0.896	0.907
	Tallow	-0.368c	-0.588	0.545	0.778ab	0.847	0.880
Wheat	No fat	0.754d-	-3.626	-0.273	0.313d	0.552	0.647
	Soybean oil	0.177a	-0.331	0.397	0.794a	0.846	0.857
	Tallow	-0.066ab	-0.778	0.369	0.699b	0.738	
							0.795
SEM^{b}		0.0901	0.3589	0.0867	0.0312	0.0268	0.0217
Main effects							
Grain type							

Maize	1.091-	-1.175	0.331a	0.679	0.808a	0.839a
Wheat	0.214-	-1.578	0.164b	0.602	0.712b	0.766b
Fat source						
No fat	1.679-	-3.052b	-0.149b	0.394	0.617c	0.689c
Soybean oil	0.061-	-0.395a	0.434a	0.789	0.871a	0.882a
Tallow	0.217-	-0.683a	0.457a	0.738	0.793b	0.837b
Probabilities, P						
Grain type	0.001	0.179	0.025	0.005	0.001	0.001
Fat source	0.001	0.001	0.001	0.001	0.001	0.001
Grain type x fat source	0.001	0.198	0.605	0.035	0.320	0.653

.(Means in a column not sharing a common letter (a-d) are significantly different (P < 0.05

.[a] Each value represents the mean of six replicates of eight birds per replicate

.[b] Pooled standard error of mean

4.4.5 Gross energy digestibility

A significant ($P < 0.05$) interaction between the grain type and fat source was observed for CAD of GE in gizzard (Table 4.6). In wheat-based diets, there was no effect of fat source on GE digestibility ($P > 0.05$), but in maize-based diets, soybean oil increased ($P < 0.05$) CAID of GE compared to the diets with no fat inclusion. Feeding maize-based diets resulted in higher ($P < 0.05$) GE digestibility in both segments of the ileum. Fat source had a significant effect on GE digestibility in the upper jejunum ($P < 0.01$) and upper ileum ($P < 0.05$). Whilst only tallow improved ($P < 0.05$) digestibility of GE in upper jejunum, both fat sources increased ($P < 0.05$) CAD of GE at the upper part of ileum

Table 4.6:

Influence of grain type and fat source on coefficient of apparent digestibility of gross energy[a] in different segments of the digestive tract in male broilers at 21 days post-hatch[b]

Grain type	Fat source	Gizzard	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum
Maize	No fat	-1.242c	0.232	0.572	0.682	0.755
	Soybean oil	-0.959b	0.249	0.539	0.702	0.770
	Tallow	-1.081bc	0.366	0.560	0.712	0.761
Wheat	No fat	-0.415a	0.144	0.487	0.600	0.676
	Soybean oil	-0.517a	0.201	0.525	0.656	0.703
	Tallow	-0.516a	0.327	0.556	0.632	0.675
SEM[c]		0.0678	0.0463	0.0212	0.0128	0.0105
Main effects						
Grain type						
Maize		-1.094	0.282	0.557	0.699a	0.762a

Wheat	-0.483	0.224	0.522	0.629b	0.685b
Fat source					
No fat	-0.829	0.188b	0.529	0.641b	0.716
Soybean oil	-0.738	0.225b	0.532	0.679a	0.736
Tallow	-0.799	0.347a	0.558	0.672a	0.718
Probabilities, P					
Grain type	0.001	0.136	0.056	0.001	0.001
Fat source	0.407	0.005	0.330	0.013	0.114
Grain type x fat source	0.024	0.850	0.132	0.305	0.664

Means in a column not sharing a common letter (a-c) are significantly different ($P < 0.05$).

[a] Gross energy content could not be analyzed in duodenum due to low quantity of digesta samples.

[b] Each value represents the mean of six replicates of eight birds per replicate.

[c] Pooled standard error of mean.

4.4.6 Amino acid digestibility at the ileal level

Influence of dietary treatments on ileal digestibility of indispensable and dispensable AA is shown in Tables 4.7 and 4.8, respectively. The main effect of grain type was significant ($P < 0.01$ to 0.001) for the digestibility of all AA, except proline. All AA were more digested ($P < 0.05$) in birds fed maize-based diets compared to those fed wheat-based diets. Neither the main effect of fat source nor the grain type x fat source interaction was significant ($P > 0.05$) for any of the AA.

Table 4.7: Influence of grain type and fat source on coefficient of apparent ileal digestibility of indispe

21 days post-hatch[a]

Grain type	Fat source	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl ne
Maize	No fat	0.909	0.856	0.833	0.851	0.844	0.858	0.868
	Soybean oil	0.910	0.857	0.840	0.856	0.853	0.853	0.878
	Tallow	0.914	0.869	0.851	0.866	0.865	0.862	0.883
Wheat	No fat	0.850	0.800	0.789	0.794	0.782	0.788	0.826
	Soybean oil	0.858	0.815	0.798	0.807	0.785	0.786	0.838
	Tallow	0.856	0.810	0.805	0.810	0.794	0.795	0.836
SEM[b]		0.0099	0.0101	0.0112	0.0107	0.0139	0.0153	0.0093
Main effects								
Grain type								
Maize		0.911a	0.861a	0.841a	0.858a	0.854a	0.858a	0.876a

Wheat	0.855b	0.808b	0.797b	0.803b	0.787b	0.790b	0.833b
Fat source							
No fat	0.879	0.828	0.811	0.822	0.813	0.823	0.847
Soybean oil	0.884	0.836	0.819	0.831	0.819	0.820	0.858
Tallow	0.885	0.839	0.828	0.838	0.830	0.829	0.860
Probabilities, P ≤							
Grain type	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Fat source	0.827	0.513	0.339	0.361	0.470	0.837	0.320
Grain type x fat source	0.931	0.704	0.986	0.912	0.937	0.988	0.904

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).

[a] Each value represents the mean of six replicates (eight birds per replicate).

[b] Pooled standard error of mean.

Table 4.8: Influence of grain type and fat source on coefficient of apparent ileal digestibility of dispe broilers at 21 days post-hatch[a]

Grain type	Fat source	Alanine	Aspartic acid	Cysteine[b]	Glycine[b]	Glutamic acid	P
Maize	No fat	0.831	0.843	0.762	0.789	0.884	0
	Soybean oil	0.831	0.845	0.748	0.795	0.886	0
	Tallow	0.845	0.853	0.769	0.810	0.898	0
Wheat	No fat	0.737	0.779	0.712	0.734	0.863	0
	Soybean oil	0.750	0.791	0.731	0.747	0.875	0
	Tallow	0.755	0.788	0.731	0.751	0.869	0
SEM[c]		0.0134	0.0110	0.0137	0.0125	0.0075	0
Main effects							
Grain type							
Maize		0.835a	0.847a	0.759a	0.798a	0.889a	0
Wheat		0.747b	0.786b	0.725b	0.744b	0.869b	0

Fat source							
	No fat	0.784	0.811	0.737	0.761	0.873	0
	Soybean oil	0.790	0.818	0.740	0.771	0.881	0
	Tallow	0.800	0.820	0.750	0.781	0.884	0
Probabilities, P ≤							
Grain type		0.001	0.001	0.004	0.001	0.003	0
Fat source		0.493	0.685	0.614	0.309	0.382	0
Grain type x fat source		0.886	0.848	0.502	0.903	0.479	0

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).

[a] Each value represents the mean of six replicates (eight birds per replicate).

[b] Semi-indispensable amino acids for poultry.

[c] Pooled standard error of mean.

4.5 Discussion

4.5.1 Dry matter digestibility

It has been established that maize has superior dry matter digestibility as compared to wheat. (Lei et al., 2017). This is likely due to the high amount of indigestible NSP in wheat as compared to maize (Annison, 1991). In the current experiment, digestibility data in most intestinal segments indicated a higher CAD for maize over wheat, which is consistent with the higher concentration of viscous NSP in wheat than maize (Angkanaporn et al., 1994). These NSP will increase the total dry matter content of the feed, but are largely indigestible in poultry (Smits and Annision, 1996). As a result, the overall dry matter digestibility of wheat should be lower than maize in all diets. NSPs such as ß-glucans and arabinoxylans have been shown to increase digesta viscosity and lower bird growth rates as well (Petersen et al., 1999). These NSP may also cause inhibition of absorption of a number of dry matter components, and thus the wheat diets would have lower overall dry matter digestibility than the maize. The observed results do indicate lower overall DM absorption in wheat, due to the lack of inclusion of enzymes meant to break down the NSP (Angkanaporn et al., 1994; Lei et al., 2017)

For all diets, increasing rates of absorption occurred as digesta moved along the lower intestinal segments, reaching the highest rate of digestibility at the lower ileum. It has been observed that the jejunum and ileum are the major sites of dry matter absorption (Renner, 1961; Vanderklis, 1990). The results of the current experiment reflect this, as dry matter digestibility generally peaked at the lower ileum in all diets.

The upper ileum showed significant differences in digestibility among the diets with different fat sources. There was no significant difference between tallow-containing diets and soybean oil-containing diets in DM digestibility, however, both showed improved digestibility over the diets with no added fat. This effect could be due to increased digesta retention time in digestive tract as a result of fat inclusion (Imbeach and Sauer, 1991). Imbeach and Sauer (1991) reported an increased AA digestibility and decreased rate of passage in pigs fed increasing level of dietary fat in the form of soybean oil and canola oil. In contrast, Li et al. (1990) found a decrease in DM digestibility in pigs fed supplementary coconut oil, soybean oil and white grease as opposed to the control. It is, however, unknown how applicable these results are to chickens, which notably have a faster digesta passage rate than pigs (Pan and Yu, 2014) with a total tract retention time of only 3.5 hours (Hughes, 2008). Li et al. (1990) place importance on the composition of the fat added in terms of the effect of nutrient digestibility, however in this experiment, there was no significant difference in DM digestibility between tallow or soybean oil. Harkins et al. (1964), in a study with rats, found that the long-chain fatty acids, including soybean oil, showed longer gut retention time than short-chain fatty acids more similar to tallow. A study in chickens by Jimenez-Moreno et al. (2009) found a higher DM digestibility in chickens fed diets containing soybean oil than yellow grease. The yellow grease is similar in composition, though not identical, to tallow, being composed largely of short-chain fatty acids and saturated fats. It is known that nutrient digestibility is markedly influenced by the age of the birds**.** Jimenez-Moreno et al. (2009) reported a significant interaction between bird age and fat source. Their results indicated an increase in DM digestibility with age in the yellow grease diet exceeding that of the soybean oil diet. If this observation also holds true for tallow, it is possible that the use of older birds (22 d) in the present experiment compared to 5 and 15 d old birds in the study by Jimenez-Moreno et al. (2009), allowed for the DM digestibility of tallow to be similar to that of soybean oil at upper ileum.

The DM digestibility results were all negative in gizzard, indicating net secretion. This makes sense, as the gizzard is primarily a site of digestion, not absorption (Hurwitz et al., 1979; Sklan et al., 1973). Observed gizzard effects thus, are less relevant and might also be attributable to limitations of the measurement techniques.

4.5.2 Nitrogen digestibility

In the upper and lower ileum, maize-based diets had superior digestibility values as compared to wheat-based diets. Larsen et al. (1994) found that NSP content did not seem to affect the N digestibility of rats fed high-fiber diets. However, there was an increase in digestive enzyme secretion caused by the NSP content. Romero et al. (2014) found lower N digestibility in high-NSP diets. They speculated that increases in endogenous secretions caused a decrease in digestibility values, despite digestibility itself not being directly affected by the NSP.

In general, tallow-included diets had the highest N digestibility values than soybean oil and no fat diets. In most cases the digestibility values of the soybean oil were not significantly different than the no-fat diets. Allahyari-Bake and Jahanian (2017) compared the effect of soybean oil and palm oil on protein digestibility in the ileum of broilers, and reported higher protein digestibility in soybean oil diets as compared to the palm oil. This contradicts the present results, as palm oil is, like tallow, a fat source high in saturated fatty acids. The results of the present study also contradict the study by Jimenez-Moreno et al. (2009) who reported a higher nitrogen digestibility for the soybean oil diets over yellow grease containing diets. It is unclear why these differences existed. It should be noted that although tallow, yellow grease and palm oil are all primarily composed of saturated fatty acids, their component fatty acids do feature other differences, such as chain length and structural composition. It is possible that these differences were more important in explaining these differing results than saturation.

Tallow's composition is higher in saturated fatty acids than soybean oil. Saturated fatty acids are less readily digestible by chickens than unsaturated fatty acids, with tallow being less easily digestible than soybean oil (Tancharoenrat, et al., 2014). This is due to the fact that unsaturated fatty acids can more easily form micelles in the intestine (Baião and Lara, 2005). Keeping this in mind, tallow's lower ability to form micelles, it has been speculated that fat sources like tallow increase total gut transit time more than fat sources like soybean oil (Knarreborg, et al., 2002). Overall, it has been observed that fat will increase overall gut retention time in monogastric animals (Hunt and Knox, 1968). This likely translates into an increased digestibility of many nutrients, including protein (Merriman et al., 2016). This increased transit time might allow for more time for protein to be observed by the intestinal lumen, and this increase overall digestibility.

4.5.3 Starch digestibility

As with previous nutrients, the superior digestibility of starch in maize diets in the ileum was expected (Weurding, et al., 2001). Starch digestibility is particularly compromised by phytate (Adedokun, et al., 2011). Wheat is known to contain higher amounts of NSP than maize (Ravindrin et al., 2016).

4.5.4 Fat digestibility

Higher fat digestibility for maize-based diets over wheat-based diets that was observed in upper jejunum and both ileum segments might be mostly explained by the higher NSP content in wheat-based diets. In all segments, diets containing no additional fat had lower fat digestibility, indicating that fat digestibility increases by inclusion of dietary fat. This is likely due to an increased secretion of lipase and other pancreatic secretions in response to higher detected fat levels in the gut (Krogdahl, 1985). Interestingly, there was no difference in fat digestibility between soybean oil and tallow in duodenum and upper jejunum. However, fat originated from soybean oil was more digested in upper and lower ileum than tallow or the diet with no fat, a finding corroborated by the literature. Sklan et al. (1973) reported higher fat absorption in jejunum and ileum of 19-day-old broilers fed a diet containing soybean oil over a diet without soybean oil. These researchers also reported an increased bile acid, fatty acid and cholesterol secretions in the duodenum in the diets with high fat inclusion, secreted from the pancreas and gall bladder.

These results indicate that while there was no significant difference in soybean and tallow digestibility in maize-based diets, wheat-based diets showed inferior tallow digestibility. This is in alignment with results from Tancharoenrat et al. (2015). The superior digestibility of soybean oil over tallow in wheat-based diets was thought to be due to an interaction between NSP present in wheat and saturated fats in tallow. NSPs are known to create insoluble gels, blocking digestion (Hughs, 2008). Saturated fats are less easily digested than unsaturated fats due to being less able to form micelles (Ravindran et al., 2016). It was speculated that these two effects compounded, making digestion of tallow less efficient, but only in the presence of NSPs contained in wheat, as maize contains fewer insoluble NSP (Tancharoenrat et al., 2015).

4.5.5 Gross energy digestibility

Observed grain effects in the gizzard, upper ileum and lower ileum are once again consistent with previous data, with higher gross energy digestibility being displayed in the maize-based diets. It is known that wheat-based diets have a slower rate of passage in the gut (Hughs, 2008), due to NSPs in the wheat forming viscous gels that block enzymatic action (Hughes, 2008, Smits and Annison, 1996). Hughs (2008) did in fact find that increased gut transit time afforded by a wheat diet did increase total metabolized energy, indicating that increased digesta viscosity and gut transit time may have nutritional benefits, likely due to increases in digestive secretions (Smits and Annison, 1996). NSP also increase gut transit time, and it has been observed that certain NSP components actually increase growth performance by this mechanism (Jimenez-Moreno et al., 2009) while other specific NSP increase viscosity while also decreasing nutrient digestibility (Angkanaporn et al., 1994).

When looking at the effect NSP have on gross energy digestibility, there are two simultaneous effects. All NSPs slow the overall rate of passage, giving the intestine more time to absorb digesta, and increasing gross energy availability of the digesta. On the other hand, some of these NSP, such as arabinoxylans and ß-glucan, form viscous gels within the gut, which decreases gut transit time, but also inhibits digestive action (Hughs, 2008). Other NSPs, or dietary fat, might increase transit time without forming these inhibitory gels, decreasing transit time without blocking the action of digestive enzymes. In the Hughs (2008) experiment, the cultivar of wheat used was particularly low in insoluble NSP, so while gut transit time decreased in wheat diets, there was less inhibition of digestion due to lower gel formation.

Significant effects from the fat source were observed in the gizzard, upper jejunum and upper ileum. An interaction between grain source and fat type was observed in the gizzard. In the upper jejunum, tallow and no-fat diets showed the greatest and lowest energy digestibility, respectively, with soybean oil being intermediate. This contradicts previous results from Thacker et al. (1994) who showed that diets containing canola oil displayed better GE digestibility than those containing tallow. It should be noted that the Thacker experiment used the fecal sampling method as opposed to the ileal sampling method. With a significant amount of energy metabolism occurring in the hindgut, these results may be less applicable to in this situation. A number of studies in pigs (Li et al., 1990; Merriman et al., 2016) also indicated greater GE digestibility when using fat sources closer in composition to soybean oil over sources more resembling tallow. It is unknown as to why the results in the present study differ from these studies; however, factors such as species and age might be partly responsible for these discrepancies. This study was also conducted in pigs, and these effects may differ in chickens. Moreover, fat sources used in these studies, while similar to tallow and soybean oil in composition, are not identical, and, therefore, these compositional differences may factor in to these discrepancies.

A difference in gut transit time may also explain the fact that diets with added fat had higher gross energy digestibility as compared to the no-fat diets. The study by Hughs (2008) showed a positive linear relationship between gut transit time and gross energy digestibility, meaning that the longer it took gut contents to pass through the intestinal tract, the more energy was absorbed by the bird. This study did use added NSP as the dietary ingredient that increased gut transit time, however dietary fats have also been known to slow gut transit time in rats and pigs (Harkins et al., 1964; Merriman, et al., 2016). In humans, dietary fats are also found to slow gastric emptying due to interactions with intestinal receptors (Hunt and Knox, 1967). If such receptors exist in the chicken intestine, this delayed gastric emptying would also allow more time for enzymatic activity to break down starches, and more time for the intestine to absorb gut contents, increasing energy digestibility.

4.5.6 Amino acid digestibility at the ileal level

With the exception of proline, ileal digestibility of all AA was higher in maize- than wheat-based diets. This is in line with previous literature showing that wheat-based diets have inferior protein digestibility to maize-based diets (Liu et al., 2014). Proline is an AA common in mucoproteins (Ravindran and Hendriks, 2004). This could be explained by both maize and wheat having similar effects on mucus secretion. The lack of fat source effect on AA digestibility at lower ileum is in line with the N digestibility in the current study, where there was no difference between different fat sources. Interestingly, there were significant effects of fat sources on N digestibility in both jejunum segments and the upper ileum, but these differences disappeared at the distal ileum. The majority of absorption of all nutrients, including AA, occurring in the jejunum (Sklan et al., 1973), with smaller amounts in the upper ileum and even less absorption in the lower ileum. It is possible that fat source may have governed the absorption of AA in the jejunum or upper ileum, but the effect was less detectable in the lower ileum.

4.5 Conclusion

The main focus of the present study was to examine nitrogen, dry matter and specific amino acid digestibility, particularly in the jejunum and ileum. Due to the fact that the gizzard and duodenum are primarily sites of digestion, and not absorption (Hurwitz et al, 1979; Sklan et al, 1973) and due to limitations of the titanium oxide marker method, results from these segments are less applicable to the overall results.

The general trend indicates that the presence of tallow in the diet increases digestibility significantly compared to diets without additional fat. The soybean oil diets generally had intermediary digestibility between the tallow and no-fat diets. Previous studies with similar fat sources indicated the opposite would be true. Differing digestibility of the different fat sources could likely be governing this effect. Future studies could involve birds of differing ages, as well as a more diverse set of fat sources to better determine which factors are affecting these digestibility rates.

The speculated mechanism of action for this increased digestibility is an increase in digesta transit time caused by the increased fat content. Future studies could confirm this by using a marker technique to trace the rate of passage in the digestive tract between the different diets.

Differences and digestibility between maize and wheat diets were expected, but it was unknown if there would be any major interactions between grain type and fat source in the diet in nitrogen and dry matter digestibility. The present study found no interaction. The decreases in digestibility seen by high NSP ingredients like wheat is likely related to the creation of insoluble gels in the gut (Smits and Annison, 1996). Thus, while fat and these gels may both increase the viscosity of gut contents, the composition of the viscous contents differ substantially, with the fat having less of an inhibitory effect on amino acid digestibility. The present study found no substantial interaction effects in the lower digestive segments between the grain effects (caused by NSP content) and fat effects. This indicates that, while the addition of both can alter digestive viscosity, there is no inhibitory effect exerted by either on the other.

No significant effect of fat source was found on amino acid digestibility. This may be due to the fact that these samples were taken from the lower ileum, and the majority of amino acid absorption may have taken place in more proximal segments.

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