

FURTHER EVALUATION OF A SLOPE-RATIO PRECISION-FED ROOSTER ASSAY FOR
RELATIVE BIOAVAILABLE ENERGY VALUES FOR FATS AND OILS

BY

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THESIS

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ABSTRACT

Five slope-ratio precision-fed rooster assays and one limit-fed chick growth assay were conducted using different fats/oils at several increasing dietary levels to determine relative bioavailable energy values. In Experiment 1, roosters were precision-fed 0, 5, or 10% of five different fats as part of a ground corn diet totaling 30 g of diet per rooster. Refined soy oil (the reference oil), poultry fat, crude soy oil, crude corn oil, and palm oil 1 were all evaluated. In Experiment 2, roosters were precision-fed 0, 5, or 10% of either a refined corn oil set at 100% (RCO), a vegetable acid oil, or a soy acid oil as part of a ground corn diet. In Experiment 3, roosters were precision-fed 0, 5, 10, 15, or 20% of either RCO, palm oil 1, tallow, or choice white grease as part of a ground corn diet. In Experiment 4, roosters were precision-fed 0, 5, 15, or 20% of either a vegetable-blend acid oil or palm oil 2 as part of a ground corn diet to determine if 20% lipid inclusion was physically possible to feed and if a linear response in nitrogen-corrected true metabolizable energy (TMEn) would be obtained. Experiment 5 was a limit-fed chick growth assay conducted using Ross 308 broilers. The broilers were limit-fed to 70% of their expected daily intake from 11 to 20 d of age and four different lipid sources (RCO, palm oil 2, choice white grease, and an acid oil) were tested at 0, 5, and 10% inclusion levels. Diets consisted of graded levels of supplemental lipid added to a corn-soy basal diet at the expense of cellulose. The TMEn of diets (rooster assays) or body weight (chick assay) gain of chicks were regressed on supplemental lipid level or intake using multiple linear regression. Relative bioavailability values (RBV) for each lipid were calculated as the regression coefficient of the test lipid divided by the regression coefficient of the reference lipid (RSO or RCO). In Experiment 1, only the RBV of palm oil 1 (82.5%) was significantly lower ($P < 0.05$) than soy oil (100%). In Experiment 2, no significant

differences ($P > 0.05$) in RBV were observed for the vegetable acid oil or the soy acid oil and RCO. In Experiment 3, both the RBV of tallow (74.4%) and palm oil 1 (82.9%) were significantly lower ($P < 0.05$) than RCO (100%), while the RBV of choice white grease (98.7%) was not significantly different ($P > 0.05$) than RCO. It was observed that the differences among fat sources were often greater at the higher inclusion levels of 15 and 20% in the rooster assay. In Experiment 4, high linearity was observed for 0-20% levels of palm oil 2 and the vegetable blend acid oil ($R^2 = 0.94-0.97$). In Experiment 5, the RBV of palm oil 2 (80.5%) and choice white grease (85.4%) were significantly lower ($P < 0.05$) than the 100% for RCO, whereas the RBV of the vegetable blend acid oil (92.7%) was not significantly ($P > 0.05$) different from 100%. In general, the rooster results agreed well with the results obtained in the chick growth assay; however, using higher inclusion rates of 15 or 20% of the fats may increase the sensitivity of the rooster assay in detecting differences among lipid sources. A slope-ratio precision-fed rooster assay was also conducted to determine the relative metabolizable energy (ME) values of RCO and four test lipids, canola oil, poultry fat, oxidized canola oil, and oxidized poultry fat. Oxidation of the lipids was achieved by cyclic heating of the lipids for 60 h at 90 °C with ambient air being percolated through the lipids at 12 liters per min. The RBV of the lipids varied from 86.3% to 104%, but none of the test fat RBVs were significantly lower ($P < 0.05$) than the refined corn oil reference (100%); although the RBV of both the canola oil and poultry fat were numerically reduced by 11 percentage units by oxidation. Thus, the results of this study indicated that oxidization of the canola oil and poultry fat did not significantly reduce the RBV of the lipids.

DEDICATION

This thesis is dedicated first and foremost to my wife, Alexandra Jebe von Schaumburg, for her consistent love and aid. It is also dedicated to my mother and father, Terri and Gregory von Schaumburg. Thanks to their support of my education and constant caring, I am able to achieve my goals. Without the three of them, none of this would have been possible.

“The scientist only imposes two things, namely truth and sincerity, imposes them upon himself and upon other scientists.”

- Erwin Schrodinger

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CHAPTER 1:

THE USE OF LIPIDS IN POULTRY DIETS: A LITERATURE REVIEW

INTRODUCTION

While lipids have experienced some undesirable critiques in recent years, the benefits provided by inclusion into poultry diets is undeniable. Dietary fats provide a concentrated source of energy, supply essential fatty acids, serve as carriers for fat soluble vitamins, reduce dustiness, improve palatability, lower particle separation in mash diets, and lubricate feed mill equipment (Ravindran et al., 2016; Das et al., 2014a). Typically, the broiler industry uses about 0.5 to 4% lipid supplementation, the layer industry uses about 0 to 3% lipid supplementation, and the turkey industry uses about 3 to 12% lipid supplementation. Ideally, all lipid sources would be equally utilized and available to the bird, and methods of discerning differences between the sources would be unnecessary. Unfortunately, this is not the case, and lipids vary in energy content and ability to improve growth or egg production (Kim et al., 2013). Due to the complex nature of fats and oils, methods to quickly and easily evaluate differences in utilization between lipid sources are needed. Contained in this review is a brief overview of information that may provide insights on the complexity of lipids and challenges in generating consistent energy values for fats and oils for use in feed formulation.

ROLES OF LIPIDS IN POULTRY NUTRITION

Fat soluble vitamins are necessary in decreasing susceptibility to infection, particularly vitamin A, which may play a secondary role in systemic immune response (Das et al., 2014b). Perween et al. (2016), found that a high caloric (3000 kcal ME/kg) and protein (19% crude protein,

CP) diet may aid in improving immune response in Vanaraja chickens. As the level of protein and energy increased, the level of antibody titer against Newcastle disease virus gradually increased. However, the improved immune response may be due to increased nutrient utilization, which in turn leads to better weight gain and health. This may not be as applicable to developed poultry industries, since birds are commonly vaccinated against Newcastle disease. Nonetheless, this study may lead to further research on the role of lipids in other immune-compromising diseases.

The use of lipids that are high in vitamin A and E, like palm, soybean, and fish oil (Das et al., 2014b,c), have antioxidant benefits that aid to their stability (Das et al., 2014c). The use of antioxidants and stabilizers are gaining popularity in the industry because lipid oxidation is a major problem, especially in poultry meat, in both the industry and for consumers (Kang et al., 2001). It increases the development of rancid flavors and odors, as well as shortens the shelf-life of the products (Bayraktar et al., 2011; Kang et al., 2001). Plasma malondialdehyde levels, which is the end product of the breakdown of lipids due to oxidative stress, were slightly increased in broilers fed oxidized oil compared to fresh oil (Bayraktar et al., 2011). Though, it did not seem the basal diet had sufficient vitamin E to ensure optimum protection against the negative effects of oxidized oil. Supplementation of 200 mg/kg of vitamin E, however, alleviated some of the negative effects of oxidized oils, as well as increased the color stability in meat by delaying the oxidation of red oxymyoglobin (preferred) to brown metmyoglobin (not preferred) (Bayraktar et al., 2011).

Inclusion of supplemental lipids, often, increase digestion of all other nutrients in the diet. This is caused by increased gut transit time (Baião & Lara, 2005).

While the importance of fat soluble vitamins and increased digestion of nutrients is undeniable, the main incentive for supplemental lipid inclusion is that lipids contain the highest caloric value among all ingredients and are a dense source of energy (Das et al., 2014c). Dietary lipid supplementation is commonly recognized to improve feed efficiency and to reduce dust formation, as well as increase weight gain. However, not all lipids are equal in their ability to improve feed efficiency and growth (NRC, 1994), which further reiterates the need for a reliable and repeatable method of determining the differences in available energy content among various lipid sources.

The inclusion of supplemental lipids has not always been viewed to have beneficial effects in poultry nutrition. For optimum intake of nutrients through feed consumption, a proper calorie to protein balance is necessary (Perween et al., 2016). If the balance between energy and crude protein (CP) is not held constant, then the dietary inclusion of lipids may have a detrimental effect on weight gain, as well as, feed conversion rates. This is due to the fact that birds will eat to meet their energy requirement. Since lipids have a much higher energy content than carbohydrates or proteins, the birds will meet their energy threshold but will be undernourished in other key nutrients. If the balance between energy and protein is held constant, then the bird will eat to meet the energy requirement and be sufficient in other key nutrient areas (Leeson et al., 1996). In high heat and humidity conditions, 30-35 C° and 50-60% humidity, a reduction of 0.50% in CP and 50 kcal/kg ME did not depress growth, decreased production cost and increased profits (Sarwar et al., 2015).

Supplemental lipids are an efficient way of increasing the birds' ability to convert feed into a useable product such as meat or eggs. As mentioned previously, poultry generally eat to

meet their energy requirement, and diets containing supplemental lipids will, generally, have greater energy density per unit feed consumed than diets without supplemental lipid (Aardsma & Parsons, 2017). Therefore, less feed will need to be consumed per unit of production. While it is viewed as minor importance in the poultry industry, there is also interest in the increased palatability of diets based on lipid inclusion.

There has been increased interest in the use of supplemental lipids to produce functional foods. Essentially, the inclusion of certain lipids high in n-3 and n-6 fatty acids would lead to higher deposits in muscle tissue and eggs. This in turn would benefit the human food industry because of the nutritional factors associated with them, such as the crucial role n-3 fatty acids have in the immune system (Koppenol et al., 2015). Though controversial on total blood cholesterol levels, humans consuming n-3 fatty acid rich eggs over a span of two weeks through six months indicated an increase in serum alpha linoleic acid and DHA, as well as a decrease in triglycerides (Cherian, 2017).

Given that 30% of the broiler chicks' total lifespan is spent inside the hatching egg, it must rely heavily on the nutrients deposited in the egg by the breeder hen. Which means formulators must consider how the pre-hatch period will improve chick viability, hatchability, and embryonic health. (Cherian, 2015). Due to the fact that the first week of life is the most vulnerable period for a chick, the chick must go through many stressors that can affect its health. When hatched from n-3 fatty acid enriched eggs, the chicks retained higher levels of EPA, DHA, and total n-3 fatty acid in the tissues and cells when faced with an n-3 fatty acid deficient diet during growth (Cherian, 2015). These results show evidence that in ovo supply of n-3 poly unsaturated fatty acids (n3-PUFA) may extend the chicks' post-hatch life by 50% via passive immunity (Cherian,

2015). Further interest in the deposit of certain lipids into the egg via the mother hens nutrition can have an impact on human nutrition. If the deposition methods are understood more clearly, then those pathways may have similarities with a pregnant human and her deposition of fat into the breast milk (Cherian, 2015). However, Koppenol et al. (2015), found no interactions on performance between maternal supplemented and post-hatch n3-PUFA supplemented offspring. Feeding n3-PUFA post-hatch resulted in increased daily weight gain and feed intake, which suggests post-hatch supplementation of n3-PUFA is more beneficial and efficient when compared to maternal supplementation (Koppenol et al., 2015). Further studies using dietary n3-PUFA supplementation via maternal and post-hatch are needed to evaluate the effect of these fatty acids on performance, metabolic development, and immune organ weight; as well as, on chicks' immune system with induced inflammation.

As briefly discussed previously, the dietary inclusion of supplemental lipids on laying hens improves feed efficiency and could potentially increase egg weight (March & MacMillan, 1990). Though there has been a debate about whether the increase in egg weight was a cause of linoleic acid inclusion or if it was due to the increase in energy. As estrogen is known to regulate protein synthesis in the oviduct, a possible explanation of the effects of supplemental lipid on egg weight could involve estrogen. Chen et al. (2014) looked at the effects of estrogen treated capons, a castrated rooster. It seems as if the estrogen had a lipogenic effect based on the increased abdominal fat and breast subcutaneous fat content compared to other groups, and that estrogen changes the distribution of lipoproteins. Females and the estrogen treated capons had higher levels of very low-density lipoproteins (VLDL), which is the main transporter for triacylglycerol in the systemic circulation (Hermier, 1997), and this may explain the increase in fat content.

Estrogen also dramatically enhances hepatic lipogenesis in order to meet the demand for vitellogenesis in laying hens (Hermier, 1997). This leads to an increase in VLDL secretion and can lead to hepatic accumulation of triglycerides, and subsequently fatty liver, which occurs when the increase in lipogenesis exceeds the capacity of synthesis and secretion of lipoproteins (Hermier, 1997). It appears that the enzymes associated with hepatic lipogenesis may be inhibited with the inclusion of n-6 poly unsaturated fatty acids, especially linoleic acid. Royan et al. (2011) set out to discover the effects of fish oil, palm oil, and soybean oil on Peroxisome Proliferator-Activated Receptors (PPARs). The PPAR γ , is a transcription factor primarily responsible for increasing fat deposition, while PPAR α , is a transcription factor primarily responsible for increasing fatty acid oxidation. Palm oil showed an increase in adipose PPAR γ , which significantly increased abdominal fat pad deposition. It seems as though the polyunsaturated fatty acids of the soybean oil and fish oil had inhibitory effects on PPAR γ . Fish oil, or fish oil + conjugated linoleic acid, caused an up-regulation of PPAR α gene expression in liver tissue, suggesting that fish oil n3-PUFA can bind and activate PPAR α . This, in theory, leads to a lower accumulation of fat in the liver, and hopefully lessens the chance of fatty liver syndrome.

Just how different lipids have different impacts on feed efficiency, different lipids can have drastic differences on weight gain. It is even harder to understand the differences associated with weight gain compared to feed efficiencies. Young growing animals can be supplied with adequate amounts of all nutrients but restricted in energy. This would allow the animal to grow in direct proportion to added available energy, regardless of source. Though with this method, chicks need to be feed restricted due to the fact that they will eat to meet their

energy requirement. Theoretically, improved growth rates in diets supplemented with lipids shouldn't be different if the diets have the same energy to protein balance. However, that is not often the case. A partial explanation of this occurrence may be that the lipids provide a more palatable diet and/or have a greater energy density. Thus, the chicks might receive a higher intake of ME in lipid supplemented diets compared to non-supplemented diets. Turkey poult offered diets with supplemental animal-vegetable blended fat (10%) had significantly increased feed intake from 3 to 11 d of age compared to diets with no supplemental lipid, 88.3 g/bird and 71.1 g/bird at 9 to 11 d of age, respectively (Turner et al., 1999). This suggests that lipid supplementation may have at least a partial effect on palatability. However, if the factor of palatability is removed by equalizing feed intake, what remains is the possibility of extra caloric effects, extra metabolic effects, or an unidentified factor as the cause of improved growth.

Improved growth may also be a result of less energy lost as heat for broilers fed diets containing supplemental lipid (extra caloric effect), or increased digestibility on the non-supplemental portions of the diet when in the presence of supplemental lipid (Aardsma et al., 2017). The increased digestibility of non-supplemental portions of the diet while in the presence of a supplemental lipid is known as the extra metabolic effect (EME) of lipids. These factors will be covered in greater detail further on in this review.

The commercial poultry industry has long realized the benefits and importance of lipid supplementation in poultry nutrition. Feed efficiency, feed intake, and growth are all often positively affected by lipid supplementation. Lipids are also a viable source of fat-soluble vitamins and essential fatty acids, and are a concentrated source of energy, whose value in diet formulation is substantial. With so many benefits and no significant negative impacts on product

quality, when protein: fat ratios are maintained, it is no surprise that the beneficial effects of lipids in poultry diets are receiving increased recognition in the industry. Lipids need to continue to be studied to further our understanding of their complex nature and to discover more applicable uses.

CHEMICAL AND BIOCHEMICAL PROPERTIES OF LIPIDS

Among the different types of lipids taken in by the chick embryo, phospholipids serve as the essential structural precursors for membrane lipid bilayers, whereas triacylglycerol serves as a source of energy (Speake et al., 1998). While many biological molecules can be classified as lipids, this review will focus on the ones that are most applicable to poultry nutrition, namely fatty acids and triacylglycerols.

Different types of fatty acids can be identified by the length of the hydrocarbon chain and the number of double bonds. They all share a similar basic structure consisting of a carboxyl group and hydrocarbon chain. A triglyceride is composed of a glycerol backbone, and fatty acids are attached by ester bonds in three locations, sn-1, sn-2, and sn-3 (Small, 1991). The location of the ester bond can impact the available energy content of the lipid source, and it is thought that long-chain saturated fatty acids bound on the sn-2 position are absorbed more efficiently than those bound on the sn-1 and sn-3 position (Smink et al., 2008).

Saturation level is another way to distinguish lipid sources. Unsaturated indicates the presence of one or more double bonds, and saturated indicates the absence of double bonds in the hydrocarbon chain of fatty acids (Baião & Lara, 2005). Lipid sources from animals are usually coined “fats”, while lipids from plant sources are termed “oils”. Fats derived from animals tend to be more saturated in nature, meaning they contain less double bonds than oils, and appear to

have a lower available energy than plant sources. This is mainly due to the fact that plant sources usually contain a higher proportion of (poly) unsaturated fatty acids, as they are well utilized and absorbed by the animal as a source of energy (Huyghebaert et al., 1988). Recently, more evidence has indicated that the ratio between unsaturated and saturated (U:S) fatty acids within a lipid source, as well as in a mixed diet, is a reliable indicator of available energy content. This concept will be explained in further detail later in this review.

While the biological importance of essential fatty acids can be seen through the maintenance of selectively permeable membrane barriers, linoleic acid (18:2 n-6) and alpha linolenic acid (ALA 18:3 n-3) have to be supplied via the diet and are therefore deemed essential fatty acids (Cherian, 2015; Navidshad et al., 2015). However, the only essential fatty acid for which a dietary requirement has been demonstrated for is linoleic acid, and it is recommended that a minimum of 1% of linoleic acid is incorporated into chicken diets (NRC, 1994). Plant sourced lipids, corn or soy oil, are usually high in linoleic acid, and should be included in the diet to prevent any negative effects associated with linoleic deficiency. The most pronounced effect of a linoleic acid deficiency can be seen in the reproductive success of laying hens. Inclusion of a source of linoleic acid increased egg production, mass, fertility, and hatchability (March & MacMillan, 1990). While industry standard corn-soy diets usually provide sufficient linoleic acid content, the cereal grain base (e.g. corn, wheat, sorghum) can vary in linoleic acid content. Therefore, it is advised that at least 1% supplemental lipid originating from plant sources, corn or soy oil, be added to all poultry diets to ensure the linoleic acid requirement has been met (NRC, 1994). It should be noted that there is a debate over whether the inclusion of linoleic acid per se is the

cause of the previously observed increased production, or rather if just the increase in supplemental dietary energy was the cause.

Even though the energy content of lipids vary by source, on average lipids contain 2.25 times as much gross energy as carbohydrates and protein (NRC, 1994). This concentrated form of energy can be utilized in a myriad of ways, whether it be to sustain maintenance, growth, or production. Energy from lipids is indistinguishable from carbohydrates, on a per unit basis, and the increased energy concentration in lipids has distinct advantages. When a diet does not contain any supplemental fat, a significant portion of the lipid fraction is entrapped within the cell structure of the feed ingredients, making it less accessible to enzyme activity. This is especially true if the diet is in mash form (Mandalawi, 2017). Using supplemental lipids in diet formulation allows the diet to have a higher energy by weight than a diet without supplemental lipids, meaning that less feed will be required to obtain the same energy content on a weight basis.

LIPID DIGESTION AND ABSORPTION

Due to the hydrophobic nature of lipids, they require specific molecules and enzymes in order to be digested and absorbed from the intestine, which is an aqueous environment. To compensate for the limitation of a shorter digestive tract, birds utilize gut refluxes to increase feed retention time and to provide more time for digestion (Ravindran et al., 2016). The primary site of lipid digestion and absorption is in the small intestine, but the process of digestion technically starts in the gizzard, where monoglycerides from digesta are refluxed from the duodenum (Ravindran et al., 2016). Bile salts from the gallbladder, which are flat amphipathic molecules (one side non-polar and hydrophobic, the other side polar and hydrophilic), enter the

duodenum to initiate fat emulsification by acting as surfactants in the formation of emulsions with water (Ravindran et al., 2016). The quality of the surface of the emulsion depends on the source of monoacylglycerols, with monoacylglycerols added by hydrolysis of triacylglycerols being preferential to supplemental monoacylglycerols (Small, 1991). This is an essential stage because pancreatic lipase requires a water-lipid interface and is preferential to fatty acids in the sn-1 and sn-3 positions of the glycerol backbone (Ravindran et al., 2016). The positional distribution of fatty acids within the glyceride molecule is known to be an important factor contributing to differences in digestibility among lipid sources, due to pancreatic lipases specificity (Ravindran et al., 2016). The pancreas releases pancreatic lipase and colipase, necessary for optimum lipase activity, into the small intestine. Since colipase is rich in hydrophilic and hydrophobic amino acids, it allows lipase to maintain an active configuration at the lipid-water interface, which allows the enzyme to reach its substrate (Ravindran et al., 2016). In the duodenum of the chicken, triacylglycerol digestion starts with the hydrolysis of the molecule via pancreatic lipase, which results in two free fatty acids and a 2-monoacylglyceride (Small, 1991; Mandalawi, 2017). However, this process is not as successful in a young chick, who lacks the necessary amounts of bile salts and pancreatic lipases for adequate lipid digestibility. Short-chain fatty acids and glycerol have the benefit of being directly absorbed, while medium-chain fatty acids, long-chain unsaturated fatty acids, and monoglycerides need to form micelles with the aid of bile salts. These mixed lipid-bile salt micelles are key to the absorption of lipolysis end-products (Ravindran et al., 2016). The formation of micelles and their subsequent dispersal in the intestinal lumen, allows saturated fatty acids and other lipid-based materials that are not able to spontaneously form micelles, to be integrated into the micelles. While the micelle lipid contents

usually enter the enterocyte via passive diffusion (Zentek et al., 2011), there is some speculation of whether or not binding- and transport-proteins assist in the process. Regardless of the method, once the enterocyte is penetrated, the lipid contents are re-synthesized into triacylglycerols, which are assembled into chylomicrons, lipoprotein particles, and secreted into the lymph (Small, 1991; Zentek et al., 2011). Now that the lipoproteins are aqueously stable, they will be transported in the circulatory system and ultimately end up in the liver via the portal vein. This is because, in laying birds, more than 90% of lipid metabolism takes place in the liver (Zhang et al., 2011). However, specifically with unsaturated fatty acids, it may be speculated that as these fatty acids circulate through the blood, they are preferentially utilized as immediate energy source by the muscle tissue rather than being stored in the adipose tissue (Sanz et al., 2000). Depending on the status of the bird, once in the liver, the lipoproteins will either be catabolized to meet the immediate energy needs of the bird, or be re-directed and deposited as storage in adipose tissue. In birds, particularly during the laying period, VLDL are very important. This is because they will transport the fat from the liver to the ovary, to be used for egg yolk synthesis (Baião & Lara, 2005). If there is an abundance of exogenous lipid, fatty acid synthesis in the liver may be suppressed. This may also lead to direct fat deposits in the liver (Zhang et al., 2011), leading to fatty liver syndrome. Post-digestive process, the bile salts remain within the intestinal lumen where they are destined for one of two fates. The bile salts may either be recaptured and pass through the process of entero-hepatic recirculation (essentially the salts are absorbed and sent to the liver), or they pass through the digestive tract and are excreted in the feces. About 95% of bile salts are thought to be re-circulated post-process, and this recirculation is crucial for efficient digestion and absorption of fats (Ravindran et al., 2016).

LIPID CHARACTERISTICS THAT MAY IMPACT DIGESTION AND ABSORPTION

As mentioned throughout the earlier parts of this review, not all fatty acids are utilized equally in a lipid source. Fatty acid profile has profound effects on the available energy content of lipids, due in part to the synergistic and antagonistic relationships that different fatty acids display towards each other that impact total fatty acid absorption from the intestine (Aardsma et al., 2017). The main relationships to be discussed are between unsaturated and saturated lipid ratios. Complex relationships exist between fatty acids that affect the uptake and absorption of one another. Due to these synergistic and antagonistic interactions among fatty acids and the profile of surrounding fatty acids, the profiles of not only the lipid sources, but also of the diet itself, should be analyzed. While the lipid content of the carbohydrate-base of most poultry diets is minimal, it still contains fatty acids that may affect the energy availability value. The chemical composition of the dietary fat fraction, as well as degree of saturation, (supplied from supplemental plus non-supplemental lipid) have been shown to account for about 75% of the variation of AMEn values for lipids in 2-week old broilers (Ketels & De Groote, 1989) and is a major determinant of available energy content of lipids. In general, longer fatty acid chain length, higher lipid inclusion levels, and higher degree of saturation will all lower the digestibility of supplemental fats (Ravindran et al., 2016).

Chain Length

The presence of a double bond decreases the melting point, while the increase in the length of the carbonic chain of saturated fatty acids increases the melting point (Baião & Lara, 2005). Medium-chain fatty acids are saturated C6:0 to C12:0 fatty acids (Turner et al., 1999), and occur naturally as medium-chain triglycerides; they have a comparatively high solubility in water

and a low melting point. Mainly because of their size and solubility compared to long-chain fatty acids, medium-chain triglycerides are more easily digested and absorbed (Turner et al., 1999). Ketels and DeGroot (1988) showed this when coconut oil (44% C12:0) was 90% utilized in 2-week old broiler chicks compared to butter oil (22% C16:0; ~9% C18:0), which had a utilization of 80%. The medium-chain triglycerides allow rapid provision of energy for both enterocytes and intermediary hepatic metabolism and are effectively hydrolyzed by pancreatic and gastric lipases (Zentek et al., 2011). It has been suggested that medium chain fatty acids may be able to inactivate pathogenic viruses, parasites, and bacteria. Though, it can be assumed that the antibacterial effect of medium-chain fatty acids would be limited to the stomach and duodenum because they are normally found in the dissociated form at neutral pH and are rapidly absorbed (Zentek et al., 2011). Further speculation is that medium-chain triglycerides increase epithelial function, villus length, and crypt depth of the upper small intestine. The consequence behind the latter is that an increased absorptive surface could increase uptake and utilize nutrients more efficiently (Zentek et al., 2011), which could prove to be useful for a young bird whose digestive tract has not fully developed and may help an older bird maximize utilization of dietary compounds.

Turner et al. (1999) evaluated a commercial source of medium-chain triglycerides, containing approximately 76% of C8:0 fatty acids, in diets for turkey poults. This diet resulted in an apparent digestibility of lipid greater than 90%. In contrast to a 60% corn high-carbohydrate diet and a 10% animal-vegetable blend supplemented diet which had a percentage digestibility of lipid of 76.4% and 70.8%, respectively, at 6 to 8 d of age. The lower percentage digestibility of lipid in the carbohydrate diet and animal-vegetable blend diet can probably be attributed to the

concentration of C16:0 (70.4, 52.7%) and C18:0 (58.4, 26.8%), respectively. Most likely because at a young age, birds have a low bile salt and lipase production leading to poor solubilization and absorption of fatty acids, especially saturated (Turner et al., 1999; Ravindran et al., 2016).

Saturation Degree

One factor that distinctly affects fat utilization parameters is the degree of lipid saturation (Ketels & De Groote, 1989). Regarding the degree of saturation of dietary fatty acids, a few factors that affect variation of fat deposition are differential tissue uptake or metabolism of fatty acids and/or different signals for feedback regulation of endogenous synthesis (Sanz et al., 1999). It has been shown through digestibility balance experiments that the degree of saturation of a fat generally exerts a greater impact than the molecular structure of the fat (Vilarrasa et al., 2015). Saturation degrees are usually measured by iodine values (estimation of the double bonds present in the lipid (Baião & Lara, 2005), melting points, and/or fatty acid profiles. Typically, animal based lipid sources tend to have a higher saturation level and lower iodine value compared to their plant based counterparts. While the saturation level may provide useful information for predicting the available energy content of the lipid, the information is crude and imprecise. For example, research has shown that the arrangement of fatty acids on the glycerol portion of triglycerides of lipid sources with a similar saturation level may be utilized differently (Sibbald & Kramer, 1977; Smink et al., 2008) Saturation level is just one of the many factors that impact available energy content and bird performance.

One distinction of saturated fatty acid utilization in the bird is that availability is determined at least partially by other fatty acids in the diet. Unsaturated fatty acids are independently utilized, regardless of the surrounding fatty acids. The presence of saturated fatty

acids is not negative per se, as their utilization can be improved by altering the U:S fatty acid ratio in which they are fed (Aardsma et al., 2017)

With adequate inclusion of unsaturated fatty acids relative to saturated fatty acids in the diet, the utilization of the saturated fatty acids can be improved. When taking into account the contribution of the fatty acids in the basal portion of the diet, the AMEn and standardized lipid digestibility of lipids was maximized when the U:S ratio in the total diet was slightly greater than 4 (Ketels & De Groote, 1989; Aardsma et al., 2017). The latter indicates that the greatest utilization of long chain saturated fatty acids, such as palmitic and stearic acids, occurs when in an environment with a greater inclusion of unsaturated fatty acids, which is due to the excellent emulsifying capacities of the latter (Ketels & De Groote, 1989). The presence of unsaturated fatty acids and conjugated bile salts are required for proper saturated fatty acid digestion and absorption, especially for palmitic and stearic acids. This is because they are both non-polar and are unable to form mixed micelles spontaneously (Ravindran et al., 2016). One study concluded that ad libitum feeding of a diet rich in n-6 polyunsaturated fatty acids, rather than saturated fatty acids, increased the apparent digestibility of total dietary fatty acids and digestibility of crude fat (Wongsuthavas et al., 2011; Smink et al., 2008). However, Shin et al. (2011), found that when substituting conjugated linoleic acid or n-6 fatty acids for n-3 fatty acids, a decrease in absorption of linoleic and arachidonic acids in the small intestine occurred. This is most likely due to the n-3 very long chain fatty acids exerting a negative effect on delta-6-desaturase activity, which converts linoleic acid to arachidonic acid (both n-6 fatty acids).

Dietary Free Fatty Acid Content

While there are conflicting reports, generally, high levels of dietary free fatty acids are considered to have negative effects on bird performance and available energy content. Generally, studies where chicks were fed high levels of free fatty acids in the diet reported negative effects on either lipid digestibility, ME, or growth performance (Aardsma et al., 2017). Wu et al. (2011), showed a decrease in body weight gain as the free fatty acid level in yellow grease increased, probably due to a decrease in feed intake. Though, it seems that a low inclusion of free fatty acids does not seem to have any negative effects, as the low inclusion (~3% FFA) yellow grease diet yielded an overall body weight gain of 62.7 grams compared to the reference soy oil of 63.0 grams. This suggests that the energy in the medium (~13% FFA) and high (19% FFA) yellow grease inclusion diets in the Wu et al. (2011) study were not utilized as efficiently, and one possible explanation could be due to palatability issues. It should be noted that the low inclusion of yellow grease still resulted in a lower dressing percentage compared to the soy oil, most likely due to the higher concentration of saturated fatty acids.

A number of factors influence the effect that free fatty acids have on the available energy and on the bird itself. Free fatty acids are more difficult to absorb than other types of lipids, with a lower age causing a further decrease in the ability of the bird to tolerate and utilize free fatty acids. There is also speculation that there may be a potential negative interaction between the level of saturation and amount of free fatty acids due to the lack of monoglycerides to aid in the digestion of the saturated lipids. If the inclusion of free fatty acids is low enough, especially in the presence of unsaturated lipids, no negative effects may be seen. However, if the supplemental lipid has a high inclusion of free fatty acids, as well as a high level of saturation,

then negative growth effects may happen. In the presence of sufficient triglycerides, and subsequent monoglycerol molecules, saturation level of the lipid should be of less concern. Due to all these varying criteria, free fatty acid content is not a reliable predictor of available energy content in a lipid source or in poultry diets.

That is not to say that free fatty acid content of a lipid source should not be taken into account when formulating diets. Depending on the lipid content of the other ingredients, the free fatty acid content may be crucial information. Since common lipid sources that are high in free fatty acid content (acidulated oils, fryer grease) can usually be acquired for relatively cheap prices, usually 10 to 20% less expensive than refined lipids (Mendoza & van Heugten, 2014; Wu et al., 2011; Reaney, 2002). Soybean oil soapstock in the U.S. is generated at a rate of ~6% of the volume of crude oil produced, which ends up being about 1 billion pounds of soapstock produced annually (Reaney, 2002). However, the fatty acid profiles of recycled soybean oil, acidulated soybean oil soapstock, and fryer oils, depend on the processing and heating conditions applied during recycling and refining (Irandoust et al., 2012). Typically, acidulated oils are produced as a by-product to the soybean/vegetable oil industry. It is obtained through the alkaline neutralization of the raw oil, producing an unstable raw soap. This unstable product is then converted to acidulated soapstock after a treatment of sulfuric acid in a hot aqueous bath, which is then allowed to settle and the top layer is the desired fatty acid rich lipid product (Baião & Lara, 2005; Reaney, 2002). When processed properly, frying oils and acidulated oils should show no detrimental effects when supplemented into the diet. Unfortunately, the processing is not always done under sound industrial practices and commercial nutritionists should be attentive to a few things. If the diet will contain high levels of free fatty acids, then the diet should have moderate

to high levels of intact triglycerides. Though free fatty acid content can have greater negative effects on digestibility of saturated lipids, the ratio of unsaturated to saturated lipids should always be maintained to at least 4. Finally, as the free fatty acid content increases, either the total amount of supplemental lipid mixture needs to be increased to account for the reduced availability, or a lower available energy value needs to be attributed to the supplemental lipid source (Ravindran et al., 2016).

Position of Fatty Acid Attachment on Glycerol Molecule

The location of the glycerol portion of triglycerides can affect available energy content, even in lipid sources with similar fatty acid profiles. Digestibility of the component fatty acids and the degree/site of their attachment to the glycerol molecule largely governs the utilization of fats and oils by the chicken (Huyghebaert et al., 1988). Fatty acids can be attached at the sn-1, sn-2, or sn-3 position, and long-chain saturated fatty acids on the sn-1 and sn-3 positions are thought to be absorbed less efficiently than those bound to the sn-2 position, which is common in lard (Smink et al., 2008). Fatty acid incorporation into mixed micelles is enhanced by sn-2-monoacylglycerols because they are amphiphilic and natural emulsifiers (Ravindran et al., 2016). When palm oil was segregated to have a greater proportion of C16:0 located on the sn-2 position, the digestibility of C16:0 was calculated to be 89.5%; whereas native palm oil had a digestibility of 51.0% for C16:0 which was mainly on the sn-1 and sn-3. The latter can be explained by the more hydrophilic character of the sn-2 monoglyceride in comparison with, by lipase hydrolyzed, fatty acids from the sn-1 and sn-3 position of the glycerol backbone (Smink et al., 2008). In the event that pancreatic lipase is missing or insufficient, such as a very young chick, the position of the fatty acids on the glycerol moiety can impact the uptake of lipids.

Quality Characteristics

Moisture, impurities, and unsaponifiables, which are commonly reported together as MIU values, as well as peroxide values and stability values are common methods to measure quality characteristics of lipid sources (Wan, 2000; Gunstone, 2008). The MIU content is important as these components dilute the energy content of the lipid (Leeson & Summers, 2001). Peroxide values are a measure of how much oxidation (rancidity) has already occurred, and stability values indicate how susceptible the sample is to further oxidation (Gunstone, 2008). The major cause of loss of quality of an ingredient or ration is the process of oxidative rancidity or peroxidation and the consequent rancidification (Baião & Lara, 2005). Ideally, due to the oxidative capacity of the hydroperoxides, the peroxide values should be kept low (Huyghebaert et al., 1988). Though palatability is usually a lower concern in poultry than many other animals, samples with high levels of oxidation can have off tastes and unpleasant odors, as well as a reduced available energy content. Though many factors need to be considered, Bayraktar et al. (2011) noticed that body weight and feed conversion rates were not adversely affected by oxidized oil, which implies the oil did not alter digestibility of the diet, palatability, or odor. Use of fryer oils in diet formulation has seen an increase in recent years. When using these oils, the formulator should closely monitor the AME content, since the values tend to be more variable and lower; the decrease largely depends on the temperature of heating, the extent of time heated, and the fatty acid profile of the lipid itself (Huyghebaert et al., 1988). Unsaturated fatty acids are more susceptible to oxidative damage on the double bond of triglycerides, which can severely impact their available energy content (Ravindran et al., 2016; Huyghebaert et al., 1988). Fryer sources with long-chain unsaturated fatty acids may have greater saponification value due to the breakdown

of C18:2 fatty acids (Huyghebaert et al. 1988). If a lipid source has high oxidative damage, or is susceptible to high oxidative damage, an anti-oxidant supplement added to the lipid source is advised. The antioxidant will minimize damages to the susceptible unsaturated fatty acids. While the presence of high MIU values or oxidative damage in a lipid source does mean that the available energy content will probably be reduced, the absence of high MIU or oxidative damage does not guarantee high available energy content.

IMPACTS OF BIRD AGE

The ability to digest and absorb dietary fat is poorly developed in the newly hatched chick (Ravindran et al., 2016). Due to the insufficient bile salts and enzyme production in the young chick, it takes a few weeks before the body matures to digest lipid maximally. With increased age comes an increased ability to utilize most nutrients. The degree to which lipid digestibility is impeded by age is dependent on the source of the lipid. Saturated lipids, such as tallow, are much more difficult for the young chick to digest compared to an unsaturated source, like corn oil (Whitehead & Fisher, 1975). This was seen when 2-week old chicks utilized tallow 5-10% less efficiently than the same chicks did at 6-weeks of age or older, which demonstrated that the ME value of tallow is greatly affected by the age of the chick (Lessire et al., 1982). Age impacted the available energy content of some, but not all lipids, further complicating the generation of available energy values for lipids (Aardsma et al, 2017). Depending on the experiment and age of the birds, the energy values determined may not be applicable in all situations.

The first few weeks of life, chicks have a limited capacity to digest and absorb lipids. This is also a critical period for how the body will respond to dietary lipids later in life (Zhan et al., 2007). When broilers were feed-restricted from 1 to 21 d of age, lipid metabolism and growth

performance were altered, as seen by the decreased carcass yield, breast muscle and fat deposition in abdomen. Though, near full compensatory growth was achieved when the feed restriction was discontinued from 22 to 63 d of age. However, the feed-restricted broilers had significantly higher levels of lipid synthesis which resulted in much more fatty and obese birds. It appears that post-feed-restriction, broilers enhanced their fat deposition when they had free access to feed (Zhan et al., 2007).

Acclimation to the mixed diet itself also appears to have some effect on the utilization of fats and oils. Lipid digestion can be impacted by physiological mechanisms and processes within the bird (Aardsma et al., 2017). When chicks were given longer time to acclimate to a diet, it seems likely that the increased acclimation time allowed for increased physiological adaptation to the diet, allowing greater utilization of the fatty acids when compared to chicks with less acclimation time. Though, the increase in utilization may have arisen from the increase in age, rather than acclimation.

It seems that the age of the chick, and how long the chicks are fed the diet, affect lipid digestion and absorption. This is especially true for saturated lipids, as digestibility of palmitic and stearic acids increased in older birds. While unsaturated lipids seem to have a slight increase in availability as the bird ages, saturated lipids had a much more pronounced effect. Shown by Whitehead and Fisher (1975), turkey poults had fat absorption of 57.3% at 2 weeks of age when fed tallow, but that value increased to 73.5% at 8 weeks of age. In contrast, age did not affect absorbability of corn oil which was 95.7% and 97.8% at 2 and 8 weeks of age, respectively (Whitehead & Fisher, 1975).

As previously stated, highly saturated lipids usually have the lowest availability, especially in very young poultry but increases with age (Turner et al., 1999). The decrease in digestion and absorption can largely be attributed to the high inclusion of C16:0 and C18:0 of relatively saturated sources (Turner et al., 1999). Since the bile production in very young chicks is so low, many consider bile to be the limiting factor in lipid digestion. Without proper bile production and quantities, lipids in the intestine cannot adequately form micelles, and a lack of micelles means a drastically impaired lipid digestion. Though bile is very important, the level of importance varies depending on the type of fatty acid. Short-chain fatty acids do not require bile at all, while long-chain fatty acids need bile salts to aid in absorption more so than medium-chain fatty acids. This is due to the insolubility of long-chain triglycerides, requiring the aid of bile salts for emulsification, leading to efficient hydrolysis; medium-chain triglycerides have a higher water solubility; thus, do not have a necessity of emulsion with bile (Zentek et al., 2011). The latter was proven by the evidence from studies in which bile salts were supplemented into the diet. Gomez and Polin (1976) added different bile sources to a tallow supplemented diet, using 1 to 3 week old chicks, resulted in a significant improvement in the chicks' capacity to utilize the tallow. The digestibility of the tallow without the dietary addition of cholic acid was 39.6% at the end of the first week, and the digestibility had risen to 68.2% when measured at the end of the third week of the chicks' life. However, when the diet was supplemented with 0.050% cholic acid, the digestibility for the tallow increased to 49.5% and 78.5% at the end of the first and third week, respectively (Gomez & Polin, 1976). The increased utilization of the diets without the supplemented cholic acid is most likely due to increased age. While the increased utilization of diets with the supplemented cholic acid is primarily due to the increased digestibility of stearic

and palmitic fatty acids, due to them being more saturated. It should be noted that the inclusion of bile salts primarily affects the utilization of lipids in the young chick; the effect becomes less pronounced as the chick ages due to the increased bile salt and lipase production in older birds. The use of dietary bile salts in diet formulation is unlikely, however, due to the high cost of bile salts (Ravindran et al., 2016).

Further evidence suggests that some other biological factor is also aiding lipid digestion as poultry age. Currently, it is believed the combination of bile salts and pancreatic lipase are the key factors for lipid digestion and absorption. At the moment, data suggest that at the 28th d of life, there is a clear increase in lipase activity, and lipase activity is higher in ducks and geese compared to chickens (Jamroz et al., 2002). It is believed that the key role of pancreatic lipase is to breakdown triglycerides into absorbable units.

It should be noted that chickens are the more susceptible to age-related utilization of lipids than some other animals. Other poultry species, such as turkey poults, are able to well digest more saturated lipids at an earlier age, as are piglets. Especially at early stages, it is plausible that poults/turkeys and ducklings/ducks have a greater capacity to produce adequate quantities of bile and lipase compared to chickens (Ravindran et al., 2016). Turkey pancreatic lipase has a higher capacity to hydrolyze the long-chain triacylglycerols more efficiently than the short-ones, compared to chicken pancreatic lipases (Fendri et al., 2007). It was discovered that concentrations of short chain fatty acids in the ceca and large intestine differed by poultry species; with chickens and geese having a higher proportion in the ceca than ducks, and ducks and chickens having a higher proportion in the large intestine compared to geese (Jamroz et al., 2002).

In summary, the ability of young poultry to utilize lipid generally increases rapidly over the first few weeks of life, but this effect is most dramatically seen with highly saturated lipids, high levels of free fatty acids in the diet, and with a combination of the two (Aardsma & Parsons, 2017; Aardsma et al., 2017).

MEASUREMENT OF AVAILABLE ENERGY CONTENT

Due to the fact that approximately 70% of the production costs of the poultry industry are feed related (Sarwar et al., 2015), with about 90% of the total feed costs consisting of protein and energy (Perween et al., 2016), there is high demand to maximize production efficiency. Since lipids tend to increase feed efficiency, due to the fact that they are a concentrated source of energy, there is demand to accurately and repeatedly determine the available energy content of various sources. Lipids tend to have intrinsic complex nature and interact with non-lipid ingredients in a diet matrix, which makes finding an accurate available energy value for various conditions difficult. However, two general points can be made that usually hold true, one being the energy of unsaturates is higher than saturates, and the second being that the energy is higher in 16:0 fatty acids compared to C18:0 (Huyghebaert et al., 1988).

While gross energy can be determined relatively easily and rapidly, the value is only the maximum amount of energy the sample contains, which bears no relationship to the amount actually available to the animal (Sibbald et al., 1960). This makes using gross energy very impractical in diet formulation. Whitehead and Fisher (1975) describe metabolizable energy content of a fat as the product of its gross energy content and its absorbability. Metabolizable energy is calculated as the gross energy consumed minus the energy excreted in the excreta (feces + urine) divided by the amount of feed consumed (Sibbald et al., 1960). The ME is much

easier to measure for most feed ingredients than digestible energy for poultry, as feces and urine are excreted together making digestibility studies of energy containing feed ingredients very difficult (Sibbald et al., 1960); thus, it is the preferred method for poultry nutrition. However, some argue that the ME system is of questionable value because it does not take into account the efficiency of nutrient utilization and subsequent partitioning into meat and eggs, waste, and excess heat production (Swick et al., 2013). It is traditionally held that ME is apparent (AME) when the endogenous losses of energy are not taken into account, and true (TME) when endogenous energy losses are taken into account (Aardsma & Parsons, 2017). Inside the intestinal lumen, there is a continuous secretion of endogenous lipids, which mix with dietary lipids and are partially digested and absorbed. In order to calculate a true digestibility of fatty acids, the unabsorbed fraction that passes beyond the ileum must be considered a loss to the animal and needs to be measured (Ravindran et al., 2016). However, ME values are at risk of factors that can make interpretation of the values difficult, mainly interactions between supplemental lipid and non-lipid portions of the diet, age of bird, and lipid characteristics. The latter present a large problem for feed formulators, due to the reported ME values of lipids being highly variable and difficulty to apply in practice (Ravindran et al., 2016; Das et al., 2014c).

Taking the amount of lipid ingested minus the amount of lipid in the excreta divided by the amount of lipid ingested can be used to estimate the energy content of lipids, also known as lipid digestibility (Ravindran et al., 2016; Aardsma & Parsons, 2017). Lipids are not excreted in the urine, meaning the digestibility of a lipid is directly related to its ME value. This allows the digestibility of the lipid to be multiplied by the gross energy of the lipid to estimate its ME content. Apparent lipid digestibility data have been able to detect differences among lipid

sources where as AME was not able to (Blanch et al., 1995). Though, both these methods make large assumptions, mainly that the supplemental lipid does not alter the utilization of other dietary components and that digestion of the lipid is independent of the diet composition (Ravindran et al., 2016). However, lipid digestibility can deviate from AME or TME values if there is an EME observed, where part of the energy measured in the classical AME or TME assays is derived from a source other than the supplemental lipid (Lessire et al., 1982).

Net energy has received increased interest as a potential method to determine the available energy content of lipids. This is mainly due to the fact that ME underestimates the amount of energy available for maintenance and growth from lipids. The latter may be caused by the fact that lipids have lower heat increments than carbohydrates. Heat increment is the heat produced by an animal in excess of what is associated with basal, or fasting, metabolism (Swick et al., 2013). Even though ME has this heat increment limitation, using net energy has its own limitations. Net energy is difficult to measure, and stage of production can influence the value.

CHALLENGES OF MEASURING ENERGY CONTENT OF LIPIDS

Lipids cannot be fed alone and must be fed in diets containing other energy-containing ingredients, which makes generating available energy values for lipids complicated. Thus, an assumption must be made that no interaction occurs between supplemental fat and dietary components (Ravindran et al., 2016). Assuming additivity of dietary components, one could calculate the separate energy contributions of the different dietary components. However, lipids have been known to violate additivity because of the synergistic and antagonistic effects among them and with non-lipid portions of the diet. A lack of additivity among lipid sources, as well as other components in the diet, means that acquired energy values would only be accurate under

the specific set of conditions that they were measured (Aardsma et al., 2017). Thus, diets with lipid containing ingredients severely complicates repeated generation and interpretation of available energy values determined for test supplemental lipids. While there are certain equations that have been developed to aid in predicting the AME of various lipids, they should be used with caution. The equations developed only work with certain types of fats, and due to the recent trend of fat blends, even more variety of compounds from many different lipid sources is seen (Huyghebaert et al., 1988).

Interactions between Basal Diet and Supplemental Lipid

Due to often increased digestion and absorption of the basal diet when lipids are supplemented, some of the energy attributed to the lipid source derives from the increased utilization of basal diet. The interaction with other dietary components is one of the main causes of complex variation in the AME values of lipids (Sibbald & Kramer, 1977). According to Lessire et al. (1982), standardized lipid digestibility data have demonstrated that the AME or TME of lipids cannot be explained in some cases only by digestibility values. How much interaction occurs between test lipid and basal diet lipids may be affected by the carbohydrate sources of the basal diet. Corn-based diets resulted in higher TME values for tallow than wheat-based diets (Sibbald & Kramer, 1978). One study done by Sibbald et al. (1960) showed similar gains and feed efficiency for chicks fed corn and wheat diets, regardless of the higher metabolizable energy content of corn diets. In a second study done by Sibbald et al. (1960), birds fed a wheat diet consistently had larger weight gains than those fed corn diets, though the differences were small. It is difficult to narrow down why these differences occur, due to the complexity of lipid interactions. A large portion of what affects the utilization of the supplemental lipid is the fatty acid profile of the

basal diet. So, it can be speculated that the metabolizable energy of corn diets are not influenced as much by age of the bird, but rather changes in the proportion of corn in the diet (Sibbald et al., 1960), and the subsequent changes in the fatty acid profile/ratio of the basal diet. The ratio of lipid contained in the basal diet to the amount of supplemental lipid also has large impacts on lipid utilization (Aardsma & Parsons, 2017; Aardsma et al., 2017); this may be caused by variation in the U:S ratio mentioned previously.

Preston et al. (2001) studied the effects of wheat inclusion and fat digestibility. Due to the high content of non-starch polysaccharides and pentosans in wheat, there are increased gel-forming properties in the gut, which led to a pronounced effect on fat digestibility in broilers. It is speculated that digestive enzymes and substrates had a hindered interaction at the mucosal surface because of slowed luminal-peristalsis. Furthermore, to ensure optimum emulsification of micelles, fat digestion needs vigorous peristalsis, which is difficult to obtain with high viscosity in the intestines (Preston et al., 2001). Increased viscosity in the intestines can lead to reduced efficiency and reabsorption of bile salts, leading to an overall lower utilization of lipids (Maisonnier et al., 2003). It is also speculated that at the highest level of inclusion, pentosans in wheat may have had an inhibitory effect on bile salts, leading to decreased fat digestibility (Preston et al., 2001). Therefore, it is important to understand the chemical composition of grains used in the basal diet and the interactions that may occur with lipid inclusion.

However, not all lipid sources will interact with the basal in predictable ways, further complicating the prediction certain diet types will have on the available energy values of different fats and oils. The idea to use completely digestible purified diets was attempted, with varying success, however. This method requires the assumption that the carrier, usually starch or

glucose, is always 100% absorbed, even when it might not be, which was seen when Whitehead and Fisher (1975) used cellulose as an “inert” filler.

Non-additivity between Lipid Sources

Depending on the amounts and sources of other fatty acids in the diet, the chicken may utilize specific fatty acids differently. This was shown by Preston et al. (2001) who found the fat digestibility in high wheat based diets for soy oil, tallow: soy blend, and tallow were 75%, 64%, and 55%, respectively. Fat digestibility was improved by decreasing wheat inclusion and consequently digesta viscosity; the fat digestibility values for soy, blend, and tallow were 89%, 81%, and 70%, respectively (Preston et al., 2001). Interestingly, the highest fat digestibility of tallow, 73%, occurred with the medium inclusion of wheat, 350 g/kg. So, not only do individual lipid energy values need to be determined, but any number of lipid mixtures values must also be determined.

For ideal synergism, the ratio between the lipid contained in the basal diet (from non-lipid ingredients) and the supplemental lipid should be 1:1 (Fuller & Dale, 1982). With that being said, the fatty acid profiles of the basal diet can influence the utilization of the supplemental lipid. The latter is primarily because the utilization of long chain saturated fatty acids in supplemental lipid sources can be increased by the unsaturated fatty acids in the basal (Fuller & Dale, 1982). This is due to the fact that not all lipids respond in the same synergistic manner.

Effects of Age

Since poultry, especially chickens, have great variability in their ability to digest and absorb lipids depending on age, available energy values calculated for lipids will vary depending on the birds' age. Values determined for older birds are not applicable to young broilers,

especially during the first week of life, which means the use of a single AME value for all growth phases is impractical (Ravindran et al., 2016). This contradicts one essential assumption that must be met for energy values of feed ingredients; the values derived must not vary significantly with age of bird (Sibbald et al., 1960). While the values found may still be applicable to birds of the same age, the values may be impractical for all other ages. This clearly would complicate industry wide utilization of determined energy values.

Generally, young birds are more susceptible to level of inclusion of the lipid in the diet, but mainly with saturated lipids. That is not to say older birds are not affected by saturated lipid inclusion levels in the diet, but they are affected to a lesser extent due to the increased ability to digest and absorb saturated lipids. Rate of inclusion seems to be of minor importance and is mainly confined to younger birds (Wiseman & Salvador, 1991).

Level of Supplementation

Determined AME and fat digestibility can be affected by level of fat inclusion (Ravindran et al., 2016; Huyghebaert et al., 1988). As mentioned previously, the U:S ratio is important. That being said, as the inclusion level of supplemental lipid increases, the ratio between the non-lipid portion of the basal diet and the supplemental lipid decreases (Aardsma et al., 2017). While inclusion level of saturated lipids can affect availability of the lipid, with careful consideration to the U:S ratio between basal lipid content and supplemental lipid, these effects may be minimized. Since the predominant fatty acids in corn-soybean meal diets are mainly of the unsaturated variety, moderately low inclusion levels of supplemental saturated lipids may not incur any negative effects. As long as the overall U:S ratio is at least 4, then optimum digestion of the saturated fatty acids can occur. However, utilization of the lipids contained in the basal portion

of the diet may be more difficult to access than supplemental lipids, which can affect the U:S ratio. For instance, if the lipids inside other dietary ingredients are inaccessible due to a cell wall matrix, then they are not exposed to digestive enzymes and will not be accessible for use in the bird.

While there may be differences in available energy values for some lipids at different inclusion levels, determining accurate available energy values at low levels of inclusion would require a large number of replicates. Thus, most trials use lipids at higher levels of inclusion than in practical diets to reduce the number of replicates needed for statistical significance. However, this allows for some to argue that the values determined at these high inclusion levels are inaccurate. A counterpoint to this argument would be if the levels of free fatty acids are kept low, and the U: S ratios held constant, then the available energy values of most lipids will not vary greatly (Wiseman & Salvador, 1991).

Extra Metabolic Effect

Extra metabolic effect usually refers to the improvements in energy utilization related to the increased digestion and absorption of basal diet components that result from supplemental lipid inclusion. This is different than the extra caloric effect, which usually consists of the increased feed intake and lower heat increment effects. Speculation of EME first occurred when ME values of a test lipid were determined to often be greater than the GE of test lipids, which is not reasonable (Mateos & Sell, 1981).

As with many other factors affecting lipid availability, EME is not the same for all lipids. It varies source by source and probably varies based on a number of other factors; diet, age of bird, etc. Usually, EME is greatest at low inclusion levels, suggesting that the EME decreases as lipid

inclusion increases. Some speculation is that EME primarily results from some sort of synergism between basal diet and supplemental lipid inclusion (Aardsma & Parsons, 2017; Aardsma et al., 2017).

The EME can also be affected by the type of cereal grains that are used in the basal diet; diets that used corn had a greater chance of experiencing EME than wheat-based diets (Aardsma et al., 2017). This is because corn has a higher level of unsaturated fatty acids than wheat (NRC, 1994), one can speculate that EME is due to the synergism between unsaturated basal lipids and saturated supplemental lipids, especially at low inclusion levels. The EME is complex. It may not be seen at all levels or with all types of lipids, and the non-lipid portions of the diet contribute to EME, as well. If the 1:1 basal lipid to supplemental lipid ratio is maintained, which should occur with a practical-type diet containing a large proportion of corn, then synergism between fatty acids found in the basal diet and the supplemental lipid may contribute to the observed EME. Consideration should also be given to the common dietary 4:1 ratio of unsaturated to saturated fatty acids in the overall diet, which may contribute to the EME synergism between fatty acids.

Lipid supplementation can decrease the rate of passage of intestinal digesta, but not all lipids reduce the rate of passage equally. Depending on the source and inclusion level of the lipid, transit retention times may vary. Golian and Polin (1984) found that an inclusion of 0, 5, and 10% of corn oil led to average transit times of 142, 157, and 168 mins, respectively. Soy oil was also used in a separate experiment by Golian and Polin (1984); inclusion of 4% of soy oil led to the longest transit time at 180 mins. It has been speculated that this decreased rate of passage by lipids allows for greater carbohydrate utilization (mainly increased digestibility of starches) and also increased digestibility of dietary proteins, contributing to EME (Mateos & Sell, 1981).

Extra Caloric Effect

Fat has been reported to enhance feed efficiency via “extra caloric” effect (Turner et al., 1999). There is considerable confusion between what is deemed EME and what is considered extra caloric. Extra caloric effect (ECE) can be described as reduced heat increment or increased feed intake of diets containing supplemental lipids leading to an improvement in growth and/or feed efficiency due to more efficient utilization of absorbed energy for growth (Aardsma et al., 2017). The ECE differs from EME because ECE refers to how well the bird can efficiently utilize the absorbed energy. This is usually measured in a tangible product, like body weight gain. Though this distinction between EME and ECE is not always clearly seen, and in some instances. That effects attributed to ECE may in fact have been unnoticed EME, and vice versa. A timely and costly method of determining true ECE would be the use of direct/indirect calorimetry or tissue energy retention to evaluate reasons for unexplained growth responses to supplemental lipids (Aardsma et al., 2017).

Interactions with Dietary Minerals

After being released during fat digestion, free fatty acids can potentially react with divalent minerals, forming either soluble or insoluble soaps (Ravindran et al., 2016). Through the formation of insoluble soaps, which renders both the fatty acids and the minerals unavailable, lipid utilization may be impacted by mineral inclusion (Atteh & Leeson, 1984). Through this, energy utilization of the fat can be jeopardized and also interfere with the birds’ bone and eggshell quality, and mineral retention (Abdulla et al., 2016). Though the data are conflicting and some studies even report an increase in calcium digestibility with dietary fat supplementation, further research is needed to truly understand this interrelationship. Along those same lines, Ca

and P requirements for bone health and maximum lipid utilization may need to be re-defined (Ravindran et al., 2016), due to the fact that modern genetic strains are vastly different than those of the past (Abdulla et al., 2016).

Interactions with Enzymes

Feed enzymes have become an area of great interest recently. Studies have shown an increase in lipid utilization when coupled with enzyme supplementation. The increase in utilization probably arises from the decreased viscosity of the intestinal contents leading to higher peristalsis (Preston et al., 2001), and increased bile salt reabsorption which led to an increased bile salt concentration (Maisonnier et al., 2003). This was seen when an inclusion of xylanase led to a 10% improvement in gain: food ratio in a wheat based diet containing supplemental tallow. The latter increase can be attributed, at least in part, to xylanase hydrolyzing arabinoxylans, hence decreasing the digesta viscosity (Preston et al., 2001).

CONCLUSION

It is clear that the use of lipids in poultry diets has many positives. They are a dense source of energy, aid in providing essential nutrients, and can increase the utilization of other components of the diet. However, there are a number of factors that can complicate how much energy is available in the lipid source. These factors include the ratio of unsaturated to saturated lipids in the diet, the interaction between the basal portion of the diet and the supplemental lipid position of the fatty acids on the glycerol bond, and the bird age; just to name a few. Due to the fact that not all lipids are utilized to the same extent, there is a need to quickly and repeatedly measure the available energy content of lipid sources accurately. This should maximize the efficiency of utilization of a diet and lead to the most economic lipid inclusion level to use in

commercial diet formulation. While there are a few methods available for determining the digestibility and/or ME values of lipids, a number of factors come into play that can impact, and even nullify, the results.

The primary objective of this thesis was to further evaluate a relatively simple in vivo assay that can determine relative differences in metabolizable energy content of different lipid sources. Since there are so many factors that can affect the utilization of lipids, rather than try to account for and control all those factor, and determine specific numerical metabolizable energy values for lipids, a relative energy bioavailability approach was used. A known highly digestible lipid source, such as corn or soy oil was used as the reference standard. Theoretically, any reduction in utilization of a test lipid source relative to the reference standard would indicate an inferior lipid source, leading to a lower available energy content in commercial diets. Thus, using a simple relative method to identify which lipids are inferior and the degree of inferiority should meet the needs of the commercial industry. A combination of multiple regression slope-ratio methodology and the precision-fed rooster assay was utilized to analyze a large range of lipid supplementation (0 to 20%). In addition, a multiple-regression slope-ratio assay based on growth rate was also used to determine relative metabolizable energy values of several lipids for broiler chicks and to compare those to those determined in roosters.

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CHAPTER 2:

FURTHER EVALUATION OF A SLOPE-RATIO PRECISION-FED ROOSTER ASSAY AND A LIMIT-FED BROILER CHICK GROWTH ASSAY FOR RELATIVE BIOAVAILABLE ENERGY VALUES OF FATS AND OILS

ABSTRACT

Three slope-ratio precision-fed rooster assays were conducted using different fats and oils at several increasing dietary levels to determine relative bioavailable energy values. In Experiment 1, roosters were precision-fed 0, 5, or 10% of five different fats as part of a ground corn diet. Refined soy oil (the reference oil set at 100%), poultry fat, crude soy oil, crude corn oil, and palm oil 1 were all evaluated. In Experiment 2, roosters were precision-fed 0, 5, or 10% of either a refined corn oil (RCO), a vegetable acid oil, or a soy acid oil as part of a ground corn diet. In Experiment 3, roosters were precision-fed 0, 5, 10, 15, or 20% of either refined corn oil, palm oil 1, tallow, or choice white grease as part of a ground corn diet. In Experiment 4, roosters were precision-fed 0, 5, 15, or 20% of either a vegetable blend acid oil or palm oil 2 as part of a ground corn diet. Multiple regression slope-ratio analysis of nitrogen-corrected true metabolizable energy (TMEn) regressed on dietary fat level was used in Experiments 1-3 to estimate relative bioavailability values (RBV). The RBV were calculated as the regression coefficient of the test lipid divided by the regression coefficient of the reference refined soy oil or RCO (set at 100%). In Experiment 1, only the RBV (82.5%) of palm oil 1 was significantly lower ($P < 0.05$) than soy oil (100%). In Experiment 2, no significant differences in RBV were observed for the vegetable acid (98.1%) or the soy acid oil (98.5%) and RCO (100%). In Experiment 3, both the RBV of tallow (74.4%) and palm oil 1 (82.9%) were significantly lower ($P < 0.05$) than RCO, while the RBV of choice white grease (98.7%) was not significantly different than RCO. It was observed that the

differences among fat sources were often greater at the higher inclusion levels of 15 and 20%. In Experiment 4, high linearity was observed for 0-20% levels of palm oil 2 and the vegetable blend acid oil ($R^2 = 0.94-0.97$). In addition to the slope-ratio precision-fed rooster assays, a limit-fed chick growth assay was conducted using Ross 308 broilers. The broilers were limit-fed to 70% of their expected daily intake from 11 to 20 d of age and four lipid sources (RCO, palm oil 2, choice white grease, and a vegetable blend acid oil) were tested at 0, 5, and 10% inclusion levels. Multiple regression slope-ratio analysis of body weight gain regressed on dietary fat level indicated that the RBV of palm oil 2 (80.5%) and choice white grease (85.4%) were significantly lower than the 100% for RCO ($P < 0.05$), whereas the RBV of the vegetable blend acid oil (92.7%) was not significantly different from 100%. In general, the rooster results agreed well with the results obtained in the chick growth assay; however, using higher inclusion rates of 15 or 20% of the fats may increase the sensitivity of the rooster assay in detecting differences among lipid sources.

INTRODUCTION

The use of lipids in poultry nutrition has many positives such as aiding in providing essential nutrients and providing a dense source of energy. However, not all lipid sources are utilized to the same degree within the bird due to a number of factors. These factors include the age of the bird, the position of the fatty acids on the glycerol bond, and the ratio between saturated and unsaturated lipids in the diet. For that reason, there is need for rapid, repeatable, and cost effective methods to measure the available energy content of lipid sources accurately. Aardsma and Parsons (2017) recently developed a relatively simple in vivo precision-fed slope-ratio rooster assay that was able to determine the relative differences in metabolizable energy

content of different lipid sources. Due to the number of factors that can affect utilization of lipids, a relative energy bioavailability approach was used. Bioavailability, as applied to animal nutrition, can be defined as how well a particular source of an ingested nutrient is absorbed in a form that can be utilized in metabolism by the animal (Littell et al., 1997; Ammerman et al., 1995) The Aardsma and Parsons (2017) method uses a highly digestible lipid source, such as refined corn or soy oil as the reference standard and the test lipids were fed at levels of 5 and 10%. Any reduction in utilization of the test lipid relative to the control lipid source should indicate inferiority of the test lipid. Furthermore, since the precision-fed assay uses adult chickens, which have often been shown to utilize lipids (especially saturated lipids) better than young chicks, Aardsma et al. (2017) developed and validated a limit-fed slope-ratio broiler chick growth assay to determine RBV of several lipids in young broiler chickens and compared those values with RBV determined with the precision-fed rooster assay. Excellent agreement was obtained for RBV between the two assays. The objectives of the current study were to further evaluate and compare the precision-fed rooster and limit-fed broiler chick assay for additional lipids and to evaluate the precision-feeding of lipids at levels higher than 5 and 10% to roosters.

MATERIALS AND METHODS

The protocols used in conducting these experiments were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign.

Experimental Design and Procedures

Four experiments were conducted with similar designs that determined TMEn values via the precision-fed rooster assay (Parsons et al., 1982). Each diet consisted of ground corn with lipids added at varying levels (0-20%) at the expense of corn. In Experiment 1, roosters were

precision-fed 0, 5, or 10% of refined soy oil, poultry fat, crude soy oil, crude corn oil, and palm oil 1 with 4 roosters per treatment. In Experiment 2, roosters were precision-fed 0, 5, or 10% of either a refined corn oil, a vegetable blend acid oil, or a soy acid oil with 4 roosters per treatment. In Experiment 3, roosters were precision-fed 0, 5, 10, 15, or 20% of either refined corn oil, palm oil 1, tallow, or choice white grease with 4 roosters per treatment. In Experiment 4, roosters were precision-fed 0, 5, 10, 15, or 20% of either a vegetable blend acid oil or palm oil 2 with 4 roosters per treatment. In all four experiments, treatment diets were tube-fed to adult Single Comb White Leghorn roosters following the general guidelines of Parsons et al. (1982) and Sibbald and Wolynetz (1986). The roosters were housed in an environmentally controlled room with a 16 h light to 8 h dark cycle in individual cages with wire floors. To empty their digestive tracts, the roosters were fasted for 26 h, then were tube-fed 30 g of diet, returned to their individual cages, and excreta (feces + urine) were quantitatively collected for 48 h on plastic trays placed underneath the cages.

The reference refined corn oil and refined soy oil were both obtained from a commercial grocery retail outlet. The poultry fat, crude soy oil, crude corn oil, and palm oil 1 all came from the same company. The vegetable acid oil and soy acid oil were commercially produced blends for use in livestock production. The tallow and choice white grease, an unrefined fat with a fatty acid composition similar to lard (Cullen et al., 1962), were feed grade lipids produced for animal production. The vegetable blend acid oil and palm oil 2 were both produced by separate commercial lipid production companies.

In Experiment 5, the limit-fed chick growth assay, the diets consisted of a basal diet (Table 2.1) with lipids added at 0, 5, and 10% at the expense of cellulose. The test lipids consisted of a

vegetable blend acid oil, choice white grease, and palm oil 2, with refined corn oil as the reference standard. To ensure that amino acids were not more limiting than energy, the basal diet was supplemented with 30% more amino acids than the breeder recommendation. Male Ross 308 broiler chicks were purchased from a commercial hatchery (Hoover's Hatchery, Rudd, IA). Chicks were housed in an environmentally controlled room with a 24 h light cycle in heated Petersime battery brooders with ad libitum access to water and a nutritionally complete common starter diet. Prior to the start of the trial at 10 d of age, chicks were fasted for 12 h overnight then weighed individually. Chicks closest to the center of the weight distribution were then selected, individually wing banded, and allotted to treatment groups. Chicks were allotted to treatments in such a way as to equalize initial body weight across treatments. Treatment replicate groups consisted of 5 chicks, in individual pens of battery brooders in a completely randomized design with 5 replicate groups per dietary treatment and 9 dietary treatments. Trough-style feeders were altered with fitted poster-board inserts that limited the feeder space to 20 cm to facilitate chicks to consume all feed present each day. Chicks were fed treatment diets once per day at approximately the same time each morning, and the amount of feed given was measured to within ± 0.5 g of the daily allotment (Table 2.2). Feed allotment for the first feeding (10 d of age) was determined by multiplying published daily intakes (Aviagen, 2012) of birds of equivalent weight by 0.7 (30% feed restriction). Each subsequent day's allotment was calculated as the next published day's intake multiplied by 0.7, increasing the ration daily. No mortality occurred during the study. On day 20 post-hatch, chicks were weighed individually 24 h after their previous feeding. Body weight gain, total feed intake, and feed efficiency were calculated, with individual pens of birds as experimental units.

Chemical Analysis

After excreta collection for 48 h in the rooster assays, the excreta were frozen, freeze-dried, weighed, and ground in a coffee grinder. Subsamples of lipids, excreta, and feed samples were analyzed according to procedures of the Association of Official Analytical Chemists International (AOAC International, 2006). Lipids, feed, and excreta were analyzed for gross energy at the University of Illinois using an adiabatic bomb calorimeter (Model 1261, Parr Instruments, Moline, IL) that had been standardized with benzoic acid. All other analyses were performed at the Experiment Station Laboratories, University of Missouri-Columbia. Excreta and feed were analyzed for total nitrogen via combustion (AOAC International, 2006; method 990.03). Feed samples were also analyzed for dry matter (AOAC International, 2006; method 934.01). All lipids were analyzed for fatty acid composition (AOAC International, 2006; methods 996.06, 965.49, and 969.33; AOCS, 2003; method Ce 2-66), free fatty acids (%) (AOCS, 2003; method Ca 5a-40), iodine value (AOCS, 2003; recommended practice Ja 14-91), moisture (%) (AOCS, 2003; method Ca 2c-25), insolubles (%) (AOCS, 2003; method Ca 3a-46), unsaponifiabiles (%) (AOCS, 2003; method Ca 6a-40), and total moisture, insolubles, and unsaponifiabiles (MIU) (%) (Table 2.3).

Statistical Analysis

For the first four experiments, the TMEn of the diets was calculated as described by Parsons et al. (1982), while the TMEn of the lipids were calculated by difference using the Han et al. (1976) method. Each experiment was analyzed individually using the GLM procedures of SAS (SAS Institute Inc, 2011). Multiple linear regression of TMEn (Y) on supplemental lipid inclusion level (X) was conducted using the methods of Littell et al. (1995; 1997) for the slope-ratio method.

In order for the slope-ratio method to be valid, the response must be linear for each nutrient source (Littell et al., 1997). The RBV of test lipids were calculated by dividing the regression coefficient of the test lipids by the regression coefficient of refined corn oil or soy oil. To determine if the test lipids were significantly different than the reference lipid (refined soy oil for Experiment 1, refined corn oil for Experiments 2-5) pair-wise comparisons were made between the regressions coefficients of the test lipids and the reference lipid using the method of Littell et al. (1995). If the regression coefficients were significantly different, then it was assumed the RBV were also significantly different since the RBV is a ratio of the regression coefficients (Littell et al., 1995). Statistical significance was accepted at a probability of $P < 0.05$. Influential outliers were identified using the Diagnostic Plots of the GLM Procedure with the Unpack and Label options of SAS. Cook's D test was used as an objective measure of outlier influence (Cook, 1977). Outlying data points were removed if careful analysis of the original data revealed a methodological or biological explanation for the data according to described methods (Freund & Littell, 2000).

For Experiment 5, multiple linear regression of body weight gain (Y) on supplemental lipid inclusion intake (X) was again conducted (Littell et al. 1995; 1997) using the slope-ratio method. As with Experiments 1-4, pair-wise comparisons were made between the regression coefficients of the test lipids and the reference refined corn oil to determine significance, with the assumption that the RBV would be significantly different if the regression coefficients were significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Composition of Lipid Sources

Table 2.3 shows the fatty acid profiles and compositions of the lipids used. Profiles of fatty acids are important indicators of the quality of the lipid and how well they are likely to be absorbed by the bird, as well as how the quality of the fat will influence fat deposition in bird carcasses (Baião & Lara, 2005). Tallow had the highest proportion of saturated fatty acids, particularly stearic and myristic, followed by both palm oils. Refined corn oil had the highest linoleic percentage. Choice white grease and poultry fat both had a balanced mix of unsaturated to saturated fatty acids. The free fatty acid content of all three acid oils or soapstocks were similar and by far the largest of all the lipid sources. This was as expected based on how acidulated soapstocks or acid oils are produced. Refined soy oil had the largest iodine value at 130.98, acid oil and refined corn oil followed with values of 126.31 and 122.85, respectively. This agrees with the literature because iodine values are an estimation of the double bonds present in the lipid (Baião & Lara, 2005). Crude soy oil had the highest MIU content with 8.33%, followed by choice white grease whose value was 8.17%. The MIU content is important because these components may dilute the energy content of the lipid (Leeson & Summers, 2001). However, most of that dilution most likely comes from the unsaponifiable component of the MIU. The higher the percentage of unsaponifiabiles, the lower the energetic value of the lipid (Baião & Lara, 2005). Vegetable acid oil had the highest percentage of unsaponifiabiles at 1.8%.

Experiment 1

Experiment 1 results are shown in Table 2.4. A linear increase in TMEn for the mixed diets was observed with addition of 5 and 10% of all of the test lipids. The TMEn of the diets containing

the 10% inclusion levels were significantly different than their 5% levels ($P < 0.05$). The R^2 value is the standard statistical index of goodness of fit of a regression model, with better fitting models having larger R^2 values (Littell et al., 1995). The R^2 of the multiple linear regression was 0.91, showing a very good linearity of TMEn as the lipid inclusion level increased. The TMEn values of the lipids calculated by difference (Table 2.3) showed that several lipids exhibited extra-metabolic effects (EME), which is when the TMEn of the lipid exceeds the gross energy of the lipid (Mateos & Sell, 1981). The greatest EME occurred with both levels of refined soy oil and crude corn oil, as well as the 10% crude soy oil. The EME at both levels of crude corn oil and the 10% level of crude soy oil, but not the 5% of crude soy oil, contradicts previous published data that showed larger EME at lipid inclusion levels of 5% or lower (Sibbald & Kramer, 1977; 1978). The results of this experiment, however, are consistent with Aardsma and Parsons (2017) who found substantial EME at all levels of the refined corn oil reference. Multiple regression of TMEn of the mixed diets on lipid inclusion level yielded slope-ratio RBV for the poultry fat and crude soy oil that were slightly lower but not significantly different than the 100% for refined soy oil, showing a high utilization by the bird. Only palm oil 1 had a RBV (82.4%) that was significantly different ($P < 0.05$) than the refined soy oil, which is most likely due to the higher degree of saturation found in palm oils. Available energy content of lipids may be reduced with increased degree of fatty acid saturation (Ketels & De Groote, 1989; Ravindran et al., 2016; Young et al., 1963). The RBV of 95.0% for poultry fat is in excellent agreement with the Cullen et al. (1961) values, who found an apparent lipid absorbability of 94% for their poultry grease. Aardsma et al. (2017) also reported a similar RBV of 96% for poultry fat using the multiple regression slope-ratio assay with precision-fed roosters.

Experiment 2

The results for Experiment 2 are shown in Table 2.5, and compares two acid oils (vegetable acid oil and soy acid oil) against refined corn oil (reference source). The TMEn of one rooster fed 10% refined corn oil was identified as an influential outlier and was removed from the analysis due to a low excreta weight. The low excreta weight can be explained by the incomplete or partial clearance of the digestive tract by the rooster or excreta being lost during collection (Dale et al., 1985). The primary reason for this experiment was to determine how well acid oils are utilized by the bird. Due to the high level of free fatty acids (~50%) in acidulated oils, concerns have been raised about how effectively acid oils are absorbed since high free fatty acid levels may reduce the ME values of the lipids (Baião & Lara, 2005). The multiple regression of mixed diet TMEn values regressed on lipid inclusion had an R^2 of 0.94 showing excellent linearity of TMEn as lipid level increased. The RBV of both the vegetable acid oil and the soy acid oil were high at 98.1% and 98.5%, respectively, indicating that the acid oils were well utilized. The latter shows promise for the use of affordable acid oils in commercial feed formulation since soapstock (which acidulated oil is derived from) is generated at a rate of about 6% of the volume of crude oil produced in the U.S. This ends up being about one billion pounds of soapstock produced annually in the U. S. with a price that can be as low as one-tenth that of refined vegetable oil (Reaney, 2002). The TMEn (calculated by difference) of 10% refined corn oil, 10% vegetable acid oil, and both levels of soy acid oil showed EME. The 10% level vegetable acid oil showed the greatest EME with the TMEn of the diet being 10.201 (kcal/g DM) and the gross energy of the vegetable acid oil being 8.6743 (kcal/g). Again, this is contrary to what was expected since EME supposedly decreases with increasing inclusion level (Sibbald & Kramer, 1977, 1978); thus, the

TMEn of the vegetable acid oil would have been expected to be higher at the 5% inclusion than at 10% inclusion.

Experiment 3

In Experiment 3, inclusion levels were increased up to 20% to determine if corn-lipid diets containing higher lipid levels could be physically tube-fed and if higher lipid inclusion levels would increase the sensitivity of the rooster assay for detecting differences in RBV among lipid sources. Adding 5, 10, 15, or 20% lipid to the ground corn basal diet at 100% expense of the basal yielded significant linear ($P < 0.05$) increases in diet TMEn for all lipid types (Table 2.6). When the TMEn of the diets was regressed on lipid level (multiple linear regression), the regression coefficients and RBV for palm oil 1 and tallow were significantly lower ($P < 0.05$) than the refined corn oil, while those for the choice white grease were not significantly different from the refined corn oil. The calculated RBV of the palm oil 1, tallow, and choice white grease were 82.9, 74.4, and 98.7%, respectively. The lower values for the tallow and palm oil 1 were most likely due to the higher saturation degree of the tallow and palm oil 1.

Tallow, being a highly saturated lipid source, is much more difficult for the bird to utilize and is rarely used in poultry diets (Preston et al., 2001; Whitehead & Fisher, 1975). It was included as a test source in this experiment to provide a lipid that would be expected to have a low RBV compared with refined corn oil. The RBV of 74.4% is consistent with Aardsma et al. (2017), who reported a RBV of 72% for tallow, indicating the validity of the assay for detecting lipids that are less utilized by poultry. Although choice white grease also contains a large amount of saturated fatty acids, it was utilized well by the roosters, indicated by the high RBV of 98.7%. The high utilization may be due in part to the fact that choice white grease typically has a greater

proportion of fatty acids on the sn-2 position, which are thought to be absorbed more efficiently than fatty acids on the sn-1 or sn-3 position (Smink et al., 2008); most likely because sn-2-monoacylglycerols are amphiphilic and natural emulsifiers that enhance fatty acid incorporation into mixed micelles (Ravindran et al., 2016). The high RBV of the choice white grease was also similar to the results found by Cullen et al. (1961), who found an absorbability value, computed from dietary fat and total fecal fat, of 95% for their choice white grease sample. The palm oil 1 had an RBV of 82.9%, which was higher than the tallow, but still significantly lower ($P < 0.05$) than the refined corn oil. The high RBV of the palm oil 1 compared to tallow is probably partially due to palm oil 1 having a higher proportion of C16:0 located on the sn-2 position. Smink et al. (2008) discovered when palm oil was segregated to have a higher sn-2 proportion compared to native palm oil, the RBV increased, with the segregated palm oil having a digestibility of 89.5% and the native palm oil being 51.0%. As mentioned earlier, the lower RBV of palm oil 1 compared with refined corn oil was probably mainly due to the higher degree of saturation of the palm oil.

The majority of the lipids showed EME at most levels of the inclusion; however, palm oil 1 did not exhibit EME at 20% inclusion, and tallow did not show EME at the 15 or 20% inclusion levels. In contrast, the largest occurrence of EME was found at the 5% tallow inclusion, showing a TMEn of 10.74 (kcal/g DM) while its gross energy was only found to be 8.776 (kcal/g DM). The TMEn values for the tallow are generally consistent with the previous research findings that EME occurs mostly at 5% or lower inclusions and decreases with increasing supplemental lipid inclusion (Sibbald & Kramer, 1977; 1978). As the lipid inclusion levels increased in the current study, greater differences in TMEn values among the lipids was seen. For example, there were no significant differences between the TMEn of refined corn oil compared with palm oil 1 and

tallow at the 5 and 10% inclusion levels. However, the TMEn values of the palm oil 1 and tallow were significantly ($P < 0.05$) lower than refined corn oil at the 15 and 20% inclusion levels. This indicates that with higher inclusion levels (15 or 20%), greater differences among fats may be seen and the sensitivity of the rooster assay for detecting differences among lipid sources may be increased. Although 15 and 20% lipid inclusion are much higher than levels fed in commercial poultry diets, the roosters are only precision fed 30 g of experimental diets, which, at 20% lipid inclusion, is equal to only 6 g of fat per rooster for a 48 h period.

Experiment 4

The purpose of this experiment was to determine if it would be physically possible to tube-feed up to 20% lipid inclusion of a vegetable blend acid oil and a second palm oil and to determine if linear responses in TMEn would be obtained. The TMEn value for one rooster fed 20% palm oil 2 was found to be an influential outlier due to high excreta weight and was removed from the analysis. The high excreta weight may have been caused by undetected regurgitation. Both the vegetable blend acid oil and palm oil 2 yielded excellent linear increases in mixed diet TMEn as inclusion level increased, as well as a significant difference ($P < 0.05$) between each lipid level (Table 2.7). The R^2 values for the vegetable blend acid oil and palm oil 2 simple linear regressions were 0.97 and 0.94, respectively. These results indicated that up to 20% vegetable blend acid oil and palm oil 2 could be tube-fed and linear responses in TMEn could be obtained. Since no reference corn oil was included in this experiment, RBV were not computed from multiple regression analyses. When averaging the TMEn values for the vegetable blend acid oil and palm oil 2 (calculated by difference) for all inclusion levels, the mean TMEn of palm oil 2 was

89% of that of the vegetable blend acid oil. This result suggested that the RBV of palm oil 2 was about 10% less than the vegetable blend acid oil.

The vegetable blend acid oil demonstrated EME at all levels of inclusion, while the palm oil 2 only showed EME at one inclusion level, 10%. The 5% vegetable blend acid oil inclusion was clearly in excess of the gross energy, with a TME_n of 10.65 (kcal/g DM) and a gross energy of 8.606 (kcal/g DM).

Experiment 5

The diets used in the limit-fed chick growth assay (Table 2.8) contained higher amino acid levels (30% increase) than the breeder recommendations to ensure that only energy was the growth limiting factor. Previous work done by Aardsma et al. (2017) deemed the method of multiplying published daily intakes of Ross 308 broilers (Aviagen, 2012) of equivalent weight by 0.7 (30% feed restriction) was adequate for this assay. Those researchers had conducted preliminary experiments using both 20 and 30% feed restriction and found the 20% restriction to be inadequate due to the fact that several pens of birds did not consume all the feed each day (Aardsma et al., 2017). Our findings in this assay are consistent with theirs with feed being present in the troughs for most of the day, and no feed refusals or leftover feed occurring in any of the pens when using the 30% feed restriction in the current study.

A highly linear response in growth was obtained from adding 5 and 10% refined corn oil, indicating that energy was the growth-limiting factor. One pen of chicks from treatment one, 0% added lipid control, was identified as an influential outlier and was removed. That pen was found to have a chick with a very low body weight, which probably resulted in the remaining 4 chicks in the pen consuming more feed than intended. Arguably, this increased feed intake may have led

to a higher proportion of energy being deposited as fat compared to the other treatments. There were no feed refusals throughout the 10 day study; thus, feed intakes were identical for all pens/treatments.

Body weight gain increased with increasing supplemental lipid inclusion for all test lipid sources, again indicating that energy was the growth-limiting factor which was the aim of the assay and required for it to be successful. The RBV of the lipids increased in order of the expected energy content, with the vegetable blend acid oil being the highest at 92.7%, followed by choice white grease at 85.4%, and lastly palm oil 2 with a value of 80.5%. The regression coefficients of the choice white grease and the palm oil 2 were lower ($P < 0.05$) than the refined corn oil reference, with the vegetable blend acid oil not being significantly different than refined corn oil. The low RBV of palm oil 2 (80.5%) was probably mainly due to its high saturated fatty acid content, especially because young broiler chickens have been reported to inefficiently digest saturated lipids (Lessire et al., 1982). The choice white grease results were somewhat unexpected, considering how efficiently the roosters were able to utilize it. Again, this may have been largely due to differences in bird age and the inability of the younger chicks to digest the saturated fatty acids in the choice white grease as well as the roosters. Finally, the vegetable blend acid oil results were also somewhat surprising since it has been reported that the high level of free fatty acids in acidulated oils may impair the birds' utilization of the lipid (Wu et al., 2011). However, both the rooster and chick assays in the current study showed that the birds were able to well utilize the vegetable blend acid oil and its RBV was similar to refined corn oil. The latter has large economic consequences for the poultry industry because the price of soapstock, which can be converted to acidulated oil with the addition of sulfuric acid in hot aqueous solution, can

be as low as one-tenth the price of refined vegetable oil (Baião & Lara, 2005; Reaney, 2002). Overall, in agreement with Aardsma et al. (2017) the limit-fed chick growth assay was successful in detecting differences in RBV among the test lipids.

Comparison of the precision-fed rooster and limit-fed chick assays

There was general agreement between the precision-fed rooster assay and the limit-fed chick assay, for the vegetable blend acid oil and the palm oil. However, there was some difference between the two assays for the choice white grease. In the rooster assay, the RBV of choice white grease was 98.7%, whereas the chick assay yielded an RBV of 85.4%. This difference may have been caused by an insufficient supply of bile salts and pancreatic lipases in the chick's digestive tract which led to reduced digestion and absorption of the choice white grease (Ravindran et al., 2016). The roosters, being much older, probably had a sufficient level of bile salts and pancreatic lipases to digest and absorb the choice white grease. While the rooster assay and the chick assay often yield comparable results, it is still necessary to consider the qualities of the lipids (particularly degree of fatty acid saturation) and the age of the bird at the time of feeding, as these factors may impact the agreement between the two assays.

General Conclusions and Implications

In agreement with previous studies by Aardsma and Parsons (2017) and Aardsma et al. (2017), the slope-ratio precision-fed rooster assay was able to detect differences among test lipids and the available energy content of varying sources of lipids. This can be useful to the commercial poultry industry due to the fact that lipids can be evaluated rapidly and inferior lipids can be quickly identified. Since formulation and purchasing is done to ensure the maximum bird performance and economy, this relatively inexpensive assay can aid in achieving these goals. The

original design of using two levels of supplemental lipid, 5 and 10% (e.g., in Experiments 1 and 2), seem satisfactory for most lipids. However, the results of Experiment 3 indicated that, having higher levels of supplemental lipid such as 15 and 20% may yield larger differences for inferior lipid sources that are in better agreement with the RBV obtained with chicks. Thus, in future experiments, it might be useful to precision feed 5, 10, and 20% of the test lipids to provide a high level of test lipid that might exhibit a difference that would not be observed at the lower levels of 5 and 10%. As mentioned earlier, due to the small amount of feed that is tube-fed to roosters in the precision-fed assay, the lipid intake is quite low and it may be desirable to tube-feed a high level such as 20% even though this level is much higher than that fed in commercial diets. Tube-feeding a high level of lipid also reduced the confounding EME effects on the interpretation of the ME values for fats and oils.

Overall, the RBVs determined in these experiments compare well with lipid research that has been done previously. As mentioned previously, the RBV of poultry fat, tallow, and choice white grease, 95.0%, 74.4%, and 98.7%, respectively, are similar with the work of Cullen et al. (1961). The absorbability values from their lab for poultry grease, tallow, and choice white grease were 94.0%, 78.9%, and 95.3%, respectively. When Sibbald and Kramer (1977) precision-fed roosters 25 g of an experimental diet with 15% lipid added, the TME values of tallow and acidulated soapstock in their Experiment 1, as well as lard in Experiment 2, were similar in ranking to the values derived for the same type of lipids in the Experiment 3 and 4 of the current study. That is, Sibbald and Kramer (1977) reported values of 8.13, 9.05, and 9.39 kcal/g DM for tallow, acidulated soapstock, and lard, respectively, while the current study found values of 8.75, 9.22, and 9.78 kcal/g DM for tallow, acid oil, and choice white grease, respectively. However, Sibbald

and Kramer (1977) reported different TME values for palm oil than the values found in the current study. Their value for palm oil was 7.71 kcal/g DM, while the values obtained in the current study were 9.037 and 9.06 kcal/g DM for palm oil 1 and palm oil 2, respectively. The higher values obtained in the current study may be because of improved processing of our palm oils compared with Sibbald and Kramer (1977).

Both refined corn and soy oil are suitable as reference lipids; however consideration must be taken to ensure the quality of the reference oil is high and is of sound quality. If the reference lipid has become rancid, then it would not be suitable to use as a reference standard, which will be discussed later on in Chapter 3. In most of the rooster experiments, considerable EME was seen for most lipids. It is well known that the EME is a result of an interaction between the fat and other dietary components that vary with levels of fat inclusion (Sibbald & Kramer, 1978). Utilizing the RBV approach is an attempt to enhance the practical value and application of the assay results while also decreasing the impacts of EME. It is important to remember, however, that the energy value of any one type of fat when fed to chickens is not an absolute single value and can vary depending on grade, source, and type of bird being fed (Cullen et al., 1961).

TABLES

Table 2.1 Ingredient composition and calculated analysis of the basal diet used in the chick growth assay (Experiment 5 of Chapter 2) on an as-is basis.

Item	% unless otherwise indicated
Fine ground corn	41.56
Soybean meal	39.98
Solka floc ¹	10.00
Pork meal (MBM)	3.00
Dicalcium phosphate	2.33
Limestone	0.79
DL-Methionine	0.55
Sodium chloride	0.47
L-Threonine	0.26
Lysine 78	0.24
Valine	0.22
Isoleucine	0.13
Arginine	0.07
Choline chloride (60%)	0.05
Vitamin premix ²	0.20
Mineral premix ³	0.15

Table 2.1 (continued)

Item	% unless otherwise indicated
Calculated analysis:	
AME (kcal/kg)	2432
CP	24.00
Ca	1.24
NPP	0.55
Lys	1.58
Met	0.91
Cys	0.38
Thr	1.18

¹Cellulose (International Fiber Corp., North Tonawanda, NY).

²Provided per kilogram of complete diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μ g; dl- α -tocopheryl acetate, 11 IU ; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; d-Ca-pantothenate, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

³Provided per kilogram of complete diet: Mn, 75 mg from MnO; Fe, 75 mg from FeSO₄•7H₂O; Zn, 75 mg from ZnO; Cu, 5 mg from CuSO₄•5H₂O; I, 0.75 mg from ethylene diamine dihydroiodide; and Se, 0.1 mg from Na₂SeO₃.

Table 2.2. Feed allotment for broiler chicks to obtain a calculated feed intake of 70% of ad libitum intake in Experiment 5.

Day of experiment	Expected body weight (g/chick)	Expected feed intake ¹ (g/chick/day)	Feed allotment ² (g/chick/d)
0	230	41	29.0
1	262	46	32.2
2	303	52	36.4
3	345	57	39.9
4	393	63	44.1
5	442	69	48.3
6	495	74	51.8
7	551	80	56.0
8	610	86	60.2
9	672	92	64.4

¹Aviagen. 2012. Ross 308 Broiler Performance Objectives. Aviagen Inc., Huntsville, AL.

²Calculated as expected feed intake multiplied by 0.7.

Table 2.3. Analyzed composition of lipid sources.¹

Lipid source	Fatty acid (%)						
	C14:0 Myristic	C16:0 Palmitic	C16:1 Palmitoleic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic
RSO ¹	0.08	10.80	0.08	3.72	20.55	53.35	7.35
CSO ¹	0.18	14.49	0.16	3.09	25.66	40.67	3.97
CCO ¹	0.06	13.19	0.11	1.81	25.69	54.97	1.35
PF ¹	0.72	19.12	2.47	5.48	27.69	35.86	1.70
PO 1 ¹	0.94	37.98	0.19	4.04	41.48	12.28	0.25
RCO ¹	0.04	11.64	0.11	1.81	28.02	55.03	0.96
VA ¹	0.11	9.77	0.20	2.98	34.78	37.15	5.68
SA ¹	0.11	12.05	0.13	3.54	21.52	49.18	7.37
T ¹	2.92	25.03	2.04	21.45	31.30	2.11	0.28
CWG ¹	1.39	23.36	2.11	13.46	36.72	13.33	0.58
PO 2 ¹	1.05	43.03	0.17	4.47	38.61	9.34	0.18
AO ¹	0.10	15.34	0.11	3.90	14.99	54.29	6.77

¹Abbreviations: Refined soy oil (RSO), crude soy oil (CSO), crude corn oil (CCO), poultry fat (PF), palm oil 1 (PO 1), refined corn oil (RCO), vegetable acid oil (VA), soy acid oil (SA), tallow (T), choice white grease (CWG), palm oil 2 (PO 2), acid oil (AO).

Table 2.3 continued. Analyzed composition of lipid sources.¹

	Free Fatty Acids (%)	Iodine Value	Moisture (%)	Insolubles (%)	Unsaponifiables (%)	Total MIU ¹ (%)	Gross Energy (kcal/g DM)
RSO ¹	0.04	130.98	0.00	0.00	1.55	1.55	9.45
CSO ¹	6.60	109.66	4.28	4.03	0.02	8.33	9.19
CCO ¹	9.46	121.94	0.07	0.00	0.33	0.4	8.98
PF ¹	0.70	97.55	0.00	0.03	0.00	0.03	9.10
PO 1 ¹	1.83	58.76	2.75	0.00	0.00	2.75	8.60
RCO ¹	0.16	122.85	0.00	0.00	0.27	0.27	9.31
VA ¹	67.94	106	1.65	0.19	1.80	3.64	8.67
SA ¹	62.27	117	1.93	0.27	1.25	3.45	9.04
T ¹	4.18	35.84	0.72	0.00	0.00	0.72	8.78
CWG ¹	7.74	64.29	0.01	7.83	0.33	8.17	9.06
PO 2 ¹	0.41	50.98	0.00	0.00	0.00	0.00	9.14
AO ¹	69.63	126.31	1.53	0.04	0.87	2.44	8.61

¹Abbreviations: Refined soy oil (RSO), crude soy oil (CSO), crude corn oil (CCO), poultry fat (PF), palm oil one (PO 1), refined corn oil (RCO), vegetable acid oil (VA), soy acid oil (SA), tallow (T), choice white grease (CWG), palm oil 2 (PO 2), acid oil (AO), sum of moisture, insolubles, and unsaponifiables (MIU).

Table 2.4. Effects of refined soybean (soy) oil, poultry fat, crude soy oil, crude corn oil, and palm oil 1 lipid types and lipid level on nitrogen-corrected true metabolizable energy (TMEn) of diets and lipids, as well as relative bioavailability (RBV) estimates in Experiment 1¹.

Dietary treatment ²	TMEn of diet (kcal/g DM)	TMEn of lipid ³ (kcal/g DM)	RBV ⁴
0% added lipid	3.97 ^d	--	
5% refined soy oil	4.27 ^c	9.87	
10% refined soy oil	4.57 ^a	9.94	100
5% crude soy oil	4.21 ^c	8.75	
10% crude soy oil	4.58 ^a	10.05	97.8
5% crude corn oil	4.28 ^c	10.06	
10% crude corn oil	4.56 ^a	9.90	100
5% palm oil 1	4.21 ^c	8.70	
10% palm oil 1	4.46 ^b	8.88	82.5*
5% poultry fat	4.24 ^c	9.45	
10% poultry fat	4.54 ^{a,b}	9.67	95.0
SEM	0.034	0.525	-

*Different ($P < 0.05$) than RBV of refined soy oil.

^{a-c} Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by a least significant difference comparison.

¹Values represent least squares means of 4 roosters per dietary treatment.

²Lipid was added at the expense of a 100% ground corn basal.

³The TMEn values of the lipids were calculated by difference using the values of the 0% added lipid diet and the diets containing the test lipids.

⁴RBV = relative bioavailability value as determined by the slope ratio method. The multiple linear regression of TMEn (Y, kcal/g DM) on lipid level (g/g diet) from refined soy oil ($X_1 \pm SE$), crude soy oil ($X_2 \pm SE$), crude corn oil ($X_3 \pm SE$), palm oil 1 ($X_4 \pm SE$), or poultry fat ($X_5 \pm SE$) yielded the equation $Y = 3.954 + 6.151 \pm 0.402X_1 + 6.015 \pm 0.402X_2 + 6.154 \pm 0.402X_3 + 5.074 \pm 0.402X_4 + 5.842 \pm 0.402X_5$ with an R^2 of 0.91.

Table 2.5. Effects of refined corn oil, vegetable acid oil, and soy acid oil lipid types and lipid level on nitrogen-corrected true metabolizable energy (TMEn) of diets and lipids, as well as relative bioavailability (RBV) estimates for each lipid type in Experiment 2¹.

Dietary treatment ²	TMEn of diet (kcal/g DM)	TMEn of lipid ³ (kcal/g DM)	RBV ⁴
0% added lipid	3.94 ^c	--	
5% refined corn oil	4.25 ^b	9.18 ^a	
10% refined corn oil ¹	4.60 ^a	10.05 ^a	100
5% veg acid oil	4.18 ^b	7.89 ^b	
10% veg acid oil	4.61 ^a	10.20 ^a	98.1
5% soy acid oil	4.27 ^b	9.65 ^a	
10% soy acid oil	4.57 ^a	9.80 ^a	98.5
SEM	0.0331	0.371	-

^{a-c} Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by a least significant difference comparison.

¹ Values represent least squares means of 4 roosters per dietary treatment, 10% refined corn oil had one rooster removed as an influential outlier due to low excreta weight.

² Lipid was added at the expense of a 100% ground corn basal.

³ The TMEn values of the lipids were calculated by difference using the values of the 0% added lipid diet and the diets containing the test lipids.

⁴ RBV = relative bioavailability value as determined by the slope ratio method. The multiple linear regression of TMEn (Y, kcal/g DM) on lipid level (g/g diet) from RCO ($X_1 \pm SE$), veg acid oil ($X_2 \pm SE$), or soy acid oil ($X_3 \pm SE$) yielded the equation $Y = 3.915 + 6.767 \pm 0.466 X_1 + 6.638 \pm 0.432 X_2 + 6.666 \pm 0.432 X_3$ with an R^2 of 0.94.

Table 2.6. Effects lipid types and lipid level on nitrogen-corrected true metabolizable energy (TMEn), as well as relative bioavailability (RBV) estimates for each lipid type as compared to refined corn oil (RCO) in the precision-fed rooster assay in Experiment 3¹.

Dietary treatment ²	TMEn of diet (kcal/g DM)	TMEn of lipid ³ (kcal/g DM)	RBV ⁴
0% added lipid	3.97 ^f	-	-
5% RCO	4.27 ^e	9.96 ^{a,b,c}	100
10% RCO	4.54 ^d	9.62 ^{b,c,d}	
15% RCO	4.90 ^b	10.14 ^{a,b}	
20% RCO	5.09 ^a	9.54 ^{b,c,d,e}	
5% palm oil 1	4.27 ^e	9.97 ^{a,b,c}	
10% palm oil 1	4.50 ^d	9.27 ^{c,d,e,f}	82.9*
15% palm oil 1	4.73 ^c	9.04 ^{d,e,f}	
20% palm oil 1	4.90 ^b	8.60 ^f	
5% tallow	4.31 ^e	10.74 ^a	
10% tallow	4.58 ^d	10.08 ^{a,b,c}	
15% tallow	4.69 ^c	8.75 ^{e,f}	74.4*
20% tallow	4.71 ^c	7.68 ^g	
5% choice white grease	4.31 ^e	10.68 ^a	
10% choice white grease	4.57 ^d	10.00 ^{a,b,c}	
15% choice white grease	4.84 ^b	9.78 ^{b,c,d}	
20% choice white grease	5.07 ^a	9.48 ^{b,c,d,e}	
SEM	0.043	0.305	-

*Different ($P < 0.05$) than RBV of RCO.

Table 2.6 (cont.)

^{a-g} Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by a least significant difference comparison.

¹Values represent least squares means of four roosters per dietary treatment.

²Lipid was added at the expense of a 100% ground corn basal.

³The TMEn values of the lipids were calculated by difference using the values of the 0% added lipid diet and the diets containing the test lipids.

⁴RBV = relative bioavailability value as determined by the slope ratio method. The multiple linear regression of diet TMEn (Y, kcal/g) on lipid level (g/g diet) from RCO ($X_1 \pm SE$), palm oil 1 ($X_2 \pm SE$), tallow ($X_3 \pm SE$), or choice white grease ($X_4 \pm SE$) yielded the equation $Y = 4.059 + 5.186 \pm 0.219 X_1 + 4.305 \pm 0.219 X_2 + 3.863 \pm 0.219 X_3 + 5.121 \pm 0.219 X_4$ with an R^2 of 0.93.

Table 2.7. Effects of an acid oil and palm oil 2 lipid types and lipid level on nitrogen-corrected true metabolizable energy (TMEn) of diets and lipids in Experiment 4¹ with linear regressions⁴.

Dietary treatment ²	TMEn of diet (kcal/g DM)	TMEn of lipid ^{3, 4} (kcal/g DM)
0% added lipid	4.01 ^f	--
5% acid oil	4.34 ^d	10.65 ^a
10% acid oil	4.57 ^c	9.56 ^b
15% acid oil	4.79 ^b	9.22 ^b
20% acid oil	5.08 ^a	9.34 ^b
5% palm oil 2	4.18 ^e	7.35 ^c
10% palm oil 2	4.55 ^c	9.35 ^b
15% palm oil 2	4.77 ^b	9.06 ^b
20% palm oil 2 ¹	4.98 ^a	8.86 ^b
SEM	0.045	0.342

^{a-e} Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by a least significant difference comparison.

¹ Values represent least squares means of 4 roosters per dietary treatment, 20% palm oil 2 had one rooster removed as an influential outlier due to high excreta weight.

² Lipid was added at the expense of a 100% ground corn basal.

³ The TMEn values of the lipids were calculated by difference using the values of the 0% added lipid diet and the diets containing the test lipids.

⁴ The linear regression of TMEn (Y, kcal/g DM) on lipid level (g/g diet) from acid oil ($X_1 \pm SE$) yielded the equation $Y = 4.043 + 5.160 \pm 0.199X_1$ with an R^2 of 0.97. The linear regression of TMEn (Y, kcal/g DM) on lipid level (g/g diet) from palm oil 2 ($X_2 \pm SE$) yielded the equation $Y = 3.990 + 5.084 \pm 0.317X_2$ with an R^2 of 0.94.

Table 2.8. Effects of lipid type and lipid intake on growth performance of male broiler chicks from 10 to 20 d post-hatch, as well as relative bioavailability estimates for each lipid type as compared to refined corn oil (RCO) in Experiment 5¹.

Dietary treatment ²	n ³	BW gain (g/chick)	Feed intake ⁴ (g/chick)	Gain:Feed (g/kg as-is)	RBV ⁵
0% added lipid control	4 ³	336.50 ^d	462.60	714.84 ^d	-
5% RCO	5	371.36 ^c	462.60	802.78 ^c	
10% RCO	5	412.00 ^a	462.60	890.60 ^a	100
5% palm oil 2	5	363.92 ^c	462.60	786.68 ^c	
10% palm oil 2	5	397.32 ^b	462.60	858.90 ^b	80.5*
5% choice white grease	5	371.32 ^c	462.60	802.68 ^c	
10% choice white grease	5	398.32 ^b	462.60	861.06 ^b	85.4*
5% acid oil	5	363.08 ^c	462.60	784.86 ^c	
10% acid oil	5	409.00 ^{a,b}	462.60	884.14 ^{a,b}	92.7
SEM	-	3.85	-	8.53	-

*Different ($P < 0.05$) than RBV of RCO.

^{a-d}Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by the least significant difference test.

¹Values represent the least squares means.

²Lipid was added at the expense of cellulose to the 0% lipid added diet.

³Experimental unit was a pen containing 4 or 5 birds, one pen from 0% added lipid control found to be an influential outlier and removed due to a low chick body weight.

⁴Feed intake was the same across all treatments to ensure that energy was the limiting factor.

⁵RBV = relative bioavailability value as determined by the slope ratio method. The multiple linear regression of BW gain (Y, g) on lipid intake (g) from RCO ($X_1 \pm SE$), palm oil 2 ($X_2 \pm SE$), choice white grease ($X_3 \pm SE$), or acid oil ($X_4 \pm SE$) yielded the equation $Y = 335.19 + 1.640 \pm 0.108 X_1 + 1.322 \pm 0.108 X_2 + 1.403 \pm 0.108 X_3 + 1.517 \pm 0.108 X_4$ with an R^2 of 0.88. The regression coefficients for the choice white grease and the palm oil 2 are different ($P < 0.05$) than that of RCO, whereas the regression coefficient of acid oil is not significantly different than RCO.

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CHAPTER 3:

EVALUATION OF OXIDIZED LIPID SAMPLES USING THE SLOPE-RATIO PRECISION-FED ROOSTER ASSAY FOR RELATIVE BIOAVAILABLE ENERGY VALUES OF FATS AND OILS

ABSTRACT

A slope-ratio precision-fed rooster assay was conducted to determine the relative bioavailable energy values of one reference lipid and four test lipids. Roosters were fasted for 26 h and then were precision-fed 0, 5, or 10% of the different lipids at 100% expense of the ground corn. Refined corn oil, canola oil, poultry fat, oxidized canola oil, and oxidized poultry fat were the lipids evaluated. Oxidation of the lipids was achieved by cyclic heating of the lipids for 60 h at 90 °C with ambient air being percolated through the lipids at 12 liters per min. Multiple regression slope-ratio analysis of nitrogen-corrected true metabolizable energy (TMEn) on dietary fat level was used to estimate relative bioavailability values (RBV). The refined corn oil was used as the reference source (set at 100%). The RBV were calculated as the regression coefficient of the test lipid divided by the regression coefficient of the reference refined corn oil. Oxidation of the canola oil and poultry fat generally had a modest effect on fatty acid composition. However, initial peroxide value, Active Oxygen Method (AOM) stability and p-Anisidine (measure of aldehyde content) value were greatly increased by oxidation. The RBV of the lipids varied from 86.3% to 104% but none of the test fats RBVs were significantly lower ($P < 0.05$) than the refined corn oil reference (100%) (Although RBV of both the canola oil and poultry fat were reduced by 11 percentage units by oxidation). Thus, the results of this study indicated that oxidization of the canola oil and poultry fat did not significantly reduce the metabolizable energy of the lipids.

INTRODUCTION

Lipids have many positive effects in commercial poultry diets by providing a concentrated source of energy, supplying essential fatty acids and functioning as carriers for fat soluble vitamins. However, over time, exposure to certain elements (e.g., UV light, high temperatures, oxygen, processing and storage conditions) can oxidize lipids, affecting their taste and odor, as well as decreasing their energy content. It has been shown that the inclusion of oxidized dietary lipids in broiler diets may also promote the destruction of fat-soluble vitamins (i.e., A, D, E, and K) and consequently lead to a depressed vitamin retention (Lin et al., 1989a; Engberg et al., 1996). The process of oxidative rancidity can be a major cause of loss of the nutritional quality of an ingredient or ration for poultry (Baião & Lara, 2005), however, limited research has evaluated the dietary effects of oxidized lipids on poultry growth performance is limited.

The few studies that have been conducted on the effects of oxidized oils in poultry diets have yielded conflicting reports on the impact that they have on growth performance. When Racanicci et al. (2008) fed 4% oxidized poultry fat to birds from 10 until 47 d of age, the detrimental effects on composition and quality aspects found in the oxidized fat were not sufficient to impair the growth performance of the broilers. However, Engberg et al. (1996) showed a decrease in live weight when birds were fed an oxidized vegetable oil blend compared to a fresh vegetable blend, but there were no differences in feed intake and feed conversion ratio. Lin et al. (1989a) observed a 4.2% and 7.3% significant decrease in body and carcass weights, respectively, when they fed 5.5% of oxidized sunflower oil to three week old broilers compared to the broilers fed unoxidized oil. Polyunsaturated and unsaturated fatty acids are more susceptible to oxidative damage on the double bond of triglycerides, which may impact the

utilization of the lipid by the bird and the overall quality of the lipid (Ravindran et al., 2016; Huyghebaert et al., 1988).

The purpose of this study was to determine the effects of oxidation of canola oil and poultry fat on the relative bioavailable energy value of the lipids, compared with refined corn oil using a precision-fed rooster assay.

MATERIALS AND METHODS

The procedures used in conducting this experiment were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign.

Experimental Design

This experiment was designed to determine the TMEn of five different lipids using the precision-fed rooster assay (Parsons et al., 1982). Each diet consisted of ground corn with lipids added at varying levels (0-10%) at the expense of the ground corn. The roosters were fasted for 26 h and then were precision-fed 0, 5, or 10% of refined corn oil, canola oil, poultry fat, oxidized canola oil, and oxidized poultry fat with four roosters per treatment. Treatment diets were tube-fed at a level of 30 g to adult Single Comb White Leghorn roosters following the general guidelines of Parsons et al. (1982) and Sibbald and Wolynetz (1986). Excreta (feces + urine) were quantitatively collected for 48 h after feeding on plastic trays placed underneath the wire floors. The roosters were housed in an environmentally controlled room with a 16 h light to 8 h dark cycle in individual cages with wire floors.

The poultry fat and canola oil used in this experiment were food grade in origin, the poultry fat was stabilized with rosemary extract, and the canola oil was refined, but not stabilized. Both lipid sources were oxidized via the same method; prior to heating, the unopened barrels of

fat and oil were allowed to warm up to room temperature. Once they were at room temperature, the lipids were heated for 60 h at 90 °C and were cycled between 8 h hot and 16 h cold. Throughout the cooking process, ambient air was percolated through the lipids at a rate of 12 liters per min.

Chemical Analysis

The excreta collected from the rooster assay was frozen, freeze-dried, weighed, and ground in a coffee grinder. Subsamples of the lipids, excreta, and feed samples were analyzed according to procedures of the Association of Official Analytical Chemists International (AOAC International, 2006). Lipids, feed, and excreta were analyzed for gross energy in an adiabatic bomb calorimeter (Model 1261, Parr Instruments, Moline, IL) that had been standardized with benzoic acid. Excreta and feed were analyzed for total nitrogen via combustion (AOAC International, 2006; method 990.03). Feed samples were also analyzed for dry matter (AOAC International, 2006; method 934.01).

All lipids used in Chapter 3 were analyzed at the University of Missouri-Columbia for fatty acid composition (AOAC International, 2006; methods 996.06, 965.49, and 969.33; AOCS, 2003; method Ce 2-66), free fatty acids (%) (AOCS, 2003; method Ca 5a-40), iodine value (AOCS, 2003; recommended practice Ja 14-91), moisture (%) (AOCS, 2003; method Ca 2c-25), insolubles (%) (AOCS, 2003; method Ca 3a-46), unsaponifiabiles (%) (AOCS, 2003; method Ca 6a-40), and total moisture, insolubles, and unsaponifiabiles (MIU) (%). In addition, the unoxidized and oxidized canola oil and poultry fat samples were analyzed for initial peroxide value (AOAC International, 2006; method 965.33) AOM stability at 20 h (AOCS International, 2006; method Cd. 12-57), and p-Anisidine value at Barrow-Agee Laboratories, Memphis, TN (Table 3.1).

Statistical Analysis

The TMEn of the diets was calculated by the method of Parsons et al. (1982), while the TMEn of the lipids were calculated by difference using the Han et al. (1976) method. Each experiment was analyzed individually using the GLM procedures of SAS (SAS Institute Inc, 2011). Multiple linear regression of TMEn (Y) on supplemental lipid inclusion level (X) was conducted using the methods of Littell et al. (1995; 1997) for the slope-ratio method. To determine if the test lipids were significantly different than the reference lipid (refined corn oil), pair-wise comparisons were made between the regression coefficients of the test lipids and the reference refined corn oil (Littell et al., 1995). If the regression coefficients were found to be significantly different, then it was assumed the RBV were also significantly different since the RBV is a ratio of the regression coefficients (Littell et al., 1995). The experiment was completely randomized and statistical significance was accepted at a probability of $P < 0.05$. Influential outliers were identified using the Diagnostic Plots of the GLM Procedure with the Unpack and Label options of SAS. Cook's D test was used as an objective measure of outlier influence (Cook, 1977). Outlying data points were removed if careful analysis of the original data revealed a methodological or biological explanation for the data according to described methods (Freund & Littell, 2000).

RESULTS AND DISCUSSION

Analytical Results

Table 3.1 shows the fatty acid profile and the composition of the lipids used. The levels of linoleic and linolenic acid were both reduced following the oxidation of the lipid samples. However, the palmitic and stearic fatty acids of both lipid sources were increased following the oxidation process. Both of these changes in fatty acid profile agree with Racanicci et al. (2008)

when oxidizing poultry offal fat, as well as Enberg et al. (1996) that oxidized a vegetable oil blend. There was a substantial increase in the free fatty acids percentage and an increase in the initial peroxide value, the active oxygen method (AOM) stability at 20 h, and the p-Anisidine value for both oxidized lipid sources, whereas the iodine values of the test lipids decreased post-oxidation. MIU content can be important because these components may dilute the energy content of the lipid (Leeson & Summers, 2001). As iodine values are an estimation of the number of double bonds present in a fat or oil, a higher extent of oxidation is expected to yield lower values (Baião & Lara, 2005). Thus, it is not surprising that a reduction was seen in iodine values in the current study. Peroxides are unstable intermediate substances of the oxidation process and are used to evaluate the quality of lipids, with higher levels of peroxides indicating an inferior lipid quality. The initial peroxide value is usually analyzed in a fresh sample when very few secondary products are formed, while the AOM exposes 2.0 ml of fat to a controlled temperature water bath (97.8 °C) with a stream of dry air bubbling at a rate of 2.33 ml per tube per second (Baião & Lara, 2005). The peroxide values are then measured for all four test lipid sources after 20 h of the AOM exposure when a large increase in peroxide values and volatile products are formed (Läubli & Bruttel, 1986). The p-Anisidine value is a measurement of the aldehyde content in an oil, which are the secondary oxidation products produced during oxidation, and account for almost 50% of the volatile products formed during oxidation (Tompkins & Perkins, 1999). The initial peroxide value and AOM are beneficial because results can be obtained in hours rather than days; however, there are inherent deficiencies since peroxides are unstable and decompose rapidly to more stable secondary reaction products. Peroxide concentration usually reaches a maximum for only a short time, and if that maximum point is missed, an inaccurate endpoint may occur

(Jebe et al., 1993). This is why it is important to look at the composition results as a combination of p-Anisidine, initial peroxide values, and AOM stability instead of just one solitary value. The observation herein that all three of the latter were greatly increased indicated that the oxidation procedure did indeed result in extensive oxidation of the canola oil and poultry fat.

Experiment 1

The results of the precision-fed rooster assay are shown in Table 3.2. The TMEn of one rooster fed 5% poultry fat and one rooster fed 5% oxidized poultry fat were identified as influential outliers and were removed from the analysis due to low excreta weights. The low excreta weight can be explained by the incomplete or partial clearance of the digestive tract by the rooster or excreta being lost during collection (Dale et al., 1985). The addition of 5 and 10% of all lipids to the corn diet yielded linear increases in TMEn ($R^2=0.87$ for the multiple regressions). The TMEn values of the lipids were calculated by difference and some levels of the lipids demonstrated extra-metabolic effects (EME), which is when the TMEn of the diet exceeds the gross energy of the lipid (Mateos & Sell, 1981). The 10% inclusion level of refined corn oil and canola oil and the 5% inclusion levels of oxidized canola oil and oxidized poultry fat showed EME, while both levels of poultry fat showed EME. The greatest EME was observed for the 5% inclusion of poultry fat, where the poultry fat had a gross energy of 9.1 (kcal/g DM) but the TMEn was 12.0 (kcal/g DM). A large EME was also observed for the 5% inclusion level of the oxidized poultry fat. The latter results are consistent with the previous published data that showed larger EME at lipid inclusion levels of 5% or lower (Sibbald & Kramer, 1977; 1978).

The RBV for all the lipids were high and none of the RBV were significantly different than the 100% for the refined corn oil. The RBV of the canola oil was numerically decreased from 97.7

to 87.0% by oxidation which is consistent with the previous research that unsaturated lipids are more prone to oxidization (Ravindran et al., 2016; Huyghebaert et al., 1988). The RBV of the poultry fat was also numerically decreased by 11 percentage units from 104 to 93%.

Conclusions

The results of this study indicated that the available energy content of canola oil and poultry fat were not significantly reduced by oxidation treatments as measured by TMEn using the slope-ratio precision-fed rooster assay. The lack of reduction in RBV occurred even though the peroxide, AOM stability, and p-Anisidine values clearly indicated that the lipids were indeed oxidized. However, while the energy content was similar, feed formulators should be aware of the possible effects of oxidation on concentrations of fatty acids in the oxidized oils. It was reported that the concentrations of linoleic and linolenic acids were lowered by 25-38% by oxidation, while saturated fatty acids (stearic and palmitic acids) increased compared to their fresh sources for a vegetable blend and a poultry fat (Engberg et al., 1996; Racinacci et al., 2008). Reductions in linoleic and linolenic acids were also observed by the oxidation of canola oil and poultry fat in the current study. It is important to have an accurate idea of the fatty acid composition of the lipid source so that the unsaturated: saturated ratio within a lipid source and the mixed diet stay consistent. The AMEn and standardized lipid digestibility of lipids was maximized when the unsaturated: saturated ratio in the total diet was slightly greater than 4 (Ketels & De Groote, 1989; Aardsma et al., 2017).

Oxidized lipid sources also appear to have a lower α -tocopherol concentration, a natural antioxidant, which is a concern for the broiler meat production industry. As the consumption of chicken increases, so does the demand for increased shelf-life and stability of the meat. It was

noted that meat originating from oxidized oil-fed broilers underwent rapid oxidative changes during refrigeration and frozen storage (Lin et al., 1989a). Engberg et al. (1996) found their oxidized oil diet had an α -tocopherol concentration of 44.0 mg/kg, compared to the fresh oil diets which had a concentration of 80.8 mg/kg. While the α -tocopherol concentration in the oxidized oil diet was still above the recommended level for commercial broiler feed (25 to 30 mg/kg feed) (Engberg et al., 1996), birds fed oxidized oil had a lower level of α -tocopherol in their meat. The concentration of α -tocopherol in the dark and white meat of the broilers were 2.2 mg/kg and 1.8 mg/kg, respectively, for birds fed oxidized sunflower oil, which was a reduction of approximately 25% compared to the fresh oil fed broilers (Lin et al., 1989a). The latter likely occurs because oxidized lipid molecules may be absorbed through the small intestine as a consequence of consuming oxidized oil diets, which then initiates a cascade of subsequent oxidation reactions that result in increased oxidation throughout the animal (Racan Ricci et al., 2008; Suomela et al., 2005). Thus, while the oxidized lipid sources contain a concentration of α -tocopherol that is still above recommended levels, more of the α -tocopherol will have to be used to protect tissue lipids from oxidized oil-induced free radical attack (Lin et al., 1989a), which can lead to an α -tocopherol deficiency that leaves the meat exposed to further free radical oxidation.

For the above reasons, it is vital to properly store lipids used in poultry diets. If the lipid is susceptible to high oxidative damage, or already has high oxidative damage, then an antioxidant supplement added to the lipid source is advised. The antioxidant should minimize any oxidative damage to the susceptible unsaturated fatty acids. The dietary supplementation of α -tocopherol in diets using oxidized lipid sources may also improve the oxidative stability of dark and white broiler meat during refrigeration and freezing (Lin et al., 1989b).

TABLES

Table 3.1. Analyzed composition of lipid sources.¹

Lipid Source	Fatty Acid (%)						
	C14:0 Myristic	C16:0 Palmitic	C16:1 Palmitoleic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic
RCO ¹	0.04	11.72	0.10	1.84	28.24	54.54	0.99
CNO ¹	0.05	4.04	0.22	1.75	58.34	19.27	8.48
PF ¹	0.61	23.88	5.92	5.96	37.43	19.15	0.82
OCNO ¹	0.06	4.79	0.26	2.11	64.04	13.90	3.97
OPF ¹	0.71	28.05	6.12	7.13	38.69	10.77	0.26

¹Abbreviations: refined corn oil reference (RCO), canola oil (CNO), poultry fat (PF), oxidized canola oil (OCNO), oxidized poultry fat (OPF).

Table 3.1 continued. Analyzed composition of lipid sources.¹

Lipid Source	Free Fatty Acids (%)	Initial Peroxide Value (meq/kg)	AOM ¹ Stability @ 20 h	p-Anisidine Value	Iodine Value	Moisture (%)	Insolubles (%)	Unsaponifiabiles (%)	Total MIU ¹ (%)	Gross Energy (kcal/g DM)
RCO ¹	0.16	--	--	--	122.35	0.00	0.00	0.00	0.00	9.19
CNO ¹	0.07	3.0	157.5	3.83	110.51	0.02	0.06	0.73	0.81	9.15
PF ¹	0.35	2.2	6.9	2.01	77.79	0.08	0.08	0.63	0.79	9.13
OCNO ¹	0.56	156.7	273.4	170.00	94.83	0.16	0.08	0.78	1.02	9.03
OPF ¹	0.63	185.8	270.8	143.00	61.61	0.14	0.04	0.23	0.41	9.60

¹Abbreviations: refined corn oil reference (RCO), canola oil (CNO), poultry fat (PF), oxidized canola oil (OCNO), oxidized poultry fat (OPF), active oxygen method (AOM), sum of moisture, insoluble, and unsaponifiabiles (MIU).

Table 3.2. Effects of refined corn oil, crude corn oil, poultry fat, oxidized crude corn oil, and oxidized poultry fat lipid types and lipid level on nitrogen-corrected true metabolizable energy (TMEn) of mixed diets and lipids, as well as relative bioavailability (RBV) estimates¹.

Dietary Treatment ²	TMEn of mixed diets (kcal/g DM)	TMEn of lipid ³ (kcal/g DM)	RBV ⁴
0% added lipid	4.00 ^e	--	
5% refined corn oil	4.25 ^d	9.07 ^b	
10% refined corn oil	4.55 ^a	9.59 ^b	100
5% canola oil	4.22 ^d	8.57 ^b	
10% canola oil	4.55 ^a	9.56 ^b	97.7
5% poultry fat ²	4.40 ^{b,c}	12.02 ^a	
10% poultry fat	4.52 ^{a,b}	9.27 ^b	104
5% oxidized canola oil	4.25 ^d	9.15 ^b	
10% oxidized canola oil	4.47 ^{a,b}	8.72 ^b	87.0
5% oxidized poultry fat ²	4.30 ^c	10.17 ^a	
10% oxidized poultry fat	4.49 ^{a,b}	8.90 ^b	92.7
SEM	0.045	0.609	-

^{a-d}Means within a column with no common superscript are significantly different ($P < 0.05$) as determined by a least significant difference comparison.

¹Values represent least squares means.

²Lipid was added at the expense of a 100% ground corn basal with an n = 4 roosters per dietary treatment. One rooster from 5% poultry fat and one rooster from 5% oxidized poultry fat were found to be influential outliers and removed due to low excreta weights.

³The TMEn values of the lipids were calculated by difference using the values of the corn basal and diets containing the added lipids.

⁴RBV = relative bioavailability value as determined by the slope ratio method. The multiple linear regression of TMEn (Y, kcal/g DM) on lipid level (g/g diet) from refined corn oil ($X_1 \pm SE$), canola oil ($X_2 \pm SE$), poultry fat ($X_3 \pm SE$), oxidized canola oil ($X_4 \pm SE$), or oxidized poultry fat ($X_5 \pm SE$) yielded the equation $Y = 4.02 + 5.23 \pm 0.43 X_1 + 5.11 \pm 0.43 X_2 + 5.45 \pm 0.43 X_3 + 4.55 \pm 0.43 X_4 + 4.85 \pm 0.43 X_5$ with an R^2 of 0.87.

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CHAPTER 4:

CONCLUSIONS

Overall, the slope-ratio precision-fed rooster assay was able to detect differences among several of the test lipids. The assay was able to distinguish inferior, superior, or similar test lipids compared to a known reference lipid rapidly. Many of the test lipids that are typically fed in the poultry industry (poultry fat and vegetable blend acid oil) had equivalent relative bioavailability values (RBV) as the refined corn oil or refined soy oil reference, indicating that these lipid sources are excellent sources of energy for poultry. Some other lipids, however, such as palm and tallow, had a significantly lower ($P < 0.05$) RBV compared to the reference soybean oil or corn oil. This validates the use of this assay in detecting and quantifying inferior lipid sources. Maximizing bird performance and profitability is a large consideration in poultry feed formulation and the use of this relatively inexpensive and rapid assay can be useful in determining which lipid sources should be fed and at which stage of production.

While the slope-ratio chick growth assay is much more labor intensive compared to the slope-ratio precision-fed rooster assay, the chick assay yields valuable data for growing poultry. As lipid utilization can be affected by age of the animal, it is important to see how the growing chick utilizes lipids compared with the rooster. The RBV for the roosters and chick were in good agreement for palm oil, tallow, and the vegetable blend acid oil. The rooster precision-fed choice white grease in Experiment 3 of Chapter 2 had a high RBV, while the chicks in Experiment 5 of Chapter 2 showed a lower utilization or RBV of the choice white grease. This allows a producer to see the efficiency of utilization of a lipid source in birds of different ages, while knowing that a specific lipid source may not be appropriate for younger birds. The producer would then be

able to select which lipid source is most appropriate to use based on age of bird and cost of the lipid to meet their production goals.

Lipid rancidity is a concern in diet formulation and the precision-fed rooster assay may be able to aid in detecting the effects of oxidation on lipid sources. While the RBV of oxidized test lipids were not significantly different ($P < 0.05$) compared with the reference refined corn oil, the RBV of both the canola oil and poultry fat were reduced by 11 percentage units by oxidation. This indicated that the precision-fed rooster assay may be able to detect a reduction in energy availability caused by oxidation but an increased number of replicates may be needed to detect significance. A producer would be able to determine the RBV of their oxidized oil relatively quick to determine if feeding the oxidized oil was still an option. While the precision-fed slope-ratio rooster is useful in determining RBV, it does not determine the composition of the lipid source. It is therefore important as a formulator to be aware of the possible effects of oxidation on concentrations of fatty acids in the oxidized oils, as linoleic and linolenic acids have been known to be reduced.

Further considerations for the use of the slope-ratio precision-fed rooster assay would be to use greater than two levels of non-zero supplemental fat or oil inclusion to aid in detecting differences among lipid samples. For example, it may be useful to precision-feed 5, 10, and 20% of the test lipids to provide a high level of 20% test lipid that may exhibit a difference that is not observed at the lower levels of 5 and 10%. Having a high level of inclusion may also aid in obtaining clearer results for samples suspected of high levels of saturation degree or free fatty acid content that may yield inaccurate utilization in the bird at low inclusion levels. Although 20% lipid inclusion is much higher than levels fed in commercial poultry diets, the roosters are only

precision fed 30 g of experimental diets, which, at 20% lipid inclusion, is equal to only 6 g of fat per rooster for a 48 h period. Tube-feeding a high level of lipid also reduced the confounding EME effects on the interpretation of the ME values for fats and oils.

The use of the slope-ratio precision-fed rooster assay, in conjunction with the slope-ratio chick growth assay when applicable, can provide valuable data on the relative bioavailability energy in test lipids rapidly, inexpensively, and reliably.