EVALUATING ENERGY UTILIZATION IN SOME SELECTED NON-CONVENTIONAL FEED INGREDIENTS FOR BROILER CHICKENS AND PIGS

by

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Dedicated to God and my family

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ABBREVIATIONS

AMEn	Nitrogen-corrected Apparent metabolizable energy
ATTD	Apparent total tract digestibility
BSF	Black soldier fly
BSFLM	Black soldier fly larvae meal
BW	Body weight
DE	Digestible energy
DF	Dry fat
DM	Dry matter
DMIti	Dry matter intake of test ingredient
Cr	chromium index method
Exp.	Experiment
FDPP	Flash-dried poultry protein
FHP	Fasting heat production
FI	Feed intake
G:F	Gain to feed ratio
GE	Gross energy
HFM	Hydrolyzed feather meal
HI	Heat increment
IDE	Ileal digestible energy
NE	Net energy
NSP	Non-starch polysaccharide
ME	Metabolizable energy
MEn	Nitrogen-corrected metabolizable energy
RCBD	Randomized complete block design
RD	Reference diet
RQ	Respiratory quotient
SBM	Soybean meal

SBO	Soybean oil
SCFA	Short-chain fatty acids
SRB	Stabilized rice bran
TMEn	Nitrogen-corrected true metabolizable energy
TTR	Total tract retention
TC	Total collection method
Ti	Titanium index method

ABSTRACT

The objective of this thesis was to evaluate energy utilization in some selected non-conventional feed ingredient for broiler chickens and pigs. Three studies were carried out to evaluate this objective. All studies employed the randomized complete block design with 8 replicates.

Study 1 evaluated the ileal digestible energy (IDE), metabolizable energy (ME) and nitrogen-corrected ME (MEn) of 2 feed ingredients which included dry fat (DF) and stabilized rice bran (SRB) with broiler chickens using the regression method in 2 experiments. Chickens were fed a common broiler chicken starter diet from d 0 to 17 and experimental diets from d 17 to 22 post hatching. Three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing either DF at 50 or 100 g/kg replacement in experiment (Exp.) 1 or SRB at 100 or 200 g/kg replacement (Exp. 2) of the energy-contributing ingredients in the RD. In each Exp., 192 chickens were randomly allocated to one of three dietary treatments. In Exp.1, the IDE, ME, and MEn linearly increased (P<0.001) as DF concentrations increased, while in Experiment 2, the IDE, ME, and MEn of the diets were not affected by dietary supplemental SRB. The regression-derived IDE concentration for DF and SRB were 6,047 and 3,556 kcal/kg DM, respectively. The respective ME and MEn estimates (kcal/kg DM) were 6,051 and 5,922 for DF; 3,437 and 3,193 for SRB. The results from this study showed that broiler chickens utilized between 77 to 79% and 68 to 76% of the gross energy (GE) in DF and SRB, respectively, and this suggested a strong potential for these ingredients as dietary energy sources for broiler chickens.

In Study 2, three experiments were conducted to evaluate the IDE, ME, and MEn of hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP) with broiler chickens and to determine the digestible energy (DE) and ME of HFM and FDPP for pigs. The HFM or FDPP were incorporated into a reference diet either at 3 levels (0, 75, or 150 g/kg) in Exp. 1 and 2, or 2 levels (0 or 150 g/kg) in Exp. 3 by replacing the energy-yielding ingredients. In Exp. 1, the inclusion of HFM, linearly decreased ($P < 0.05$) the nitrogen corrected metabolizability although, the ME concentration in the diets were linearly increased ($P < 0.05$). In Exp. 2, a linear decrease was observed on the ileal digestibility of DM and energy ($P < 0.05$). It was also observed that the total tract retention (TTR) of DM and energy linearly increased ($P < 0.05$). Similarly, the ME and MEn concentration linearly increased with a *P*-value of < 0.001 and < 0.01, respectively. In Exp.

3, the dietary treatments significantly increased ($P < 0.05$) the fecal energy loss. Diet substituted with HFM had significantly higher ($P < 0.001$) urinary GE loss than the RD. The TTR of GE in the RD was significantly higher than those in the test diet containing 150 g/kg of HFM. The respective IDE, ME, and MEn evaluated for HFM in the current study were 4,509, 4,250, and 3745 kcal/kg DM with corresponding values of 3,221, 4,710, and 4,081 kcal/kg DM for FDPP when fed to broiler chickens. In pigs, the respective DE and ME evaluated for HFM were 4,783 and 4,405 kcal/kg DM while estimates for FDPP were 4,553 and 4,320 kcal/kg DM, respectively.

In Study 3, energy value of partially defatted black soldier fly larvae meal (BSFLM) was determined in 2 experiments with broiler chickens and growing pigs. The Exp. 1 was conducted to evaluate IDE, ME, and MEn of BSFLM with broiler chickens while Exp. 2 was conducted to evaluate the digestible energy (DE) and ME of BSFLM in growing pigs. Total collection (TC) and two index methods using either titanium dioxide (Ti) or chromium oxide (Cr) were compared. In Exp 1 and 2, three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing BSFLM at either 100 or 200 g/kg replacement of the energy-contributing ingredients in the RD. In Exp. 1, a linear increase ($P < 0.05$) was observed in the IDE concentration of the diet. With increasing BSFLM, a quadratic and linear increase ($P < 0.05$) was also observed on the ME and MEn concentration in the diet, respectively. The regression-derived IDE, ME, and MEn concentration in BSFLM were 4,517, 4,725, and 4,238 kcal/kg DM when fed to broiler chickens. In Exp. 2, the inclusion of BSFLM resulted in linear decrease in DM digestibility and linear increase in dietary DE concentration ($P < 0.05$). The metabolizability of GE linearly decreased ($P < 0.05$) while the ME concentration quadratically increased with the increasing inclusion of BSFLM in the diet. In pigs, the regression-derived DE estimates with TC, Ti index marker, and Cr index marker were 5,010, 4,907, and 4,927 kcal/kg, respectively. The ME derived using the TC method was 4,711 kcal/kg. The result from this study is interpreted to suggest BSFLM as a potential energy feed ingredient for inclusion in diets for broiler chickens and pigs.

In summary, we could conclude that DF can be added to the list of fat source while SRB is a potential fiber source in broiler chicken diets. Also, HFM, FDPP, and partially defatted BSFLM are all potential energy sources which should be added to the list of protein sources for broiler chickens and pigs. Generally, there is a need for an overhauling of feed ingredients termed as conventional, those alternative feed ingredients that are also readily and constantly available for

livestock feeding should be driven and sensitized to farmers for consideration as conventional feed ingredient.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

World population has been predicted to grow over 9 billion people by 2050 causing the world to produce 70% more food (FAO, 2009). A direct impact of this is that livestock production especially poultry and swine will grow exponentially reaching up to double the current production. (Schiavone et al., 2018). Hence, the animal production industry is presented with the challenge to develop innovative methods that are focused to meet future environmental and economic needs (Barragan-Fonseca et al., 2017). One of the ways through which global animal scientist could combat this onus is to broaden the scope of what is acceptable as conventional feed ingredient, most importantly are those feed ingredients that are characterized to supply dietary energy. This is because animal feed is the single most expensive input in commercial livestock production, and it contributes about 50 to 70% of the total cost of production (Lawrence et al., 2008; Velayudhan et al., 2015; Alqaisi et al., 2017). Interestingly, in the United States, about 50 to 85% of the ingredients in typical swine rations are carbohydrates from cereal grains while protein contributes 15 to 20% of the total energy in the diet (Myer and Brendemuhl, 2014). This indicates that a large part of the cost of feed goes to energy contributing ingredients, thus more emphasis needs to be placed on sustainable, alternative, and novel dietary energy feedstuffs.

Dietary energy content could modulate feed efficiency via two partially dependent pathways: Firstly, as dietary energy increases, decreasing feed intake is observed, this is because animals will only eat to their energy satisfaction (Nyachoti et al., 2004; Jeffre et al., 2010). Secondly, growth rate is promoted by increasing dietary energy level (Waldroup, 1981; Plavnik et al., 1997), given that no other nutrient is limiting. For efficient monogastric production, it is therefore imperative that diets are formulated to match dietary energy supply with energy requirements for maintenance and productive functions. Hence, there are two goals to be achieved in modern monogastric production, which are efficient and sustainable production. To jointly achieve these goals while holding the world population growth in view, it is critical that the energy values of those alternative feed ingredients are precisely determined, and that the energy system that best suit the energy needs of each species are used.

For several years, many studies on nutrient digestion, partition and utilization have been conducted with growing pigs and broiler chickens providing a basis for establishing nutritional requirements under a variety of external and internal conditions (Cerniglia, 1981; Chwalibog et al., 2005). However, with progressive changes in animal production and emergence of new feed ingredients, there is still a need for continuous update on nutritional knowledge such as energy determination studies by means of animal experimentation.

1.2 Energy as a feed component: what it supplies and its importance

Energy is required to fuel body processes such as metabolic processes, physiological functions, muscular activity, heat production, growth, and synthesis of new tissues (Kil et al., 2013). It is released from feed components by oxidation. The main sources of energy in animal feed are carbohydrates, proteins, and fats. The nutrients that provide energy are commonly referred to as macronutrients (Collin et al., 2003, Swennen et al., 2005, 2007). Carbohydrates and proteins provide a similar amount of energy per gram of feed. Lipids are a concentrated source of energy and provide almost twice the amount of energy than those supplied by proteins and carbohydrates. The average amount of energy released ranges from approximately 4 kcal/g for carbohydrates or protein to 9 kcal/g for fats (FAO: WHO: UNU 2004). Dietary energy is aimed to supply adenosine triphosphate (ATP) needed for maintenance and production (Emmans, 1999; Rijnen et al., 2004), which is the molecular form in which energy is stored in animal cells (El Bacha et al., 2010).

1.3 Major energy supplying feedstuffs

There is a basic criterion for a feed ingredient to be classified as fat, protein, and carbohydrate source. De Groote (1974) employed a simplified net energy (NE) system to classify feed ingredients into categories depending on the crude protein, crude fat, starch, and sugar content. Feed ingredients with NE:ME ratio averaging 0.63 were classified as Protein-containing ingredients, cereals had values around 0.73 while fats and oils had values averaging at 0.90. Plavnik et al. (1997) stated that differences exist at the metabolic level when carbohydrates, fat, and protein are used as energy sources. Some of these attributed differences are discussed below.

1.3.1 Fat as energy source

Fat is often added to diets to increase the dietary energy density and total energy intake (Haddad and Younis, 2004). Diets supplemented with a higher fat content has a positive effect on energy utilization as well as on metabolizable energy intake (Mateos and Sell, 1980). This effect is because of the better metabolizability of fat when compared to proteins, and fat also has longer transition time through the digestive tract (Swennen et al., 2004). There are instances when fat supplements become the least-cost source of energy and when high usage levels may be justifiable (Waldroup, 1981). Less energy is produced as heat when energy in the body is derived from dietary fat as opposed to energy from the conversion of excess dietary protein (Emmans, 1994; Gous, 2010). Since fats have a lower heat increment than proteins and carbohydrates, it has been suggested that under extreme heat stress conditions, a greater portion of the dietary energy for broiler chickens should be supplied by supplemental fats especially through vegetable fat sources (Bonnet et al., 1997). Moreover, because broiler breeders have been reported to produce more heat at the end of production phase due to additional energy required to maintain the higher body weight achieved at the end of production, it has been observed that this phenomenon causes hens to resort to using fat calories to alleviate such physiological condition as this can be inferred through an observed decline in fat tissue during this phase. (Salas et al., 2017; Caldas et al., 2018). It has also been theoretically established that the lower heat increment of fat causes an extra caloric effect which is linked to an improved utilization of metabolizable energy calories (Touchburn and Naber, 1966; Jensen et al., 1970).

1.3.2 Protein as energy source

Feeding a high protein diet encourages lean meat production but, in most cases, this is not a cost-effective option as it is well known that protein-rich feed ingredients are generally more expensive. Utilization of protein as a source of energy involves more complex metabolic pathways and a higher metabolic rate. One of the implications of this higher metabolic rate can be observed through protein respiratory quotient (RQ) value. The RQ is defined as the ratio between the volume of carbon dioxide produced by an animal and the volume of oxygen used (Chepete, 2004). The RQ value for protein oxidation is known to be higher than that of fat; 0.74 and 0.71 is the respective average value reported for protein and fat especially in uricotelic animals (MacLean and

Tobin, 1987; Walsberg and Wolf, 1995). Dietary protein and amino acids contents act as significant contributors to total heat production (THP) compared to other dietary nutrients (Teeter et al., 1996). MacLeod (1997) observed that body protein turn-over increased after a higher intake of lysine; a first-limiting amino acid in broiler chickens, and this resulted in greater THP which was also closely correlated with protein accretion in broiler chickens.

1.3.3 Carbohydrates as energy source

Carbohydrates serve as the main energy source in the diet of monogastric species, with additional role of serving as biomaterial building blocks (Chang, 2012). Not only do carbohydrates serve as energy source but the concentration, type, and source of carbohydrate fractions in the diet could affect the balance of the gut microbiota, manipulate intestinal absorptive function, and immune response in monogastric animals (Cummings, 1981; Knudsen et al., 2017). Carbohydrates are majorly classified into monosaccharides, disaccharides, oligosaccharides, and polysaccharides. In terms of its abundance, starch is second to cellulose polysaccharide synthesized by plants and is the primary source of energy for many monogastric species (Choct and Kocher, 2000). Although, it has recently been shown by Baéza et al. (2015) that meat-type chickens are prone to adapt to dietary starch substitution with fat and fiber. Other polymeric carbohydrates such as non-starch polysaccharides (NSP) and resistant starch also play beneficial roles in animal nutrition (Slominski et al., 1994). The fermentative breakdown of NSP leads to the production of metabolites such as short-chain fatty acids (SCFA) and this could contribute up to 24% of the dietary energy in pigs and about 3% in broiler chickens (Choct and Kocher, 2000; Adebowale et al., 2019).

1.4 Factors affecting dietary energy utilization and deposition

Energy deposition can be defined as the net difference between energy intake and expenditure and is controlled by multiple regulatory mechanisms (Swennen et al., 2004). Utilization rate of energy-yielding feedstuffs could be dependent on age or physiological stage of the animal, species of animal, the environmental condition of raising the animal, and nutritional factors such as diet quality and composition. These factors could significantly impact energy digestibility and metabolizability (Bakker and Jongbloed, 1994; Rijnen, 2003).

1.4.1 Environmental condition of raising animals

Zootechnical performances are not only dependent on genetics, but they are also greatly affected by the environment, one of which is climatic condition (Gregory, 2010; Babinszky et al., 2011). It is well documented that poultry of different breeds respond differently to climatic variation and the interactions between genetics and environment in a specific geographic location may affect broiler chicken growth performance (Alade and Ademola, 2013; Okere, 2014). During extreme cold or hot weather, livestock can adapt and develop coping mechanisms. In hot weather, drastic reduction in feed intake has been observed (Austic, 1985; Howlider and Rose, 1987), which affects metabolism and results in reduced growth response (Quinteiro-Filho et al., 2010). Also, protein digestion is hindered during adverse heat stress, thereby resulting in low feed intake (Larbier et al., 1993). Oshi et al. (2017) and Quinteiro-Filho et al. (2010) explained that heat stress increases corticosterone which is the main glucocorticoid that manipulates metabolism via regulation of energy intake during stress conditions in broiler chickens.

On the other hand, during extremely cold temperatures, chickens can increase their body temperature. Consequently, chickens use much of the dietary energy to warm their body, therefore, diverting feed energy from growth (Oshi et al., 2017). Likewise, ambient temperature is a major factor that manipulates the maintenance energy requirements in pigs. Van Milgen and Noblet (2000) reported that the fasting heat production (FHP) of growing pigs are 16% lesser at 33°C than at 23°C, in the same manner, Close (1996) stated that pigs have 4% greater energy requirement for maintenance for each 1°C reduction of the temperature when they are kept below the lower critical temperature. This is due to a demand to increase heat production for maintaining body temperature. Contrary to broiler chickens, hot temperatures seem to have little impact on the energy requirement for maintenance in pigs (Black, 1995; Giles et al., 1998).

Another example of such environmental conditions is the animal housing. Gomez et al. (2000) reported a trend for higher digestibility of dietary energy when growing gilts were individually housed compared to group-housed growing pigs and explained that the reason why this trend was observed is due to the increased competition for feed within group-housed gilts. This competition for feed will stimulate a higher rate of feed intake, and this will consequently increase the rate of digesta passage through the gut while reducing the mean retention time resulting in a decrease exposure of digesta to digestive secretions (Metz and Dekker, 1985), hence,

the lower digestibility of dietary energy in group-housed pigs. Similarly, Rijnen (2003) reported that energy digestibility was 2.0 percentage units lower in group-housed pigs than in pigs that were contained in metabolism crates, while metabolizability was 3.7 percentage units lower. The digestive utilization of two macronutrients, protein and fat, were influenced by housing conditions (Rijnen, 2003), which could also be attributed to an increase in digesta passage rate and greater heat production caused by physical activity in group housed pigs (van Milgen and Noblet, 2000).

1.4.2 Age and physiological state of animal

Metabolic patterns for energy utilization changes as an animal matures (Barzegar et al., 2020). It has been well-reported that adult poultry such as broiler chickens, layers, and turkeys utilize the energy of feedstuffs to a greater extent with less variation than growing ones (Svihus and Gullord, 2002; Cozannet et al., 2010). Some researchers reported that at 43 weeks of age, laying birds majorly oxidize fat or protein to meet the requirement for energy when compared to the beginning of their production when energy is mostly provided through carbohydrates (Choct, 2004; Caldas et al., 2018). Also, Salas et al. (2017) found that the broiler breeder uses glucose for egg lipogenesis at the beginning of production while dietary fat is mostly used for egg lipogenesis at the end of production.

Sulistiyanto et al. (1999) reported lower availability of dietary energy at 1 and/or 3 d than at 10 d after hatching when either carbohydrate, protein, or fat was fed as sole energy-yielding source to broiler chicks. The same changes were observed for total metabolizable energy values obtained in chicks fed a formulated diet (Murakami et al., 1995; Akiba et al., 1993). The age effect was attributed to the underdeveloped digestive enzyme activity (Nitsan et al. 1991b; Akiba and Murakami, 1995) and gastrointestinal function (Nitsan et al., 1991; Noy and Sklan, 1997; Pluske et al., 2003) up to 10 d post-hatch. Interestingly, no significant age-dependency was observed in metabolizability of fat sources in chicks from age 1 to 10 days (Sulistiyanto et al., 1999) which contradicts the findings that lipase activity is low during the first few days post-hatch in chicks (Nitsan et al., 1991; Akiba and Murakami, 1995). Plavnik et al. (1997) suggested that the regulation of energy intake by broiler chicks and turkey poults are not effective due to the immature digestive capabilities especially in their first week of life. Le Goff and Noblet (2001) suggested that increases in energy digestibility with stage of maturity can be attributed largely to an increased utilization

of dietary fat and fiber and increased capacity for fermentation in adult sows when compared to growing pigs. Also, dietary fiber was more pronounced in reducing energy digestibility in growing pigs than in adult sows. In the same vein, Choct et al. (2010) reported that the digestibility of NSP increases with the age of animals, since grower and finisher pigs utilize dietary fiber better than young pigs.

1.4.3 Nutritional factors

The composition and quality of diets formulated for livestock could be of enormous influence on how dietary energy is utilized. The ratio between macronutrients (protein, lipid, and carbohydrate) has a major impact on livestock live performance (Buyse et al., 1992; Bregendahl et al., 2002; Collin et al., 2003; Swennen et al., 2007). Changing the concentration of one macronutrient in the diet varies the level of the other macronutrients, thereby any effect observed cannot be attributed to a particular macronutrient (Buyse et al., 2001). Some studies with broiler chickens often manipulate diets by replacing one macronutrient by another macronutrient, while the third macronutrient level is maintained (Swennen et al., 2004, 2005). This paired substitution assumes that the effect of the macronutrient on dietary energy content can be isolated, allowing a precise investigation of its effect on bird performance (Buyse et al., 2001; Hada et al., 2001). Meanwhile, few studies such as Swennen et al. (2010) have aimed to investigate the influence of isoenergetic substitution between the three energy delivering macronutrients in broiler chickens pre-starter diets on performance and intermediary nutrient metabolism. Furthermore, dietary fiber is considered a diluting factor in animal diets due to their ability to reduce fecal and ileal digestibility (Yin et al., 2000). The result of Le Goff and Noblet, (2001) indicated that energy digestibility in growing pigs was reduced by approximately one percentage point for every 1% additional neutral detergent fiber in the diet. To sustain production traits such as growth or breast muscle weight, fiber-rich diets can be maintained by adding fat sources such as vegetable oils (Désert et al., 2018).

1.4.4 Species of animal

There is a large difference in the energy utilization in pigs and poultry, this is commonly attributed to the difference in the gastrointestinal tract of the two species. In general, pigs are

known to better utilize energy in a feedstuff due to the larger fermentative capacity in the large intestine and a longer digesta transit time (Choct and Cadogan, 2001). In fact, an interaction between animal species and type of diet could result in significantly different utilizable energy profile for the same feed ingredient; for example, when fiber-rich diet are fed to both pigs and chickens, a large portion of NSP is digested by the large intestine microflora in the pig while the capacity of the gut microflora in the chicken is simply limited in digesting large amounts of NSP within the short transit time of the digesta (Choct et al., 2010) in the relatively shorter ceca. Also, since digestion of feed is jointly achieved by enzymes, chemical (such as acid in the stomach of pigs and crop of chickens) and microbial degradation, the stomach compartment in pigs is larger when compared to chickens' crop and this creates wider surface area for acid digestion thereby contributing to pig's ability to better utilize feed component than poultry (Choct and Cadogan, 2001; Choct et al., 2010).

1.5 Energy evaluation system

One of the important decisions to be made in feeding livestock is to determine the level of energy that optimizes growth, carcass quality, and efficiency of feed utilization with profitability of production (Waldroup, 1981). An energy system can be referred to as a method for predicting the energy value of either compound feeds or single ingredients as it relates to a given type of animal. The purpose of an energy system is to describe the energy available in the feed for maintenance, growth, and for a given level of performance (Gous, 2010; Noblet et al., 2010). In compound feeds, the calculation of most energy evaluation systems assumes additivity of the contributions by carbohydrates, fats, and proteins (NRC, 1998). Meanwhile, gross energy (GE) cannot be regarded as an evaluation system, but it is the basis for the estimation of other systems used in evaluating energy. This is because GE is totally independent of the animals and provides no indication of energy available to animals (Kil et al., 2013). Gross energy represents the maximum quantity of energy present in an ingredient or compound diet and is obtained from the complete combustion of organic materials using bomb calorimetry (Velayudhan et al, 2015).

The three common energy systems employed in poultry and swine are the digestible energy system, metabolizable energy system, and the net energy system. There have been various arguments about the efficacy of one system over the other, some of which include; that the DE or

ME overestimates energy value of protein or fibrous feeds but underestimates energy value of fat or starch rich ingredients (Noblet et al.,1994) and on the other hand, that the NE system results in formulation of diets that are lower in crude protein content with a resultant reduction in N excretion, thus minimizing the environmental impact of monogastric production (Velayudhan et al., 2015). These three major systems of evaluating energy are further discussed below.

1.5.1 Digestible energy

The digestible energy of a diet (or single feed ingredient) can be evaluated by taking the difference between the gross energy in the diet and the gross energy in the feces after consumption of said diet (Kil et al., 2013; De Lange and Birkett, 2005). Digestible energy is not readily achievable in the chicken, as avian species voids their waste as a combination of urine and feces except through introduction of artificial anus or ligation of urethra (Suzuki and Nishizaki, 1931; Rothchild, 1947). Digestible energy is regarded as an apparent measurement of the energy value because the endogenous losses of energy are not considered in its calculation (Reynolds, 2000; Kong and Adeola, 2013, 2014). The DE of most diets fed to pigs varies between 70 and 90% of GE in the diet (Sauvant et al., 2004; Kil, 2008). A similar variant of DE which is more commonly adopted in poultry is the apparent ileal digestibility of nutrients (Stein et al., 2007; Eklund et al., 2008). This can be used in measurement of energy; in which case it is referred to as the ileal digestible energy. The ileal digestible energy (IDE) is defined as the net disappearance of ingested energy from the digestive tract proximal to the distal ileum (Bolarinwa and Adeola, 2012; Kong and Adeola, 2016; Rho et al., 2017). The IDE is calculated from the difference between GE in the diet and GE in the digesta collected from approximately two-thirds of the distal ileum, defined as extending from Meckel's diverticulum to the ileo-cecal junction (Olukosi et al., 2007; Rezvani et al., 2008; Romero et al., 2014). In pigs, ileal cannulation is done to allow access to ileal digesta while in broiler chickens, birds are usually euthanized to collect the ileal digesta (Kong and Adeola, 2014).

1.5.2 Metabolizable energy

In the metabolizable energy system, the energy loss by an animal through urine is factored in. The ME of a diet is calculated by subtracting energy excreted in feces, urine and gases from

GE giving an apparent value of ME (AME). The gaseous energy losses are usually ignored in the calculation of ME because negligible quantities of gases are produced by pigs and poultry, but the correction for gaseous energy might become important when feeding a fiber-rich diet (Wenk et al., 2000; Kil et al., 2013).

Other modifications of the classical AME value are the true metabolizable energy (TME) and the nitrogen-corrected metabolizable energy (MEn; De Lange and Birkett, 2005). The TME corrects AME for endogenous losses of energy that could arise from sloughed-off cells, intestinal microbial products, and digestive enzymes (Adeola, 2001; Kong and Adeola, 2014) while MEn are used to correct AME using a respective correction factor of 7.45 or 8.22 kcal/g of N for pigs or poultry (Hill and Anderson, 1958; Harris et al., 1972; Morgan et al., 1975). This is because the energy deposited as retained protein in fast growing animals cannot be completely recovered by animals if the amino acids are degraded for energy. It is pertinent to note that the correction can also be imposed when nitrogen is lost rather than retained, in which case it is referred to as a state of negative nitrogen balance (Harris et al., 1972). To illustrate this using the pig: for each gram of nitrogen lost from the body (i.e., negative nitrogen balance), 7.45 kcal would be added to the metabolizable energy and for each gram of nitrogen retained in the body (i.e., positive nitrogen balance), 7.45 kcal would be subtracted from the metabolizable energy.

1.5.3 Net energy

Net energy is the energy available for maintenance, growth, and production. The use of NE system instead of the ME system for both feedstuff energy evaluation and diet formulation in poultry is in its early stages of development (Barzegar et al., 2020). This is contrary to what is obtainable in swine production where the NE system is more common and widely adopted because it is assumed that the NE system is more accurate in predicting the growth performance and body composition of pigs (Oresanya et al., 2008; Noblet et al., 2010). Some researchers are of the opinion that the NE system is superior to ME system because it allows the heat increment (HI) of feeding to be calculated (Gous, 2010; Liu et al., 2017; Birkett and de Lange, 2001). Zuidhof (2019) concluded that both systems are two complementary approaches of solving the same problem as NE system focuses on the efficiency of ME retention.

There are three known methodologies for the in-vivo measurements of NE value in pigs and chickens, namely, direct calorimetry, indirect calorimetry and the comparative slaughter with each method presenting their limitations (Zubair and Leeson, 1994; Chepete et al., 2004; Barzegar et al., 2020). Two major criticisms reported against the NE system by De Lange and Birkett (2005) and Barzegar et al. (2020) are the laborious nature of determining HI of ingredients especially when comparative slaughter or live animal indirect calorimetry (IC) are employed; also, an accurate calorimetry methodology requires experienced operators and a setup of flawless equipment.

1.6 Energy evaluation methodology

The fundamental procedure in an energy digestibility trial (either for compound feed or a test ingredient) often requires measuring the ingested energy and the voided energy. There are two known methods (Kong and Adeola, 2014) that can be employed for this estimation in swine and poultry diets, they are the total collection (TC) and index method (IM). To determine the difference between the energy in consumed feed and energy in the excreted components, the TC requires whole collection and record of feed intake, fecal output, and urine output while IM accommodates partial sampling but demands a precise chemical analysis of the index markers (Zhang and Adeola, 2017). In a TC for swine, pigs are usually confined to metabolic crates and adapted to feed for 4 to 6 days before proceeding to the marker- to- marker collection period that could span for another 4 to 6 days (Adeola, 2001). Sample collection starts and ends at the appearance of the colored markers added to the feed. The three popularly used colored markers are ferric oxide, chromic oxide, and indigo carmine (Wang and Adeola, 2018; Kong and Adeola, 2014).

Some modifications are usually introduced in poultry TC. There is usually a 48-h feed withdrawal after which only 25 to 30 g of the test ingredient is force-fed and the excreta voided during the exact 48 h post-feeding are collected quantitatively (McNab and Blair, 1988). The TC is more commonly used in swine than in poultry. In the index method, an indigestible inert marker is feed. The amount of marker feed and the amount voided in the output are expected to be uniform over equal periods of time since it can neither be digested nor absorbed (Adeola, 2001). Examples of index compounds used as marker are insoluble ash, chromic oxide, and titanium dioxide (Sales and Janssens, 2003; Wang and Adeola, 2018).

1.7 Lesser-known energy feedstuffs for chicken and pig diets

Soybean and corn are the two most widely used vegetable carbohydrate, protein, and oil sources for monogastric animals. Interestingly, soybean has been named as the most prevalent legume/oil seed crop in the world (FAO, 2008). Moreover, not only are they used in feeding animals, but they are also important players in feeding the fast-growing human population, and this creates an urgent demand to diversify in the choice of ingredients used in monogastric feed formulation. Also, aside from the fact that supplementation of diets with high energy sources such as corn and soybean have become increasingly costly, the choice of the energy level in practical diets should not only be based on economic considerations but should also take sustainability of the production of the feed ingredient into consideration, given the limited world land mass used in cultivation of corn and soybeans. Hence, lesser-known feed ingredient which are either by-products or co-products that could serve as alternate sources of feed ingredients should be encouraged. For the effective use of those lesser-known feed ingredients for various species of animals, it becomes essential that the feeding value of those feed ingredients are properly evaluated. Some of those lesser known but utilizable feedstuffs are described below.

1.7.1 Dry fat

Dry fat (DF) is a calcium salt of long-chain fatty acids derived from vegetable oils (Nigdi et al., 1990; Alizadeh et al., 2012). In ruminant production, DF is often categorized among other rumen-protected fats (RPF) such as prilled fat and prilled fat with lecithin (Behan et al., 2019). The RPF is widely used in ruminant nutrition to improve growth and reproductive performance, decrease fatty acid biohydrogenation within the rumen, and reduce production of methane from the rumen (Hightshoe et al., 1991; Park et al., 2010). From the name, it can be inferred that DF is an energy source from fat but in a dry granulated form. In a study conducted by Behan et al. (2019) to compare three different RPF in Dorper sheep with DF inclusive, it was reported that the different types of RPF had no unfavourable effect on the ruminal fermentation and productive parameters. Also, Haddad and Younis (2004) reported no increase in Awassi lamb performance when calcium salt of long chain fatty acids was supplemented to increase their dietary fat concentration.

Two common brands of DF used in previous studies were Megalac^{TM} and Polyfat® (Alizadeh et al., 2012; Selim et al., 2013). Megalac^{TM} is a calcium salt of mostly saturated fatty

acids produced by Essentiom, Church and Dwight Co. Inc., (Ewing Township, NJ, USA). Polyfat® also consists of calcium salts of 70% palm oil fatty acids, 25% sunflower plus corn oils and 5% soybean oil and is produced by Norel-Misr (Egypt; Boulos et al., 2011; Selim et al., 2013). Dry fat may be a useful feedstuff in monogastric nutrition and could serve as a credible addition to the list of lipid sources used in their diet.

1.7.2 Stabilized rice bran

Rice bran (RB) is a by-product from rice milling (Sayre et al., 1988; Gallinger et al., 2004). The bid to ensure better utilization of rice bran for nutritional purposes has led to the development of stabilized rice bran (Bhosale and Vijayalakshmi, 2015). For stabilized rice bran (SRB), the lipid degrading enzymes lipases are deactivated by processing with heat and friction, hence, there is a stabilization of its lipid content, thereby ensuring shelf-life longevity of the by-product (Randall et al.,1985; Sayre et al., 1987; Tao et al., 1993). Stabilized rice bran has received increasing attention as a livestock feedstuff due to its nutritional qualities, although it is higher in fiber when compared to regular rice bran (Faria, 2012). Sayre (1987) results showed that 60 % SRB diet produced similar weight gain as the 60% corn (commercial chick starter diet), while greater gain was reported when compared to raw bran.

Moreover, the presence of fiber in the intestine of the animal is not only targeted at supplying energy but could lead to increased microbial diversity and fortification of the host mucins (Desai et al., 2016). Makki et al. (2018) stated that virtually all fiber induces specific shifts in microbiota composition due to competitive interactions thereby serving as potential prebiotics. Therefore, microbial metabolism of cereal fiber such as SRB could also release ferulic acid and SCFA which could modulate gut physiology and integrity (Koh et al., 2016; Makki et al., 2018). Moreover, in a review by Adebowale et al. (2019), fiber associated with increased growth performance could favorably modulate the intestinal health and overall health status of monogastric animals especially native fiber in cereals.

1.7.3 Hydrolyzed feather meal

Feather meal (FM) is a by-product of the poultry processing industry containing about 90% protein, although the digestibility of its protein is hindered by its non-soluble keratin content

(Hadas and Kautsky, 1994). These keratins are fibrous structural proteins stabilized by disulfide bonds (Ravindran et al., 1993). Feather meals have been explored both for their use as a component in animal's diet and as organic soil fertilizers. When feather meal is subjected to pressurized steam processing, the disulfide bonds are readily hydrolyzed, hence producing hydrolyzed feather meal (HFM). The HFM is a by-product with an improved digestion by endogenous proteolytic enzymes (Bielorai et al., 1982; Moritz and Latshaw, 2001), although, excessive hydrolytic processing could decrease concentration of amino acids (AA) in HFM (Papadopoulos et al., 1985). Aside from the hydrothermal treatment, other treatments developed to increase the digestibility of feather meal include chemical (acidic, alkalic, or catalytic) hydrolysis, enzymatic hydrolysis, and steam flash explosion (Onifade et al., 1998; Coward-Kelly et al., 2006; Daroit et al., 2011; Zhang et al., 2014).

Davis et al. (1961) established the standard for pepsin digestible protein (PDP) content for feather meal and a PDP of 75% is recommended to ascertain good quality HFM. Moritz and Latshaw (2001) stated that a steam pressure of 310 kPa for 36 minutes is required to produce HFM with a bulk density of 483 kg/m^3 and an approximate PDP of 75%. Earlier, Latshaw et al. (1994) reported an average AA digestibility of 72% for all AA in feather meals processed at 202 or 322 kPa using continuous hydrolyzation in a study conducted with mature roosters. Feather meal protein has also been reported to be distinctly deficient in four major AA including methionine, lysine, histidine, and tryptophan, hence there is need for synthetic supplementation of these AA when HFM is used in diet formulation (Baker et al., 1981). In a study by Kikuchi et al. (1994) using juvenile Japanese flounder fish, feather meal was used as partial substitute for fish meal by feeding 0 to 50% of the diet as feather meal while reducing the fish meal content. The result of the study showed that juvenile Japanese flounder fish fed diets containing 12 to 25% feather meal did not differ from those feds on the control diet containing 80% fish meal.

1.7.4 Flash dried poultry protein

Flash-dried poultry protein (FDPP) is also a poultry processing by-product with an approximate crude protein content of about 65% (Kureshy et al., 2000). It is derived when poultry by-product meals are subjected to the flash-drying procedure instead of the conventional dry-rendering method (Ravindran et al., 1993). Often, meals produced from by-products such as poultry by-product meal, poultry offal meal, meat and bone meal, are highly variable in their

biochemical composition due to variable raw material composition causing high levels of ash and low digestibility (Cruz-Suárez et al., 2007). According to Kureshy et al. (2000), flash drying is an example of the enhanced processing techniques used to improve product quality and digestibility to get less variable growth performance when poultry by-products are fed to animals. Also, the flash drying procedure enhances palatability, AA stability in products, and minimizes heat damage to the ingredient (Ravindran et al., 1993; Davis and Arnold, 2000).

The FDPP amino acid profile has been described by Moser et al. (1998) to be comparable with that of fish meal. Because plant proteins are often deficient in lysine and methionine, contain antinutrients such as trypsin inhibitors, and may have poor palatability, FDPP have been used as alternative animal protein to replace fish meal (FM) in diets of shrimp, red drum fish, and weanling pigs due to its ability to supply those indispensable AA as found in FM (Moser et al., 1998; Davis and Arnold, 2000; Kureshy et al., 2000; Cruz-Suárez et al., 2007). The result obtained in weanling pigs showed that feed efficiency was similar when either a control diet or 5% FM or 5.7% FDPP were fed (Moser et al., 1998). In a study conducted by Davis and Arnold (2000), the replacement of 40–80% of the FM in the basal diet with FDPP resulted in a significant increase in weight gain and feed efficiency of shrimp.

1.7.5 Black soldier fly larvae meal

Black soldier fly larvae meal (BSFLM) is an example of insect meal derived from black soldier fly (*Hermetia illucens*). The BSFLM has shown to be an environmentally sustainable option for dietary protein due to the voracious ability of black soldier fly to convert waste to feed (Barragan-Fonseca et al., 2017; English et al., 2021). The black soldier fly has been the subject of recent attention in animal nutrition both for its fat and protein (Schiavone et al., 2018). The meal is often defatted to improve the storability of the feed and increase the protein digestibility (Surendra *et al*., 2016). When comparing BSFLM and soybean meal for essential amino acid levels, Newton et al. (2005) reported similar lysine, leucine, phenylalanine, and threonine values for the two ingredients.

Furthermore, BSFLM is characterized with high chitin content, and this presents it peculiar advantages and disadvantages. Chitin is a nitrogen containing polysaccharide which constitutes black soldier fly exoskeleton (Ravindran et al., 1993). The reported chitin content in BSFLM

ranges from 50 g/kg to 100g/kg (Diener et al., 2009; Kroeckel et al., 2012; Schiavone et al., 2017). The reason for this wide variation in chitin level has often been attributed to the substrate on which black soldier fly is grown and the stage of the larvae development (Barragan-Fonseca et al., 2017). Chitin level has been reported to negatively influence nutrient (fat and protein) digestion and absorption due to the presence of non-protein nitrogen which are nutritionally unavailable to animals (Marco et al., 2015; Marono et al., 2015; Schiavone et al., 2017). In addition, chitin found in black soldier fly is a dietary fiber that can be fermented by the microbiota in the hind gut of poultry and swine to produce SCFAs; a substrate that helps improve the compositional balance of the microbial community, hence black soldier fly meals could serve as potential prebiotics (Khempaka et al., 2011; Borrelli et al., 2017). Chitin have also been stated to exhibit anti-viral, anti-tumor, antimicrobial properties, and a bacteriostatic effect on Gram-negative bacteria, thus BSFLM could potentially serve as antibiotic feed additives (Van Huis, 2013; Piccolo et al., 2017). Higher activity of endogenous chitinase (an enzyme that hydrolyzes chitin) has been observed in some poultry (Robbins, 1997; Suzuki et al., 2002).

1.8 Summary

In summary, this literature review details important factors to consider when formulating diets to meet the energy requirement of swine and poultry, especially when it relates to effective energy deposition and utilization. Energy evaluation systems were discussed. More so, the review expatiates on methodologies developed through previous energy determination studies. The review has some of the differences between swine and poultry digestibility procedures highlighted and describes the peculiarity of the three broad categories of energy source which are fat, protein, and carbohydrate. Some lesser-known feed ingredient that could be used as alternative fat, fiber, or protein sources were also introduced in the review.

1.9 Objective

The objective of this thesis was to evaluate energy utilization in some selected non-conventional feed ingredients for broiler chickens and pigs. Three studies were carried out to evaluate this objective.

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CHAPTER 2. ENERGY VALUE OF DRY FAT AND STABILIZED RICE BRAN FOR BROILER CHICKENS

2.1 Abstract

The ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) of dry fat (DF) and stabilized rice bran (SRB) were determined in two experiments with broiler chickens using the regression method. Broiler chickens were fed a common starter diet from d 0 to 17 and experimental diets from d 17 to 22 post hatching. Three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing either DF at 50 or 100 g/kg replacement in Experiment (Exp.) 1 or SRB at 100 or 200 g/kg replacement (Exp. 2) of the energy-contributing ingredients in the RD. In each Exp., 192 broiler chickens were randomly allocated to one of three dietary treatments in a randomised complete block design, comprising eight replicate cages with eight birds per cage. In Exp. 1, the IDE, ME, and MEn linearly increased ($P<0.001$) with increasing DF concentrations, while in Exp. 2, the IDE, ME, and MEn of the diets were not affected by dietary supplemental SRB. The regression-derived IDE concentration for DF and SRB were 6,047 and 3,556 kcal/kg DM, respectively. The respective ME and MEn estimates (kcal/kg DM) were 6,051 and 5,922 for DF; 3,437 and 3,193 for SRB. In conclusion, the current data showed that broiler chickens utilized between 77 to 79% and 68 to 76% of the gross energy (GE) in DF and SRB, respectively, and this suggested a strong potential for these ingredients as dietary energy sources for broiler chickens.

Keywords: Dry fat, stabilized rice bran, digestibility, regression, metabolizability

2.2 Introduction

Dietary energy concentration is one of the main factors influencing feed intake in broiler chickens (Jeffre *et al.*, 2010). Dietary provision of adequate energy is important for efficient production and this adequacy is partly dependent on knowledge of the utilizable energy of feed ingredients used in diets (Bolarinwa and Adeola, 2012). However, these energy values can only be assigned using appropriate data derived from a reliable methodology. The regression method has been shown to be a reliable technique when a direct method is not applicable (Bolarinwa and

Adeola, 2016). Compared to the direct method, which involves adding a test ingredient as the sole source of the test nutrient in the experimental diet (Kong and Adeola, 2014), the regression method accommodates supplementation of only a portion of the reference diet (RD) with the test ingredient.

Dry fat (DF) is an energy source widely used in ruminant nutrition to improve growth, reproductive performance and decrease fatty acid biohydrogenation within the rumen (Hightshoe *et al.*, 1991; Behan *et al.*, 2019). A common brand is the Megalac^{TM}, which is a bypass fat which alleviates depression in fibre digestibility associated with oils and has shown high potential in supplying energy in ruminants. Megalac^{TM} is a calcium salt of long-chain fatty acids derived from vegetable oils with ethoxyquin added as preservative (Alizadeh *et al.*, 2012) and is highly digestible (Huang *et al.*, 2009) with the added advantage over soybean oil (SBO) of being a dry granular material. Therefore, it mixes well with all common ingredients and does not melt or freeze, irrespective of weather fluctuations. Given its potential nutritional value and ease of mixing into a complete diet, DF may be a useful feedstuff in broiler chicken nutrition and could serve as a credible addition to the list of lipid sources used in broiler chicken diet. However, the data on the energy value of DF for broiler chickens is scarce.

Stabilized rice bran (SRB) is a by-product of rice milling but, in contrast to regular rice bran, the lipases in SRB have been deactivated by heat and friction, thereby ensuring the stability of its oil content even when stored for several months (Randall *et al.*,1985; Qureshi *et al.*, 2002). Stabilized rice bran has received increasing attention as a livestock feedstuff due to its nutritional qualities, although it is relatively high in fibre and phytate. In broiler chicken diets, an inclusion level of 10–20% has been recommended to promote performance in birds (Gallinger *et al.*, 2004), possibly due to the potential prebiotic characteristics of the SRB non-starch polysaccharides in the gastrointestinal tract.

To appropriately utilize these ingredients in the diets of broiler chickens, an estimation of their energy values is necessary. Therefore, the objective of the current study was to determine the ileal digestible energy (IDE), metabolizable energy (ME) and nitrogen-corrected ME (MEn) of DF and SRB for broiler chickens using the regression method.

2.2.1 Materials And Methods

All experimental protocols were reviewed and approved by the Purdue University Animal Care and Use Committee (#1311000983), Indiana, USA.

2.2.2 Bird management, experimental design, and diets

The two experiments were conducted using the same protocol. A total of 384 male broiler chicks (Cobb 500; Siloam Spring, AR, USA) were obtained from a local hatchery at one day old. Chicks were individually tagged with identification numbers and reared in electrically heated battery cages (model SB 4 T; Alternative Design Manufacturing and Supply, Siloam Springs, AR) with temperature and lighting maintained as previously described by Aderibigbe *et al.* (2020a). Birds had *ad libitum* access to water and a standard broiler chicken starter diet from 0 to 17 d of age. In each Exp., on d 17, 192 male broiler chickens were individually weighed, and randomly assigned to one of the experimental diets in a complete block design, with body weight (BW) as a blocking factor. Each experimental diet comprised eight replicate cages containing eight birds each. The DF used as the fat source in Exp. 2 was Megalac^{TM}, a calcium salt of long-chain fatty acids (Essentiom, Church and Dwight Co. Inc., Ewing Township, NJ, USA). Dietary treatments consisted of a corn-soybean meal-based reference diet (RD) and two test diets prepared by supplementing the RD with either DF at 50 or 100 g/kg (Exp. 1); or SRB at 100 or 200 g/kg (Exp. 2), at the expense of corn and soybean meal in Exp. 1 but corn, soybean meal and dry fat in Exp. 2 (Tables 1 and 2). In both experiments, titanium dioxide was included at 5 g/kg as an indigestible marker. Birds were given *ad libitum* access to water and experimental diets from 17 to 22 days old. All diets used were fed as mash, and vitamin-mineral premix was added to all diets according to requirement (NRC, 1994).

2.2.3 Sample collection and chemical analysis

On d 19 post hatching, for both experiments, the excreta collection pans were lined with waxed paper for daily sample collection from d 20 to 22. The samples were pooled within a cage over the three day and stored in a freezer at −20°C until further analysis. The BW gain and feed intake (FI; g/bird) during the experimental periods were recorded, and the gain to feed ratio (G: F; g/kg) of each cage was calculated. On d 22, all birds were euthanized *via* carbon dioxide

asphyxiation. Ileal digesta samples were collected from the distal two-thirds of the ileum, defined as extending from Meckel's diverticulum to the ileo-caecal junction. The content in the ileum was flushed out with distilled water and pooled per cage then stored in a freezer at −20°C until further analysis. At the end of the experiments, ileal digesta and excreta samples were thawed and placed in a forced-air oven (Precision Scientific Co., Chicago, IL, USA; method 934.01; AOAC, 2006) at 55°C for 96 h. Dried samples were ground using a mill grinder (Retsch ZM 100; Retsch GmbH and Co., Haan, Germany). Gross energy (GE) of the test ingredients, diets, excreta samples, and ileal digesta samples were determined using an isoperibol oxygen bomb calorimeter (Parr 1261; Parr Instruments Co., Moline, IL, USA) with benzoic acid as a calibration standard. Dry matter (DM) content was determined by drying samples in an oven at 105°C for 24 h (method 934.01; AOAC, 2005). Nitrogen (N) concentration was determined using the combustion method (Model FP2000; Leco Corp., St. Joseph, MI, USA) with EDTA as the calibration standard. Titanium (TI) concentrations in experimental diets, excreta, and ileal digesta samples were analyzed by spectrophotometer at 410 nm of absorption (TruMac N; LECO Corp., St. Joseph, MI, USA) as described by Short *et al.* (1996). Test ingredients concentrations of crude fibre, NDF, and ADF were analyzed as described in AOAC (2006 methods 978.10 and 973.18 (A, B, C, and D). Calcium (Ca) and phosphorus (P) concentration of test ingredients were determined using method previously described by Aderibigbe *et al.* (2020b). The Ca concentration was measured by flame atomic absorption spectroscopy using Varian Spectr. AA 220FS (Varian Australia Pty Ltd, Victoria, Australia) while P concentrations were estimated by spectrophotometry and the absorbance read at 630 nm (Spectronic 21D, Milton Roy Co., Rochester, NY). Free fatty acid composition and acid hydrolyzed fat of the test ingredients were analyzed by a previously described method (Horwitz, 2000).

2.2.4 Calculations and statistical analysis

The index method was used to calculate the apparent ileal digestibility and metabolizability of DM, N, and GE in the experimental diets (Kong and Adeola, 2014). The IDE and apparent ME contents of the experimental diets were then calculated as the product of respective coefficients and the GE of diets. Apparent MEn was calculated by correction of apparent ME to zero N retention using the factor of 8.22 kcal/g of N (Hill and Anderson, 1958). The apparent ileal

digestibility or metabolizability of nutrient or GE in DF and SRB were calculated by the following equation:

$$C = [1 - (TI_i/TI_o) \times (E_o/E_i)];$$

where C was the coefficient of ileal digestibility or metabolizability of nutrient or energy; TI_i and TI_o represented the concentration of TI (g/kg DM) in experimental diets and ileal digesta or excreta samples, respectively; E_i and E_o are the concentration of nutrient or GE (kcal/kg DM) in experimental diets and ileal digesta or excreta samples, respectively. Based on C, the coefficient of ileal digestibility or metabolizability of nutrient or energy, the IDE, ME, and MEn in experimental diets were calculated as the product of C and gross energy of the diet. The IDE (kcal/kg DM) in test ingredients, IDEti, was calculated by difference procedure suggested by Adeola (2001): $IDE_{ti} = [IDE_{td} - (P_{rd} \times IDE_{rd})] / P_{ti}$,

where IDE_{ti}, IDE_{td}, and IDE_{rd} represent the IDE in test ingredients, test diets, and reference diet, respectively; P_{rd} and P_{ti} represented the proportion of reference diet and test ingredient (kg/kg) in test diets, respectively. The ME and MEn in test ingredients were calculated by replacing IDE with ME or MEn. The test ingredient intake was the product of feed DM, feed intake, and the proportion of test ingredient in test diets. Test ingredient-associated IDE, ME, or MEn intake were calculated as product of test ingredient intake and IDE_{ti}, ME_{ti}, or MEn_{ti}.

Digestibility data was analyzed as a randomised complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included experimental diets and block as independent variables. Linear and quadratic contrasts were used to compare the effects of increasing levels of DF or SRB. Regression of the test ingredient associated IDE, ME, and MEn intake in kcal *vs*. the test ingredient intake for each cage of birds was conducted using multiple linear regressions as described by Bolarinwa and Adeola (2012, 2016). The solution option was used to generate intercept and slopes. Y was test ingredient-associated IDE, ME, or MEn intake in kcal while DMintake was the test ingredient in kilograms of DM.

2.3 Result

The analyzed nutrient composition of the test ingredients and experimental diets are shown in Tables 2-1 and 2-2, respectively. There was a linear decrease ($P < 0.001$) in BW gain of the chickens (Table 2-3) with increasing dietary concentration of DF. A quadratic effect ($P < 0.05$) was also observed in BW gain and feed intake with increasing concentration of DF whereas G: F

linearly decreased ($P < 0.001$) with increasing dietary DF concentration. Increasing dietary concentration of DF resulted in a quadratic ($P < 0.05$) effect on the ileal DM digestibility but the metabolizability of nitrogen linearly increased ($P < 0.001$). Similarly, the concentration of IDE, ME, and MEn in the diet increased linearly ($P < 0.001$) as the concentration of the DF increased. When compared with the RD, the substitution of 100g/kg of DF increased IDE, ME, and MEn by 8.7%, 8.1% and 9.0% respectively. In Exp. 2, there was a linear decrease ($P < 0.001$) in BW gain and G:F with increasing dietary SRB concentration. The metabolizability of DM and energy decreased linearly ($P < 0.05$) with increasing dietary SRB concentration. The regression-derived IDE concentration for DF and SRB were 6,047 and 3,556 kcal/kg DM, respectively (Table 2-4). The respective ME and MEn concentration were 6,051 and 5,922 kcal/kg DM for DF; 3,437 and 3,193 kcal/kg DM for SRB.

2.4 Discussion

The increasing costs and undersupply of protein and energy ingredients are significant obstacles for animal production and the feed industry. In order to replace the very limited conventional feed resources, there is increasing interest in nutritionally adequate alternatives. However, utilization of these unconventional feed ingredients has been limited by the paucity of information on their nutrient and energy availability. The gross energy composition of DF was similar to that reported by Selim *et al.* (2013) for dry fat product. In the current study, the ether extract of DF could not be obtained, because the fatty acids were not in a free form but were rather part of a calcium salt containing long chain fatty acids, therefore acid hydrolysis was performed. The chemical composition for SRB were similar to a previous report by Pereira and Adeola (2016) for un-stabilized rice bran, except that the SRB had higher fibre and lower phosphorus content.

The current study showed that addition of up to 10% DF in the diet resulted in a quadratic decrease in growth performance indices in birds. This trend is similar to observations by Fascina et al. (2009) who evaluated inclusion of soybean oil and beef tallow in starter diet and showed that increasing soybean oil had a quadratic influence on growth performance. This observation is quite expected, as birds will eat to satisfy their energy requirement and typically reduce feed intake as dietary energy concentration increases (Jeffre et al., 2010). Another possible reason for a reduced feed intake might be due to a reduced palatability of the test diet containing the DF as it was observed that DF directly increased the dustiness and dryness of the feed (data not shown), which

might have led to the associative decrease in feed palatability and intake (Forbes, 2003). It is also possible that the excessively high Ca to P ratio in DF disrupted the overall feed intake response of the birds. A wide Ca:P ratio caused by high dietary calcium could adversely affect phosphorus metabolism and utilization through the formation of Ca-P complexes which is unavailable for birds in the gastrointestinal tract (Li et al., 2012; Han et al., 2016). Hence, a suppressed growth performance.

The digestibility and metabolizability coefficients of GE were not affected by increasing dietary DF. However, IDE, ME, and MEn concentrations increased with increasing DF concentrations in the diet. This observation was likely due to the high gross energy density of DF. Alternatively, it may have been as a result of the improved digestibility of the fatty acid in the calcium soap which resulted in a consequent increase in the concentration of fatty acids in the intestinal chime (Bhatt and Sahoo, 2017; Behan *et al.*, 2019). Furthermore, the dry nature of DF could have reduced fat susceptibility to oxidative peroxidation. The ME content of the diets followed a similar trend to a previous report by Cerniglia (1981) who examined the ME of SBO and tallow at 5% and 10% replacement in feed and observed that these two energy sources had significantly higher ME at 10% replacement. However, this was contrary to observations by Su *et al.* (2015) who reported that determined ME were not affected by inclusion level of SBO or palm oil. The increased dietary ME concentration suggests an increased efficiency of DF utilization by birds as this resulted in a decrease in the percentage of fat in the diet that was excreted

In addition, because energy utilization is affected by age, species, and protein quality of a feed (Bolarinwa and Adeola, 2012), the current study corrected ME for nitrogen retention, and the ME of the diets in Exp. 1 decreased by 5.5% to 6.5% when corrected to MEn. The regression-derived IDE, ME, MEn for DF were 6,047; 6,051; and 5,922 kcal/kg of DM respectively. These were relatively lower than values previously reported for SBO and tallow, where Bertechini *et al.* (2019) reported ME values that ranged from 8,229 to 8,824 kcal/ kg and MEn from 8,497 to 8,769 kcal/kg when SBO was fed to broiler chickens of different age group while Baião and Lara (2005) reported an AME value of 7,373 kcal/ kg for tallow fed to three-week-old broiler chickens. However, higher energy value was expected for SBO compared to DF, due to the presence of more double bonds, which released more energy upon dissociation in the gut. Also, respective lipid concentrations (DM basis) of 100, 96, or 88% for SBO, tallow, or DF, could have direct

implications on the respective GE and energy utilization values. Substituting the reference diet with 100 g/kg of DF increased IDE, ME, and MEn by 8.7%, 8.1% and 9.0% respectively.

In Exp. 2, the inclusion of SRB resulted in a decrease in the final BW, BW gain, and G: F ratio. Fibre content is well known to be the causative agent for the negative effect of fibrous feed stuff on growth performance and nutrient utilization for birds and inclusion of SRB typifies one of such cases (Jørgensen et al., 1996; Sklan et al., 2003; Adeola et al., 2010). A decrease was observed for DM and GE metabolizability, with increasing SRB levels. This was partly expected due to increased dietary fibre concentration as SRB replaced the more utilizable ingredients in the test diet (Bolarinwa and Adeola, 2012). The negative effect of high dietary fibre on nutrient utilization in birds has been widely reported (Jørgensen *et al.*, 1996; Sklan *et al.*, 2003; Adeola *et al.*, 2010). This is because dietary fibre affects viscosity and passage rate of digesta, which affects nutrient utilization by the birds. Although dietary fibre causes increased production of volatile fatty acids (VFA) in the hindgut of the birds (Mateos *et al.*, 2012), utilization of VFA as energy source for poultry has been associated with some inefficiencies (Langhout *et al.*, 2000). Notwithstanding, the data suggested that the IDE, ME, and MEn concentration were unaffected by SRB inclusion up to 200 g/kg replacement in the diet. This demonstrated that energy utilization in the two test diets containing SRB were similar to those in the reference diet.

The regression-derived estimates for IDE, ME, MEn values for SRB were 3,556; 3,437; and 3,193 kcal/kg of DM, respectively. These values were higher than those previously reported for regular unstabilized rice bran (RURB) in broiler chickens. Conte *et al.* (2002) reported 2,553 kcal AME/kg and 2,533 kcal AMEn/kg whereas Rostagno *et al.* (2011) reported 2,522 kcal AME/kg while Pereira and Adeola, (2016) reported 2,498; 2,691; and 2,476 kcal/kg DM for IDE, ME, MEn values of RURB, respectively. The higher energy values of SRB over RURB may be explained by the increased stability of the oil in SRB due to the inactivated lipase enzymes that would otherwise degrade the rice bran oil. Interestingly, the value obtained for SRB in the current study was similar to those reported for whole rice meal (WRM) fed to broiler chickens. Junqueira *et al.* (2009) reported 2,968 kcal AME/kg and 2,804 kcal AMEn/kg for WRM. This suggested that, although a by-product of rice milling, SRB may be a good energy source for broiler chickens.

The current data shows that DF and SRB can be utilized in the diet of broiler chickens and should be considered for energy supply, although inclusion should be based on practical inclusion levels. A 5% inclusion might be preferable for DF, while further inclusion could reduce palatability

of diet leading to an associative decrease in feed intake. Moreover, SBO inclusion in practical diets are generally not more than 5% (Bolarinwa and Adeola, 2012; Rutherfurd *et al.*, 2012) because of its high energy density.

In conclusion, the current data suggested that broiler chickens were able to utilize between 77 to 79% and 68 to 76% of the GE in DF and SRB, respectively. The respective regression-derived IDE, ME, and MEn estimates (kcal/kg DM) for dry fat were 6,047, 6,051, and 5,922. Respective estimates for stabilized rice bran were 3,556, 3,437, and 3,193kcal/kg DM.

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Table 2-1. Analyzed chemical composition of test ingredients, on an as fed basis.

Composition, g/kg	Dry fat	Stabilized rice bran
Dry matter	970.0	937.0
Gross energy, kcal/kg	7,672	4,865
Crude protein	0.0	149.3
Free fatty acids	6.2	69.9
Acid hydrolyzed fat	884	210
Crude fiber	0.0	72.2
NDF	0.0	203.0
ADF	0.0	86.4
Ash	134.0	102.5
Calcium	89.6	10.0
Phosphorus	0.1	7.3

Table 2-2. Ingredient composition, calculated composition and analyzed composition of diets

	Exp. 1			Exp. 2		
		Dry fat, g/kg			Stabilized rice bran g/kg	
Ingredient, g/kg	RD[1]	50	100	RD[1]	100	200
Corn	575.50	544.30	513.20	532.50	475.10	417.70
Soybean meal	360.00	341.16	322.32	360.00	322.60	285.19
Ground limestone	15.00	15.00	15.00	6.00	6.00	6.00
Monocalcium phosphate	13.00	13.00	13.00	15.00	15.00	15.00
Salt	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine HCl	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine	2.50	2.50	2.50	2.50	2.50	2.50
L-Threonine	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin-mineral premix[2]	3.00	3.00	3.00	3.00	3.00	3.00
Titanium dioxide premix[3]	25.00	25.00	25.00	25.00	25.00	25.00
Dry fat	0.00	50.00	100.00	50.00	44.81	39.61
SRB	0.00	0.00	0.00	0.00	100.00	200.00
Calculated composition, g/kg						
Crude protein (N × 6.25)	224	212	201	220	211	201
Ether extract	26	66	105	66	59	52
Calcium	8.9	13	18	10	10	9
Phosphorus	7	7	6	7	8	9
Non-phytate P	4.3	4.2	4.1	4.7	4.8	4.8
Analyzed nutrient, g/kg						
Gross energy, kcal/kg	3,815	3,984	4,244	4,055	4,125	4,187
Crude protein (N × 6.25)	205	194	186	208	203	190
Ether extract	20.5	45.5	39.8	44.8	52.6	56.0
Crude fiber	21.0	18.3	16.5	20.2	22.9	26.4
Neutral detergent fiber	53.3	54.8	53.2	52.8	67.9	83.6
Acid detergent fiber	19.6	17.4	16.7	16.9	20.4	34.5
Ash	47.3	55.7	60.4	55.9	62.6	69.4

[1] RD = reference diet. [2]Provided the following quantities per kg of complete diet: retinol, 3.29 mg; cholecalciferol, 0.13 mg; tocopherol, 14.74 mg; menadione, 8.76 mg; riboflavin, 11.0 mg; D-pantothenic acid, 22.0 mg; niacin, 88.2 mg; choline chloride, 1,542 mg; vitamin B12, 0.03 mg; biotin, 0.11 mg; thiamine mononitrate, 4.40 mg; folic acid, 1.98 mg; pyridoxine hydrochloride, 6.60 mg; I, 2.22 mg; Mn, 132 mg; Cu, 8.88 mg; Fe, 88.2 mg; Zn, 88.2 mg; Se, 0.60 mg. [3]1 g Titanium dioxide plus 4 g corn.

Table 2-3. Ileal digestibility and metabolizability of DM and gross energy of chickens fed diets containing dry fat and stabilized rice bran from d 17 to 22 post hatching in Exp. 1 and 2.

		Dry fat, g/kg			*P*-value	
Item[2]	RD[1]	50	100	SEM	Linear	Quadratic
Experiment 1						
Growth performance						
Initial BW, g	548	548	548	0.2	-	_
Final BW, g	872	874	839	4.2	< 0.001	0.002
BW gain, g	324	327	292	4.0	< 0.001	0.002
Feed intake, g	471	484	462	5.7	0.283	0.023
G:F, g/kg	687	675	631	5.8	< 0.001	0.039
Ileal digestibility, coefficient						
DM	0.77	0.76	0.77	0.004	0.645	0.017
Energy	0.74	0.73	0.73	0.004	0.242	0.622
IDE, kcal/kg DM	3,181	3,277	3,456	18.9	< 0.001	0.097
Metabolizability, coefficient						
DM	0.77	0.76	0.77	0.005	0.629	0.564
Nitrogen	0.79	0.83	0.84	0.008	< 0.001	0.285
Energy	0.76	0.75	0.75	0.005	0.132	0.901
Nitrogen-corrected Energy	0.70	0.70	0.70	0.005	0.580	0.887
ME, kcal/kg DM	3,250	3,358	3,516	22.7	< 0.001	0.364
ME_n, kcal/kg DM	3,040	3,148	3,315	20.8	< 0.001	0.244
		Stabilized rice bran, g/kg				
	RD[1]	100	200			
Experiment 2						
Growth Performance						
Initial BW, g	548	548	548	0.3	-	-
Final BW, g	910	902	874	6.1	< 0.001	0.228
BW gain, g	363	354	326	6.2	< 0.001	0.230
Feed intake, g	483	476	466	8.4	0.158	0.862
G:F, g:kg	751	745	702	9.1	< 0.001	0.448
Ileal digestibility coefficient						
DM	0.71	0.70	0.69	0.007	0.188	0.959
Energy	0.72	0.73	0.72	0.007	0.797	0.580
IDE, kcal/kg DM	3,248	3,322	3,286	29.4	0.397	0.152
Metabolizability, %						
DM	0.72	0.72	0.71	0.004	0.017	0.415
Nitrogen	0.72	0.74	0.75	0.015	0.279	0.783
Energy	0.76	0.75	0.75	0.004	0.038	0.818
Nitrogen-corrected Energy	0.71	0.70	0.70	0.003	0.143	0.829
ME, kcal/kg DM	3,420	3,439	3,396	15.8	0.301	0.129
ME_n, kcal/kg DM	3,217	3,231	3,186	12.4	0.102	0.071

[1] RD = Reference diet. [2] Each mean represents eight replicate cages with eight chickens per cage; IDE = Ileal digestible energy, ME = Metabolizable energy, ME_n = Nitrogen-corrected metabolizable energy.

Table 2.4. Regression equations relating test ingredient-associated gross energy intake (kcal/kg) to test ingredient intake (g/kg DM) using dry fat (DF) in Exp. 1 and stabilized rice bran (SRB)in Exp. 2[1]

Item[2]	Regression equation	R^2	SD
Exp. 1(Dry fat)			
IDE	Y = 6,047 (337.0) × DF – 4.78 (9.082)	0.94	28.2
ME	Y = 6,051 (319.3) × DF – 4.78 (8.604)	0.94	26.5
ME_n	Y = 5,922 (293.5) × DF – 4.78 (7.997)	0.95	24.4
Exp. 2 (Stabilized rice bran)			
IDE	Y = 3,556 (208.9) × SRB + 7.17(11.711)	0.93	25.8
ME	Y = 3,437 (109.7) × SRB + 4.78 (5.975)	0.98	18.9
ME_n	Y = 3,193 (88.43) × SRB + 4.78 (5.019)	0.98	15.3

[1] Values in parentheses are SE; Y is in kcal, Slope is in kcal/kg DM, Intercept is in kcal. [2] IDE = Ileal digestible energy; ME = Metabolizable energy; ME_n = Nitrogen-corrected metabolizable energy.

CHAPTER 3. ENERGY VALUE OF HYDROLYZED FEATHER MEAL AND FLASH-DRIED POULTRY PROTEIN FOR BROILER CHICKENS AND PIGS.

3.1 Abstract

Three experiments were conducted to determine the ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) contents of hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP) for broiler chickens and to determine the digestible energy (DE) and ME of HFM and FDPP for pigs. The HFM or FDPP were incorporated into a reference diet either at 3 levels (0, 75, or 150 g/kg) in experiments (Exp.) 1 and 2 or 2 levels (0, 150 g/kg) in Exp. 3 by replacing the energy-yielding ingredients. Each diet was randomly allocated to 8 replicate cages of broiler chickens (6 birds per cage) or barrows. In Exp. 1, the inclusion of HFM, linearly decreased ($P < 0.05$) the nitrogen corrected metabolizability although, the ME concentration in the diets were linearly increased ($P < 0.05$). In Exp. 2, a linear decrease was observed on the ileal digestibility of DM and energy ($P < 0.05$). It was also observed that the total tract retention (TTR) of DM and energy linearly increased ($P < 0.05$). Similarly, the ME and MEn concentration linearly increased with a *P*-value of < 0.001 and 0.01, respectively. In Exp. 3, the dietary treatments significantly increased ($P < 0.05$) the fecal energy loss. Diet substituted with HFM had significantly higher ($P < 0.001$) urinary GE loss than the RD. The TTR of GE in the RD was significantly higher than those in the test diet containing 150 g/kg of HFM. The respective IDE, ME, and MEn evaluated for HFM in the current study were 4,509, 4,250, and 3745 kcal/kg DM with corresponding values of 3,221, 4,710, and 4,081 kcal/kg DM for FDPP when fed to broiler chickens. In pigs, the respective DE and ME evaluated for HFM were 4,783 and 4,405 kcal/kg DM while estimates for FDPP were 4,553 and 4,320 kcal/kg DM, respectively.

Keywords: Broiler chickens, by-products, flash-dried poultry protein, hydrolyzed feather meal, pigs.

3.2 Introduction

Broiler chickens and pigs are prominent monogastric species particularly raised for their meat production. Necessary information on their nutrient and energy availability is pertinent to

precisely formulate diets that would meet their requirements and enable effective substitution of ingredients. Keen interest should be placed on dietary energy because of its importance in feed intake and its direct impact on bird growth and diet cost (Ahiwe *et al*., 2018). Hence, there is a continual need to evaluate feedstuffs that could serve as credible addition to the list of energy supplying feed ingredients.

Hydrolyzed feather meal (HFM) is a by-product of the poultry processing industry produced by steam-hydrolyzing fresh poultry feathers (Sulabo *et al*., 2013). Recent reports on HFM energy values are limited; for which HFM produced in recent times might be processed more efficiently due to technological advances than in the past, which might consequently affect their nutrient content. For example, in the past decade, there has been a progressive shifting away from acid or alkaline hydrolysis towards pressure cooking (Moritz S. and Latshaw., 2001) and enzymatic hydrolysis (Thazeem *et al*., 2016) of feather with this reported to result in feather meals with 75% pepsin digestibility (Csapó, and Albert, 2018). Similarly, the flash-dried poultry protein (FDPP) is also a by-product derived from the inedible portion of the poultry meat processing industry through the flash-drying procedure rather than via dry-rendering (Ravindran *et al*., 1993; Kureshy *et al*., 2000). Flash dried poultry protein is a protein-rich by-product with an amino acid profile that is comparable to fish meal (Moser *et al*., 1998). Park *et al*. (2020) reported its amino acid digestibility but information on the energy values have rarely been reported. Therefore, the objective of this study was to determine the energy value of HFM and FDPP in broiler chickens and pigs.

3.3 Materials and Methods

All experimental protocols were reviewed and approved by the Purdue University Animal Care and Use Committee (PACUC, West Lafayette, IN). The PACUC protocol number for the broiler chicken and pig experiments were #1311000983 and #1112000248, respectively. The HFM and FDPP used in all the experiments were provided by Darling Ingredients, Inc., Cold Spring, KY.

3.3.1 Animal management, experimental design, and diets

Experiments 1 and 2: Energy value of HFM and FDPP for Broiler Chickens

Experiments (Exp.) 1 and 2 were conducted using the same protocol but at different time periods. A total of 288 male broiler chicks (Ross 708; Aviagen, Huntsville, AL) were obtained from a local hatchery at day old. Chicks were individually tagged for identification purpose and reared in electrically heated battery cages (model SB 4 T; Alternative Design Manufacturing and Supply, Siloam Springs, AR) with temperature maintained at 35, 31, and 27°C from days 0 to 7, 7 to 14, and 14 to 22 or 23, respectively. Light was provided 23 h per day throughout the study. Birds had *ad libitum* access to water throughout the duration of the experiments. A standard broiler chicken starter diet was fed from days 0 to 17 or 0 to 16 in Exp. 1 or 2, respectively. In Exp. 1 or 2, 144 male broiler chickens were individually weighed on d 18 or 17, respectively, and randomly assigned to one of the dietary treatments in a randomized complete block design, with body weight (BW) as a blocking factor. Each dietary treatment contained 8 replicate cages with 6 birds per cage. Dietary treatments consisted of a corn-soybean meal-based reference diet (RD) and two test diets prepared by supplementing the RD with either HFM at 75 or 150 g/kg (Exp. 1); or FDPP at 75 or 150 g/kg (Exp. 2), at the expense of corn, soybean meal (SBM), and dry fat (Table 1). The nutrient composition of the dry fat used in the current study were according to Osunbami et al. (2021).

All diets in both experiments were formulated to maintain the ratio of corn to SBM to dry fat at 0.63:0.32:0.05. In both experiments, titanium dioxide was included at 5 g/kg as an indigestible marker in the diets. Birds had un-restricted access to experimental diets for five days. All diets were fed in mash form, and vitamin-mineral premix was added to all diets according to requirement (NRC, 1994). Excreta collection was performed during the last 3 days of the experimental period in collection pans lined with waxed paper. After 5 days of feeding the experimental diets, all birds were euthanized by asphyxiation using CO_2, weighed individually, and dissected to excise the ileum. Ileal digesta samples were collected from the distal two-thirds of the ileum, which is defined as extending from Meckel's diverticulum to the ileo-cecal junction, by flushing with distilled water. The content in the ileum were pooled per cage then stored in a freezer at −20°C until further analysis. The BW gain and feed intake (FI; g/bird) during the

experimental periods were recorded, and the gain to feed ratio (G: F; g/kg) of each cage was calculated.

Experiment 3: Energy value of HFM and FDPP for Pigs

Twenty-four barrows with initial BW of approximately 20 kg were individually housed in metabolic crates equipped with a feeder and drinker. Barrows used were crossbreed of Duroc x Yorkshire x Landrace. Pigs were assigned one of three dietary treatments in a randomized complete block design with BW as blocking factor. The reference diet was prepared to contain corn, SBM, and soybean oil (SBO) as the sole sources of energy (Table 1). The two test diets were prepared by adding HFM or FDPP at 150 g/kg at the expense of corn, SBM, and SBO in the RD. The corn: SBM: SBO ratio was kept at 0.70:0.25:0.05. Daily feed allowance was estimated as 4.5% of mean BW of pigs in each block. The daily feed allowance was portioned into 2 equal meals and fed at 0800 and 1700 h. All diets were formulated to meet or exceed the estimated vitamin and mineral requirements suggested in NRC (2012). There were 5 days of adaptation to the feed and environment. The marker-to-marker procedure was employed in sample collection (Adeola, 2001). On days 6 and 11, the first meal fed to pigs were hand mixed with approximately 3 g of chromic oxide used as a colored marker. Collection of feces started at the appearance of first marker in feces and halted at the appearance of the second marker. During the fecal collection period, urine was also quantitatively collected using plastic buckets containing 10 mL of 10% formic acid. Urine collected daily from each pig was weighed and proportionally subsampled. Feces and urine collected were immediately stored at -20°C.

3.3.2 Chemical Analysis

At the end of the experiments, ileal digesta, excreta, fecal, and urine samples were thawed and placed in a forced-air oven (Precision Scientific Co., Chicago, IL, USA; method 934.01; AOAC, 2006) at 55°C for 120 h. Dried ileal digesta samples were ground using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany) while excreta and fecal samples were ground using a mill grinder (ZM 100; Retsch GmbH, Haan, Germany). Gross energy (GE) of the test ingredients, experimental diets, ileal digesta samples, excreta samples, fecal samples, and dried urine were determined using an isoperibol oxygen bomb calorimeter (Parr 1261; Parr Instruments

Co., Moline, IL, USA) with benzoic acid as a calibration standard. Dry matter (DM) content was determined by drying samples in an oven at 105°C for 24 h (method 934.01; AOAC, 2005). Nitrogen concentration was determined using the combustion method (TruMac N; LECO Corp., St. Joseph, MI, USA) with EDTA as the calibration standard. Titanium concentrations in experimental diets, excreta, and ileal digesta samples for Exp. 1 and 2 were analyzed by spectrophotometer at 410 nm of absorption (Spectronic 21D; Milton Roy Co., Rochester, NY, USA) as described by Short *et al.* (1996). In addition, test ingredients were analyzed for crude fiber (method 978.10; AOAC, 2006), ether extract (method 945.16; AOAC, 2000), and ash content (method 942.05; AOAC, 2006).

3.3.3 Calculations and Statistical Analysis

In Exp. 1 and 2, the index method was used to calculate the ileal digestibility and total tract retention (TTR) of DM, N, and GE in the experimental diets (Kong and Adeola, 2014). The ileal digestibility or TTR of nutrient or GE in HFM and FDPP were calculated by the following equation:

$$Z = [1 - (TC_i/TC_o) \times (E_o/E_i)].$$

where Z is the coefficient of ileal digestibility and percentage ileal digestibility was derived by multiplying Z by 100, TTR of nutrient or energy; TC_i and TC_o represent the concentration of titanium (g/kg DM) in experimental diets and ileal digesta or excreta output, respectively; E_i and E_o are the concentration of nutrient or GE (kcal/kg DM) in experimental diets and ileal digesta or excreta output, respectively.

The IDE, ME, and MEn in experimental diets were then calculated as the product of Z and gross energy of the diet. The MEn was calculated by a correction of ME to zero N retention using the factor of 8.22 kcal/g of N (Hill and Anderson, 1958). The IDE (kcal/kg DM) in test ingredients, IDEti, was calculated by difference procedure proposed by Adeola (2001): $IDE_{ti} = [IDE_{td} – (P_{rd} \times IDE_{rd})] / P_{ti}$, where IDE_{ti}, IDE_{td}, and IDE_{rd} represent the IDE in test ingredients, test diets, and reference diet, respectively; P_{rd} and P_{ti} represented the proportional contribution of reference diet and test ingredient (kg/kg) in test diets, respectively. The ME and MEn in test ingredients were calculated following the same calculation steps as IDE of test ingredient. Orthogonal polynomial contrasts were performed to determine the linear and quadratic

effects of increasing levels of test ingredients. Data were analyzed using GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included diet and block as independent variables. Statistical significance was declared at $P < 0.05$ while tendencies were declared at $P < 0.10$. The test ingredient DM intake (DMIti) was the product of feed DM, feed intake, and the proportion of test ingredient in test diets. Test ingredient-associated IDE, ME, or MEn intake were calculated as product of test ingredient intake and IDE_{ti}, ME_{ti}, or MEn_{ti}. Regression analysis between the test ingredient–associated IDE, ME, or MEn intake and DMI_{ti} was conducted using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to generate a regression equation as described by Bolarinwa and Adeola (2016). In the regression equation, Y was the test ingredient-associated IDE, ME, or MEn intake in kcal while DMIti was the test ingredient in kilograms of DM.

In Exp. 3, the total collection method was used to calculate the Apparent total tract digestibility (ATTD) and metabolizability of energy following the equations suggested by Kong and Adeola, (2014):

$$\text{ATTD of GE (\%)} = 100 \times [(FI \times GE_i) – (FO \times GE_o)] / (FI \times GE_i);$$

$$\text{Metabolizability of GE (\%)} = 100 \times [(FI \times GE_i) – (FO \times GE_o) – (UO \times GE_u)] / (FI \times GE_i),$$

where FI, FO, and UO are the weight of feed intake, feces output, and urine output (kg, DM), respectively; GE_i, GE_o, and GE_u are the concentration of GE (kcal/kg, DM) in experimental diets, feces, and urine, respectively. The ATTD of nitrogen was calculated following the same equation as ATTD of GE. Based on the ATTD of energy in reference and test diets, the digestibility of energy in the test ingredients was calculated using the following equations (Adeola and Kong, 2014).

$$(D_{rd} \times P_{rd}) + (D_{ti} \times P_{ti}) = D_{td}$$

$$P_{rd} = 1 – P_{ti}$$

$$D_{ti}\,(\%) = D_{rd} + [(D_{td} – D_{rd}) / P_{ti}]$$

$$\text{Test ingredient DE (kcal/kg)} = D_{ti} \times GE_{ti}$$

Where D_{rd}, D_{td}, D_{ti} represents ATTD of energy in RD, test diet and test ingredient, respectively; P_{rd} and P_{ti} are proportional contribution of energy in the RD and test ingredient to the test diet, respectively; GE_{ti} represents gross energy of the test ingredient. The ME contributed from HFM

and FDPP was calculated by the same calculation procedure for DE. Data were analyzed using GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included experimental diets and block as independent variables. Difference between least square means were separated by pairwise comparison with Tukey's adjustment. Experimental unit was pig and statistical significance was again declared at $P < 0.05$

3.4 Results

The ingredient composition, calculated composition, and the analyzed nutrient composition of the experimental diets used in Exp. 1, 2, and 3 are shown in Table 3-1. The analyzed nutrient composition of the test ingredients is shown in Table 3-2. In Exp. 1, as the dietary concentration of HFM increased, a linear decrease ($P < 0.05$) in FI was observed (Table 3-3). Similarly, the concentration of the IDE in the diets had a tendency for a linear increase ($P = 0.090$) as the concentration of HFM increased. The inclusion of HFM tended to decrease TTR of DM linearly ($P = 0.077$) and quadratically ($P = 0.087$). As the dietary concentration of HFM increased, the ME concentration in the diets linearly increased ($P < 0.05$) with a tendency for quadratic effect ($P = 0.091$), however the TTR of nitrogen-corrected energy was observed to linearly decrease ($P < 0.05$) with a tendency for quadratic effect ($P = 0.081$). There was also a tendency for a quadratic effect ($P = 0.082$) on the MEn concentrations in the diets.

In Exp. 2, there was a tendency for a linear decrease ($P = 0.053$) in FI (Table 3-4) with increasing concentration of FDPP. Conversely, there was a linear increase in G: F ($P < 0.001$) with increasing concentration of FDPP. Also, a linear decrease was observed on the ileal digestibility of DM and energy ($P < 0.05$). It was observed that the TTR of DM linearly increased ($P < 0.05$) while there was a tendency for both linear ($P = 0.090$) and quadratic ($P = 0.078$) effect on TTR of nitrogen. As the dietary concentration of FDPP increased, ME was also observed to linearly increase ($P < 0.05$). Similarly, MEn concentration linearly increased with a P-value of < 0.01.

In Exp. 3, there was no difference in FI of pigs fed the three experimental diets. The fecal output in RD was significantly lower ($P < 0.05$) than those observed in the two test diets (Table 3-5). Also, the fecal energy loss was significantly different between dietary treatments ($P < 0.05$). Urinary GE loss was significantly different ($P < 0.001$). The metabolizability of GE in the RD was significantly higher ($P < 0.05$) than the test diet containing 150 g/kg of HFM but not different from

the test diet containing 150 g/kg of FDPP. The DE and ME concentration were not significantly different between dietary treatments. As shown in Table 3-6, In broiler chickens, the regression-derived IDE, ME, and MEn concentrations in HFM were 4,509, 4,250, and 3,745 kcal/kg while those in FDPP were 3,221, 4,710, and 4,081 kcal/kg, respectively. In pigs, the DE and ME derived from HFM and FDPP were not significantly different (P = 0.169 and 0.659, respectively). The respective DE and ME concentration in HFM and FDPP for pigs were 4,783 and 4,405 kcal/kg DM; and 4,553 and 4,320 kcal/kg DM.

3.5 Discussion

The proximate composition for DM, crude protein, ether extract, and ash in HFM were within the range of previously reported values by Cotanch *et al.* (2007) and Sulabo *et al.* (2013) where HFM from various processing plants were examined. The nutrient composition for FDPP were comparable with those reported by Park *et al*. (2020), however, the concentrations of CP in FDPP were greater than those reported by Moser *et al.* (1998). When comparing the two ingredients, HFM has a higher constituent of crude protein than FDPP, but its ash content is approximately 10 percentage points lower than that of FDPP. The ingredient composition of the reference diets used in Exp. 1 and 2 were identical but their analyzed GE were observed to be 74 kcal/g apart. The reason for the observed difference might be because diets used in the two experiments were prepared at different times and the batches of ingredient used are likely to be different. When comparing the MEn, the two reference diets were different by 169 kcal/g. This could be attributed to the disparity in their GE concentration, intrinsic bird factors, and the different environmental conditions, considering that both experiments were carried out at different time periods.

In Exp. 1, an increase in the dietary concentration of HFM could have resulted in the corresponding increase of GE concentration in test diets thereby resulting in the consequent decline in FI. Similar tendency was observed in Exp. 2. Many researchers (Waldroup, 1976; Firman *et al.*, 2010; Classen, 2017) have documented the physiological response of birds to increasing energy intake, such that birds will naturally compensate for high dietary energy with low FI, thus, creating an intrinsic energy balance. Regulation of FI by the central nervous system and peripheral tissue in poultry has been reviewed to be conveyed both by short-term and long-term systems (Kuenzel,

1994; Kuenzel *et al.*, 1999; Richards, 2003). It has been reported that the control of FI involves hormonal (which includes ghrelin, cholecystokinin, bombesin, and other peptides) and neural signals (such as leptins) that originate mainly in the gut, pancreas, and liver (Ashwell *et al*., 1999; Jensen, 2001; Blevins *et al*., 2002; Richards, 2003). Such satiety signals have been said to be produced in response to nutrient content such as dietary energy and the presence of feed or specific feed components in the gastrointestinal tract (Richards, 2003). The G:F had a tendency for a linear response in Exp. 1, and a significant linear response was observed for G:F in Exp. 2. Diets containing HFM and FDPP can be said to have fostered a superior efficiency of feed utilization relative to the reference diet and this is likely due to the similar BW gain between groups with lower and higher FI. In two studies conducted by Waldroup, (1976), it was reported that G:F ratios were improved as the energy density levels of the diet increased. The reason for the increase in energy density is because the decline in total FI is not at a rate commensurate with the increase in energy levels. As a result, total energy consumption of birds increased hereby allowing those additional calories to measure up with groups with higher total feed consumption.

Considering the objectives of this study was to determine the IDE, ME, and ME_n contents of HFM and FDPP in broiler chicken, a combination of the difference procedure and regression method was employed to get an approximate energy value which should be closer enough to the true mean values through a fitted line. The reason for using both difference procedure and regression analysis was to reduce the standard errors (SE) since it is often speculated that using the difference procedure alone results in higher SE especially when substituted test ingredient concentration is low (Oliveira *et al*., 2020; Park *et al*., 2021). The respective IDE derived from HFM and FDPP by broiler chickens were 4,509 and 3,221 kcal/kg DM. The IDE can be defined as energy available to birds from a feed ingredient before microbial fermentation of energy substrates in the ceca and the relatively short colon (Adeola *et al*., 2010). The reason for the low IDE value of FDPP when compared to its ME value is not clear but might be associated to the higher ash or mineral content in addition to the numerically higher content of its crude fiber over HFM which could have resulted in bird's failure to optimally utilize nutrient at the ileal level. Adeola *et al*. (1986) commented about the higher sensitivity of ileal digestibility in evaluating protein digestibility. Also, previous work from Zanella *et al*. (1999) and Douglas *et al*. (2000) have used the ileal digestibility as a parameter to examine birds' sensitivity to dietary enzyme supplementation. In the same manner, the variation observed in the IDE value of FDPP might be

an indicator for changes in the balance of mineral ions (Cowieson *et al*., 2006) created by the higher ash content in FDPP which could possibly be adjusted for at the total tract level.

The regression-derived ME and MEn concentrations in HFM were 4,250, and 3,745 kcal/kg, respectively. According to Pesti *et al*. (1989), the true and apparent MEn (TMEn and AMEn respectively) values should be similar, provided birds in AMEn assays maintain FI that is above the energy requirement for maintenance. Comparing previous published TMEn and AMEn values for HFM in broiler chickens with the AMEn in the current study, Pesti *et al*. (1989) reported respective mean values of 3,340 and 3,420 kcal/kg for TMEn and AMEn while Dale, (1992)) reported a TMEn value of 3,454 kcal/kg which are all numerically lower than 3,745 kcal/kg DM reported for AMEn in the present study. The reason for the increased AMEn value might be attributable to the fat content of the HFM used in the current study. In the study by Dale (1992), the author correlated the ME value of feather meal to their fat content and reported an average fat content of 7.7% for 15 different sampled feather meals which is 1.42 percentage point lower than the HFM used in the current study. Although, HFM available currently may be processed more effectively than those in the past, its fat composition depends on the extra materials processed along with the feathers, such as blood and fat trims, and those could directly vary the fat content of HFM. The DE and ME of HFM determined in the present experiment for pigs were 4,783 and 4,405 kcal/kg DM. These values were within the range reported by Sulabo *et al*. (2013) for 4 samples of HFM obtained from four different processing plants with or without blood inclusion. Although, the ME value for HFM fed to pigs in the present study were greater than the 3,031 kcal/kg DM in NRC (2012).

To the authors' knowledge, previously published data reporting the IDE, ME, and MEn value for FDPP in broiler chickens nor its DE and ME values in pigs are scarce. Comparing the ME value for FDPP in the two species, broiler chickens were observed to have utilized a higher percentage of the GE in FDPP at the ME level than pigs. This is contrary to expectation given the higher retention time and longer gastrointestinal tract in pigs. The reason for this is unclear but it might be associated with the high pepsin digestibility of the protein content in FDPP (Kureshy *et al*., 2000) and the mechanism of excreting nitrogen in the two species. When explaining the waste of carbon and energy in nitrogen excretion, Pilgrim (1954) expressed that urea is approximately as wasteful as uric acid although uric acid consists of four nitrogen atoms while urea consists of

two. Hence, the margin of energy waste in the two species might rather be highly attributed to the volume of urine versus the quantity of uric acid excreted while factoring the rate of protein catabolism initiated by the test ingredient (Salway, 2018). In broiler chickens, the respective IDE, ME, and MEn evaluated for FDPP are 3,221, 4,710, and 4,081 kcal/kg DM. In pigs, the respective DE and ME evaluated for FDPP were 4,553 and 4,320 kcal/kg DM.

In conclusion, the energy concentration in HFM can be said to have been progressively utilized with better efficiency over the last decades, which is probably due to improvement upon its processing techniques and management of poultry by-product. Also, FDPP is a credible energy source which should be added to the list of protein source for monogastric animals. Generally, there is a need for an overhauling of feed ingredients termed as conventional, those alternative feed ingredients that are also readily and constantly available for livestock feeding should be driven and sensitized to farmers for consideration as conventional feed ingredient.

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Table 3-1. Ingredient composition, calculated composition, and analyzed composition of diets in Exp. 1, 2 and 3

	Exp. 1			Exp. 2			Exp. 3		
		HFM, g/kg			FDPP, g/kg			HFM, g/kg	FDPP, g/kg
Ingredient g/kg	RD[1]	75	150	RD[1]	75	150	RD[1]	150	150
Corn	585.8	538.8	491.7	585.8	538.8	491.7	672.9	567.9	567.9
Soybean meal	310.0	285.9	261.9	310.0	285.9	261.9	245.0	206.8	206.8
Soybean oil	-	-	-	-	-	-	43.5	36.7	36.7
Dry fat	50.0	46.1	42.2	50.0	46.1	42.2	-	-	-
Ground limestone	4.0	4.0	4.0	4.0	4.0	4.0	12.0	12.0	12.0
Monocalcium phosphate	14.0	14.0	14.0	14.0	14.0	14.0	13.0	13.0	13.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
L-Lysine HCl	1.5	1.5	1.5	1.5	1.5	1.5	4.2	4.2	4.2
DL-Methionine	2.0	2.0	2.0	2.0	2.0	2.0	0.8	0.8	0.8
L-Threonine	0.7	0.7	0.7	0.7	0.7	0.7	1.2	1.2	1.2
L-Tryptophan	-	-	-	-	-	-	0.1	0.1	0.1
Vitamin premix[2]	-	-	-	-	-	-	2.0	2.0	2.0
Mineral premix[3]	-	-	-	-	-	-	0.8	0.8	0.8
Selenium premix[4]	-	-	-	-	-	-	0.5	0.5	0.5
Vitamin-mineral premix[5]	3.0	3.0	3.0	3.0	3.0	3.0	-	-	-
Titanium dioxide premix[6]	25.0	25.0	25.0	25.0	25.0	25.0	-	-	-
Hydrolyzed feather meal	0.0	75.0	150	-	-	-	0.0	150	-
Flash-dried poultry protein	-	-	-	0.0	75.0	150	0.0	-	150
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated nutrient, g/kg									
Crude protein (N × 6.25)	201	251	302	201	238	274	178	282	255
Ether extract	67	67	67	67	71	74	69	72	77
Calcium	9	9	9	9	11	13	7.5	7.8	12.1
Non-phytate P	4.3	4.0	5.0	4.3	5.0	7.0	3.9	4.0	6.1
Analyzed nutrient									

Table 3-1 continued

Gross energy, kcal/kg DM	4,578	4,709	4,846	4,649	4,688	4,732	4,709	4,878	4,790
Crude protein (N × 6.25), g/kg	184	240	299	188	239	269	180	275	252

[1]RD- reference diet for experiment

[2]Provided the following quantities per kg of complete diet: vitamin A, 5,280 IU; vitamin D_3, 528 IU; vitamin E, 35.2 IU; menadione, 1.8 mg; riboflavin, 7.0 mg; $_D$-pantothenic acid, 17.6 mg; niacin, 26.4 mg; vitamin B_{12}, 0.03 mg.

[3]Provided the following quantities per kg of complete diet: I, 0.29 mg; Mn, 13.7 mg; Cu, 7.23 mg; Fe, 155 mg; Zn, 119 mg.

[4]Provided 0.3 mg Se/kg of complete diet.

[5]Provided the following quantities per kg of complete diet: vitamin A, 10,968 IU; vitamin D3, 5,286 IU; vitamin E, 22.0 IU; menadione, 8.76 mg; riboflavin, 11.0 mg; D-pantothenic acid, 22.0 mg; niacin, 88.2 mg; choline chloride, 1,542 mg; vitamin B12, 0.03 mg; biotin, 0.11 mg; thiamine mononitrate, 4.40 mg; folic acid, 1.98 mg; pyridoxine hydrochloride, 6.60 mg; I, 2.22 mg; Mn, 132 mg; Cu, 8.88 mg; Fe, 88.2 mg; Zn, 88.2 mg; Se, 0.60 mg.

[6]5 g Titanium dioxide plus 20 g corn.

Table 3-2. Proximate composition of hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP)

Composition, (%)	HFM	FDPP
Dry matter	95.65	95.40
Gross energy, kcal/kg	5,323	5,243
Crude protein	87.8	70.00
Crude fiber	0.724	1.46
Ether extract	9.12	14.80
Ash	1.89	11.9

Table 3-3. Growth performance, ileal digestibility and total tract retention of DM, nitrogen, and gross energy of chickens fed diets containing hydrolyzed feather meal (HFM) from d 18 to 23 post hatching, Exp. 1

		HFM, g/kg			P-value	
Item[2]	RD[1]	75	150	SEM	Linear	Quadratic
Growth performance						
Initial BW, g	461	460	456	1.8	-	-
Final BW, g	692	695	685	14.2	0.644	0.678
BW gain, g	231	235	226	13.6	0.798	0.701
Feed intake, g	342	339	305	11.8	0.046	0.314
G:F, g/kg	677	694	743	24.9	0.183	0.596
Ileal digestibility, %						
DM	71.90	68.55	70.62	1.286	0.492	0.108
Energy	73.44	70.37	72.39	1.209	0.552	0.108
IDE, kcal/kg DM	3,361	3,313	3,508	56.8	0.090	0.104
Total tract retention, %						
DM	67.59	64.44	65.23	0.875	0.077	0.087
Nitrogen	51.31	45.42	46.22	2.072	0.104	0.209
Energy	70.58	68.18	69.50	0.853	0.384	0.096
Nitrogen-corrected Energy	67.49	64.69	65.23	0.722	0.044	0.081
ME, kcal/kg DM	3,231	3,210	3,368	40.1	0.030	0.091
ME_n, kcal/kg DM	3,107	3,067	3,186	34.7	0.129	0.082

[1]RD – Reference diet.

[2]Each means represents 8 replicate cages with 8 chickens per cage; IDE = Ileal digestible energy, ME = Metabolizable energy, ME_n = Nitrogen-corrected metabolizable energy.

Table 3-4. Growth performance, ileal digestibility and total tract retention of DM, nitrogen, and gross energy of chickens fed diets containing flash-dried poultry protein (FDPP) from d 17 to 22 post hatching, Exp. 2

		FDPP, g/kg			P-value	
Item[2]	RD[1]	75	150	SEM	Linear	Quadratic
Growth performance						
Initial BW, g	485	485	486	0.2	-	-
Final BW, g	730	742	762	19.3	0.269	0.856
BW gain, g	253	257	276	18.4	0.385	0.730
Feed intake, g	386	315	318	22.8	0.053	0.207
G:F, g/kg	651	810	884	32.9	< 0.001	0.3167
Ileal digestibility, %						
DM	73.68	71.65	70.13	0.861	0.006	0.496
Energy	76.02	75.08	73.56	0.841	0.025	0.798
IDE, kcal/kg DM	3,535	3,520	3,480	48.6	0.130	0.783
Total tract retention, %						
DM	70.60	71.78	71.93	0.413	0.038	0.325
Nitrogen	60.00	62.71	62.00	0.776	0.090	0.078
Energy	73.65	75.48	75.88	0.427	0.002	0.196
Nitrogen-corrected Energy	70.01	70.73	70.70	0.394	0.234	0.453
ME, kcal/kg DM	3,425	3,539	3,591	20.0	< 0.001	0.226
ME_n, kcal/kg DM	3,276	3,342	3,372	18.6	0.003	0.4461

[1]RD – Reference diet.

[2]Each means represents 8 replicate cages with 8 chickens per cage; IDE = Ileal digestible energy, ME = Metabolizable energy, ME_n = Nitrogen-corrected metabolizable energy.

Table 3-5. Apparent total tract digestibility (ATTD) and metabolizability of gross energy in pigs fed diets containing hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP) in Exp. 3[1]

Item[2]	RD[3]	HFM, 150 g/kg	FDPP, 150 g/kg	SEM	P-value
Dry matter intake, g/d	784	795	843	21.3	0.147
GE intake, kcal/d	3,691	3,877	4,039	102.3	0.088
Feces output, g/d	91[b]	104[a]	108[a]	3.0	0.004
GE in feces, kcal/kg DM	4,830[b]	5,060[a]	4,779[b]	33.5	< 0.001
Fecal GE output, kcal/d	440[b]	527[a]	514[a]	14.9	0.002
DE intake, kcal/d	3,251	3,351	3,524	101.4	0.192
ATTD of GE, %	87.98	86.39	87.22	0.474	0.093
Digestibility of N, %	87.37	86.07	87.22	0.627	0.309
DE, kcal/g DM	4,143	4,214	4,180	22.5	0.121
Urine output, g/d	1,362	1,408	1,423	139.8	0.951
GE in urine, kcal/kg	56.2	81.7	80.5	9.98	0.162
Urinary GE output, kcal/d	74.3[b]	108.8[a]	97.6[ab]	4.61	< 0.001
ME intake, kcal/d	3,177	3,242	3,427	102.7	0.238
Metabolizability of GE, %	85.94[a]	83.57[b]	84.84[ab]	0.561	0.032
ME, kcal/kg DM	4,047	4,077	4,064	26.7	0.744

[1]Each least squares mean represents 8 observations.
[2]DE = Digestible energy, ME = Metabolizable energy.
[3]RD – Reference diet.
[a,b]Means within a row without a common superscript differ at $P < 0.05$.

Table 3-6. Regression equations relating test ingredient-associated gross energy intake (kcal/kg) to test ingredient intake (g/kg DM) using hydrolyzed feather meal (HFM) in Exp. 1 and flash-dried poultry protein (FDPP) in Exp. 2. and the digestible and metabolizable energy in HFM and FDPP in Exp. 3

Item[2]	Regression equation[1]	R^2	SD
Exp. 1: HFM			
IDE	Y = 4,509 (581.5) × HFM – 14.40 (15.635)	0.732	48.5
ME	Y = 4,250 (403.0) × HFM – 5.11 (10.836)	0.834	33.6
ME_n	Y = 3,745 (349.0) × HFM – 4.97 (9.389)	0.839	29.1
Exp. 2: FDPP			
IDE	Y = 3,221(338.2) × FDPP – 2.28 (9.449)	0.805	43.0
ME	Y = 4,710 (226.9) × FDPP – 1.83 (6.342)	0.951	20.3
ME_n	Y = 4,081 (204.3) × FDPP – 0.63 (5.709)	0.948	18.3

Exp. 3:

	HFM	FDPP	SEM	*P*-value
DE, kcal/kg DM	4,783	4,553	105.9	0.169
ME, kcal/kg DM	4,405	4,320	131.7	0.659

[1] Values in parentheses are SE; Y is in kcal, Slope is in kcal/kg DM, Intercept is in kcal.
[2]IDE = Ileal digestible energy; DE = Digestible energy; ME = Metabolizable energy; MEn = Nitrogen-corrected metabolizable energy. The IDE, ME, or MEn for HFM or FDPP were estimated by the slope of the regression equation in Exp. 1 and 2.

CHAPTER 4. ENERGY VALUE OF BLACK SOLDIER FLY LARVAE MEAL FOR BROILER CHICKENS AND PIGS.

4.1 Abstract

Energy value of partially defatted black soldier fly larvae meal (BSFLM) was determined in 2 experiments with broiler chickens and growing pigs. Experiment (Exp.) 1 was conducted to evaluate the ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) of BSFLM with broiler chickens while Exp. 2 was conducted to evaluate the digestible energy (DE) and ME of BSFLM in growing pigs. Total collection (TC) and the index method using either titanium dioxide (Ti) or chromium oxide (Cr) were compared. In Exp 1 and 2, three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing BSFLM at either 100 or 200 g/kg replacement of the energy-contributing ingredients in the RD. Each diet was randomly allocated to 8 replicate cages of broiler chickens (6 birds per cage) or barrows. In Exp. 1, a linear increase ($P < 0.05$) was observed in the IDE concentration of the diet. With increasing BSFLM, a quadratic and linear increase ($P < 0.05$) was also observed on the ME and MEn concentration in the diet, respectively. The regression derived IDE, ME, and MEn concentration in BSFLM were 4,517, 4,725, and 4,238 kcal/kg DM when fed to broiler chickens. In Exp. 2, the inclusion of BSFLM resulted in linear decrease in DM digestibility and linear increase in dietary DE concentration ($P < 0.05$). The metabolizability of GE linearly decreased ($P < 0.05$) while the ME concentration quadratically increased ($P < 0.05$) with the increasing inclusion of BSFLM in the diet. In pigs, the regression-derived DE estimates with TC, Ti index marker, and Cr index marker were 5,010, 4,907, and 4,927 kcal/kg, respectively. The ME derived using the TC method was 4,711 kcal/kg. The result from this study is interpreted to suggest BSFLM as a potential energy feed ingredient for inclusion in diets for broiler chickens and pigs.

Key words: Black soldier fly larvae meal, broiler chickens, index marker, pigs, total collection

4.2 Introduction

Broiler chickens and pigs are part of a few fast growing, highly efficient animals that could rapidly satisfy the impending shortage of world protein, as they can be produced within a short time compared to other meat producing animals. The publication by FAO (2013) about edible

insects have made the likes of black soldier fly (BSF) and mealworms (Janssen et al., 2017) more popular as emerging feed ingredient in livestock production. Although black soldier fly is native to the Americas, it is prevalent throughout Australia, India, Africa, and Europe due to its ability to tolerate a wide selection of environmental conditions ranging from tropical to temperate regions (Sheppard et al., 1994; Barragan-Fonseca et al., 2017). The general notion behind the use of BSF larvae in animal feed is to partly mediate the future premonition (FAO, 2009) about the shortage of nutritious and healthy food in 2050.

The rearing of BSF is still in its early stage of technological advancement and its processing involves the extraction of its proteins, fats, chitin, minerals, and vitamins components. Such extraction processes are reported (FAO, 2013) to be costly and needs to be further developed to render them profitable. The black soldier fly larvae meal (BSFLM) is the granular protein concentrate derived from BSF processing. The cost factor could arguably impact - the cost effectiveness of using BSFLM in large-scale diet formulation, but it could also be presumed that as time goes on, further research into idealistic rearing and processing procedures will reduce the cost of BSFLM, allowing for its efficient harnessing in livestock diet. In lieu of this, accurate and reliable information about the energy value in partially defatted BSFLM for broiler chickens and pigs are necessary. Experiments on energy value of full fat BSFLM in chickens and pigs have been reported by De Marco et al. (2015), Schiavone et al. (2017), and Crosbie et al. (2020). While the full fat meal could result in rapid rancidity of the meal, the partially defatted BSFLM might be more beneficial as it is known that the fat extract of BSF is rich in medium chain fatty acids such as capric acids, lauric acids and myristic acids which are known to be active against lipid coated viruses, clostridium, and many pathogenic protozoa (Moula et al., 2018). Energy value of partially defatted BSFLM in pigs are limited and no literature has determined its energy value using various digestibility evaluation methods nor concurrently report the utilization of its energy values in broiler chickens and pigs. The first objective of the current study was to determine the ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEn) of BSFLM in broiler chickens and its digestible energy (DE) and ME in pigs. Additionally, we aimed to determine the DE of BSFLM derived using 3 methods which included total collection (TC), titanium index (Ti) and chromium index (Cr) methods in pigs.

4.3 Materials and Methods

All experimental protocols were reviewed and approved by the Purdue University Animal Care and Use Committee (PACUC, West Lafayette, IN). The PACUC protocol number for the broiler chicken and pig experiments were #1311000983 and #1112000248, respectively. The partially defatted BSFLM used in experiments (Exp.) 1 and 2 were purchased from Enterra Feed Co. (Maple Ridge, BC, Canada).

4.3.1 Animal management, experimental design, and diets

Experiments 1: Energy value of partially defatted BSFLM for Broiler Chickens

A total of 144 male broiler chicks (Cobb 500; Siloam Spring, AR, USA) were obtained from a local hatchery at one day old. Chicks were individually tagged for identification purpose and reared in electrically heated battery cages (model SB 4 T; Alternative Design Manufacturing and Supply, Siloam Springs, AR) with temperature maintained at 35, 31, and 27°C from days 0 to 7, 7 to 14, and 14 to 22 respectively. Light was provided 23 h per day throughout the study. Birds had *ad libitum* access to water throughout the duration of the experiments. All diets were fed in mash form, and vitamin-mineral premix was added to all diets to meet the nutrient requirements as recommended by the Cobb broiler management guide (2015). A standard broiler chicken starter diet was fed from days 0 to16. On day 16, birds were individually weighed and randomly designated to one of the dietary treatments in a randomized complete block design (RCBD), with body weight (BW) as a blocking factor. Each dietary treatment consisted of 8 replicate cages with 6 birds per cage. Dietary treatments consisted of a reference diet (RD) and two test diets (Table 1). The RD was a corn-soybean meal-based diet while the two test diets were prepared by substituting either 100 or 200g/kg of the RD with BSFLM at the expense of corn, soybean meal (SBM), and dry fat. The nutrient composition of the dry fat used in the current study were according to Osunbami et al. (2021). The 3 dietary treatments were formulated to maintain the ratio of corn to SBM to dry fat at 12.6:6.4:1. Titanium dioxide was included at 5 g/kg as an indigestible index marker in the diets.

Birds had un-restricted access to experimental diets from days 16 to 21. Excreta collection was performed during the last 3 days of the experimental period in collection pans lined with waxed paper. After 5 days of feeding the experimental diets, all birds were euthanized by

asphyxiation using CO_2, weighed individually, and dissected to excise the ileum. Ileal digesta samples were collected from the distal two-thirds of the ileum defined as extending from Meckel's diverticulum to the ileocecal junction. The content in the ileum was flushed with distilled water and pooled per cage before storage in a −20°C freezer until further analysis. The BW gain and feed intake (FI; g/bird) during the experimental periods were recorded, and the gain to feed ratio (G:F; g/kg) of each cage was calculated.

Experiment 2: Energy value of partially defatted BSFLM for Pigs using 3 methods.

Twenty-four barrows with initial BW of approximately 20 kg were individually housed in metabolic crates furnished with a feeder and drinker. Barrows used were crossbreed of Duroc x Yorkshire x Landrace. Three methods were employed in estimation and included TC, Ti, and Cr. Pigs were assigned one of three dietary treatments in a RCBD with BW as blocking factor. The reference diet was prepared to contain corn, SBM, and soybean oil (SBO) as the sole sources of energy (Table 1). The test diets were prepared by adding BSFLM at 100 or 200 g/kg at the expense of corn, SBM, and SBO in the RD. The corn: SBM: SBO ratio was kept at 14:5:1. Titanium dioxide and Cr_2O_3 were included each at 5 g/kg as an indigestible marker in the diets in order to estimate energy value of BSFLM through index methods. All diets were formulated to meet or exceed the estimated vitamin and mineral requirements suggested in NRC (2012). Daily feed allowance was estimated as 4.5 % of mean BW of pigs in each block and was portioned into 2 equal meals fed at 0800 and 1700 h. There were 5 days of adaptation to the feed and environment. The marker-to-marker procedure was employed in sample collection (Adeola, 2001). On days 6 and 11, approximately 200 g of the first meal fed to pigs were hand mixed with approximately 3 g of ferric oxide as a colored marker. Collection of feces started at the appearance of first marker in feces and stopped at the appearance of the second marker. Concurrently, during the fecal collection period, daily urine output was also quantitatively collected using plastic buckets containing 10 mL of 10 % formic acid. Urine collected daily from each pig was weighed and proportionally subsampled. Feces and urine collected were immediately stored at -20°C.

4.3.2 Chemical Analysis

At the end of the experiments, ileal digesta, excreta, fecal, and urine samples were thawed and placed in a forced-air oven (Precision Scientific Co., Chicago, IL, USA; method 934.01; AOAC, 2006) at 55°C for 120 h. Excreta and fecal samples were ground using a mill grinder (ZM 100; Retsch GmbH, Haan, Germany) while ileal digesta samples were ground using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany). Gross energy (GE) of the test ingredient, experimental diets, ileal digesta samples, excreta samples, fecal samples, and dried urine were determined using an isoperibol oxygen bomb calorimeter (Parr 1261; Parr Instruments Co., Moline, IL, USA) with benzoic acid as a calibration standard. Dry matter (DM) content was determined gravimetrically by drying samples in an oven at 105°C for 24 h (method 934.01, AOAC International, 2006). Nitrogen (N) concentration was determined using the combustion method (TruMac N; LECO Corp., St. Joseph, MI, USA) with EDTA as the calibration standard. Test ingredient was analyzed for crude fiber (method 978.10; AOAC, 2006), ether extract (method 945.16; AOAC, 2000), and ash content (method 942.05; AOAC, 2006). Titanium concentrations in experimental diets, ileal digesta, and excreta for Exp. 1, and diets and fecal samples for Exp. 2 were analyzed as described by Myers et al. (2004). Briefly, samples were weighed into 250 mL macro-Kjeldahl digestion tubes. Kjeldahl tablet which contained 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$ were added to each tube as catalyst, after which 13 mL of concentrated H_2SO_4 were added to each tube, and digestion was done at 420°C for 2 h. After cooling for 30 minutes, 10 mL of 30 % H_2O_2 were added to each tube and allowed to cool for another 30 minutes. A blank solution was prepared alongside to help calibrate spectrophotometer (Spark 10M; Tecan Group Ltd., Männedorf, Switzerland). Finally, total liquid weight was made up to 100 g with distilled water by filtering through Whatman No. 541 ashless filter paper to remove any precipitate. Filtrates were then plated to measure spectrophotometer absorbance at 410 nm. Chromium concentration in experimental diets and fecal samples for Exp. 2 was analyzed as described by Fenton and Fenton, (1979) with slight modifications. In short, samples were digested with nitric acid and 70 % perchloric acid. The digests were allowed to stand overnight after dilution to 100 mL with distilled water before the spectrophotometry absorbance was measured at 450 nm.

4.3.3 Calculations and Statistical Analysis

Index method

The index method (Kong and Adeola, 2014) was used to calculate ileal digestibility and metabolizability of DM, N, and GE of diets in Exp 1. The apparent total tract digestibility (ATTD) of DM, N, and GE in the experimental diets from Exp. 2 was also estimated using the index method. For an illustration, to calculate the coefficient of ileal digestibility of nutrient or GE in experimental diet, the following equation was applied:

$$Z = [1 - (C_i/C_o) \times (E_o/E_i)].$$

where Z is the coefficient of ileal digestibility and the percentage ileal digestibility was derived by multiplying Z by 100; C_i and C_o represent the concentration of index marker (g/kg DM) in experimental diets and ileal digesta output, respectively; E_i and E_o are the concentration of nutrient or GE (kcal/kg DM) in experimental diets and ileal digesta, respectively.

The product of Z and GE of the diet was used to calculate IDE, DE, ME, and MEn of diets. Correction of the ME to zero N retention using the factor of 8.22 kcal/g of N (Hill and Anderson, 1958) was used to estimate the MEn. The IDE, DE, ME, and MEn (kcal/kg DM) in test ingredient was calculated by difference procedure proposed by Adeola (2001): For an illustration, the IDE in test ingredient (IDEti) was calculated using the following equation:

$$IDE_{ti} = [IDE_{td} - (P_{rd} \times IDE_{rd})] / P_{ti}.$$

where IDE_{ti}, IDE_{td}, and IDE_{rd} represent the IDE in test ingredient, test diets (i.e., experimental diets containing test ingredient) and reference diet, respectively; P_{rd} and P_{ti} represent the proportional contribution of reference diet and test ingredient (kg/kg) in test diets, respectively. The DE, ME, and MEn in test ingredient were calculated following the same calculation steps as IDE of test ingredient.

Total collection

In Exp. 2, the TC method was used to calculate the ATTD, metabolizability of DM, GE, and N (Kong and Adeola, 2014) and the index marker recovery following the equations below:

$$\text{ATTD of GE (\%)} = 100 \times [(F_i \times GE_i) - (F_o \times GE_o)] / (F_i \times GE_i);$$

$$\text{Metabolizability of GE (\%)} = 100 \times [(F_i \times GE_i) - (F_o \times GE_o) - (U_o \times GE_u)] / (F_i \times GE_i);$$

$$\text{Index marker recovery (\%)} = 100 \times [(C_o \times F_o) / (C_i \times F_i)].$$

where F_i, F_o, and U_o are the weight of feed intake, feces output, and urine output (kg, DM), respectively; GE_i, GE_o, and GE_u are the concentration of GE (kcal/kg, DM) in experimental diets, feces, and urine, respectively. The ATTD of DM and N was calculated following the same equation as ATTD of GE. The DE and ME (kcal/kg DM) in BSFLM were calculated by the same difference procedure mentioned above.

$$DEti = [DEtd - (DErd \times Prd)] / Pti;$$

$$MEti = [MEtd - (MErd \times Prd)] / Pti,$$

where DEti, DEtd, and DErd represent the DE (kcal/kg) in test ingredient, test diet, and reference diet, respectively; Prd and Pti represent the proportion of reference diet and test ingredient in the test diet, respectively; MEti, MEtd, and MErd represent the ME (kcal/kg) in test ingredient, test diet, and reference diet, respectively.

Before statistical analysis, an outlier test was carried out and no outliers were detected in the dataset. The data were analyzed by ANOVA using GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with RCBD arrangement. The model included diet and block as independent variables. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of increasing levels of test ingredient in the experimental diet. Statistical significance was declared at $P < 0.05$. The test ingredient DM intake (DMIti) was the product of feed DM, feed intake, and the proportion of test ingredient in test diets. Test ingredient-associated IDE, DE, ME, or MEn intake were calculated as product of test ingredient intake and IDEti, DEti, MEti, or MEnti. Regression analysis between the test ingredient–associated IDE, DE, ME, or MEn intake and DMIti was conducted using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to generate a regression equation as described by Bolarinwa and Adeola (2016). In the regression equation, Y was the test ingredient-associated IDE, ME, or MEn intake in kcal while DMIti was the test ingredient in kilograms of DM. Finally, In Exp. 2, the TC method was used as a standard to measure the relativity of recovery such that the ATTD of DM, GE, and N from the 3 methods were

expressed in relative percentage to the TC method using a split plot arrangement. The whole-plot factor (n = 24) was the dietary BSFLM level, and the split-plot factor (n = 72) was the type of methods which included TC, Ti and Cr. The full model was used for analysis. The fixed effects included BSFLM level, methods, and interaction between BSFLM level and method. The random effects were block, 2-way interactions of block and BSFLM level, 2-way interactions of block and method, and 3-way interactions of block, BSFLM level, and method. The GLMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the split plot analysis. Least square means were separated by PDIFF option with the Tukey's adjustment.

4.4 Results

The ingredient composition, calculated, and analyzed nutrient values in diets fed to broiler chickens and pigs are presented in Table 4-1 while the proximate composition of the test ingredient is shown in Table 4-2. In Exp. 1, there was no effect of the dietary treatment on final BW and BWG but there was a linear decrease ($P < 0.05$) in FI and a linear increase ($P < 0.001$) in G:F with increasing BSFLM concentration (Table 4-3). There was no effect of dietary inclusion of BSFLM on the AID of DM and energy but a linear increase ($P < 0.05$) in the IDE concentration of the diet was observed. With increasing BSFLM, there were quadratic decrease ($P < 0.050$) in DM, N, and energy metabolizability. A quadratic and linear increase ($P < 0.05$) was observed on the ME and MEn concentration, respectively, with the inclusion BSFLM.

In Exp. 2, the feed and GE intake were not affected by the inclusion of BSFLM in the RD (Table 4-4). Fecal output was increased ($P < 0.05$) by approximately 14% when BSFLM was included in the RD at 200 g/kg. The GE in urine (kcal/g) and the urinary GE output (kcal/d) were linearly increased ($P < 0.05$) with increasing inclusion of BSFLM in the RD. The metabolizability of GE linearly decreased ($P < 0.05$) while the ME concentration in diet quadratically increased ($P < 0.05$) with the addition of BSFLM. When the TC or Ti method was used to determine DM digestibility of diets fed to pigs, a linear decrease ($P < 0.05$) was observed (Table 4-5). Regardless of the method used, there was no effect of increasing inclusion of BSFLM on the digestibility of GE. Using the TC method, there was a linear decrease ($P < 0.01$) in N digestibility however, when the index methods were used (Ti and Cr), there was no significant difference in N digestibility with the inclusion of BSFLM in the RD. The DE in diet increased quadratically ($P < 0.050$) when

the TC and Ti methods were used and linearly ($P < 0.01$) when the Cr method was used in evaluating digestibility. There was no effect of increasing the inclusion of BSFLM on the recovery of Ti and Cr in the experimental diets

When the 3 digestibility evaluation methods were expressed relative to the TC method, there was no interaction nor BSFLM level effect on DM and GE digestibility, but a method effect ($P < 0.001$) was observed with the TC method returning the highest value as compared to the index methods (Table 4-6). There was a significant interaction effect ($P < 0.001$) between BSFLM level and method for N digestibility. The regression derived IDE, ME, and MEn concentration in BSFLM were 4,517, 4,725, and 4,238 kcal/kg DM when fed to broiler chickens (Table 4-7). The respective regression-derived DE concentration in BSFLM using the TC, Ti, and Cr method were 5,010, 4,907, and 4,927 kcal/kg DM when fed to pigs. There was no difference amongst the three methods used to derive the test ingredient DE concentration. The regression-derived ME concentration in BSFLM using the TC method was 4,711 kcal/kg DM.

4.5 Discussion

The proximate composition of the BSFLM used in the current study is close to those reported by Cullere et al. (2016) and Renna et al. (2017) for partially defatted BSFLM, although its crude fat content showcased to be higher than what was reported by Mwaniki et al. (2018). The proximate composition of the BSFLM used in the current study represents that of a partially defatted product, in which case extrapolation of its nutritional value to other types of BSFLM such as full-fat larvae meal (Rawski et al., 2020) should be done with caution. When BSFLM is partially defatted, the process increases the CP content (Schiavone et al., 2017) of the meal alongside minimizing oxidative peroxidation (El-Sayed et al., 2014) caused by lipids. Thus, producing a protein concentrate source with an increased shelf-life. A marked linear decline in feed intake was observed to have occurred when BSFLM was included at 200 g/kg although, the corresponding G:F was the highest among the 3 treatments group. The authors acknowledge that results from digestibility studies are not always an indication of animal growth or feed efficiency (Romero et al., 2014), as diets in Exp. 1 were formulated to achieve the objective of energy evaluation through proportional substitution of test ingredient in RD. This infers that the 3 dietary treatments are not comparable for the purpose of estimating performance although, reporting their performance

response can serve as a guide to what could be expected from individual dietary treatments. As such, performance studies are required to make general inferences about the growth performance and feed efficiency of BSFLM in the broiler chicken. However, this study provides concrete information about the energy value in BSFLM which will aid in formulating diets that meet the requirement of broiler chickens when BSFLM are included.

When comparing the 3 dietary treatments in Exp. 1 based on their digestibility responses, the diet with 100 g/kg of BSFLM consistently had the highest utilization of its GE content for IDE and metabolizability. The percentage utilization of each corresponding GE concentration in the RD, and diets containing 100 g/kg BSFLM or 200 g/kg BSFLM at the IDE level were 67.62 vs 72.79 vs 70.58 %, respectively. Similarly, the respective percentage utilization of GE concentration at the ME level were 70.09 vs 73.18 vs 72.25 % while those for MEn were 66.88 vs 68.39 vs 67.71 %. In broiler chickens, the regression derived IDE, ME, and MEn concentration in BSFLM were 4,517, 4,725, and 4,238 kcal/kg DM which shows that 78 to 86 % of the GE in partially defatted BSFLM used in the current study was utilizable. These values are quite higher than studies previously reported. De Marco et al. (2015) reported a respective AME and AMEn of 4,152 and 3,965 kcal/kg for full-fat BSFLM while Schiavone et al. (2017) reported 3,882 and 3,552 as the respective AME and AMEn value for partially defatted BSFLM. The reason for this occurrence is unclear but might be attributed to improvement in quality of recently produced BSFLM.

In Exp 2, among the 3 methods used in deriving energy value of BSFLM in pigs, it is imperative to mention that only the TC method can be employed in estimating metabolizabilty. Estimation via the TC method showed that diet with 200 g/kg of BSFLM promoted highest quantity of daily fecal output and daily urinary loss of GE, cumulating in lowest metabolizability of its GE content in pigs. The higher urinary output seen to be associated with 200 g/kg inclusion of BSFLM could ultimately result in increased N excretion to the environment. The observed result might be indicating a diminishing utilization of BSFLM beyond 100 g/kg of inclusion.

To compare the ME value of BSFLM in the two species, broiler chickens and pigs have similar utilization of the test ingredient: 4,725 vs 4,711. Higher utilization in pigs could have been expected given the higher retention time and longer gastrointestinal tract in pigs. However, this result might be partially explained by the chitin content in BSFLM. Chitin content in partially

defatted BSFLM ranges from 50 to 100 g/kg (Kroeckel et al., 2012; Renna et al., 2017; Schiavone et al., 2017) while the respective AME and AMEn content of chitin reported by Hossain and Blair, (2007) were 2142 and 2116 kcal/kg in broiler chickens. Early study by Suzuki et al. (2002) showed that chickens produce gut chitinase in the proventriculus and liver while pigs' secretion of chitinase has only been identified recently in the stomach and is significantly influenced by age (Tabata et al., 2017; Kawasaki et al., 2021). Moreover, the result of Tabata et al. (2018) indicates that chicken's expression of acidic chitinase gene is about six folds higher than that of pigs. It can therefore be speculated that the presence of chitinase will invariably lead to improved digestibility of BSFLM indigestible chitin content in broiler chickens than in pigs. Recent publications have emphasized the beneficial effect of chitin found in BSFLM on cecal microbial community, health status, and it potential as a prebiotic in broiler chickens (Biasato et al., 2020; Dorper et al., 2020). However, no consensus has been reached about the BSFLM inclusion level that meets both health and growth performance needs in broiler chickens. Furthermore, insects are regarded as a natural nutrient source to poultry (Józefiak et al., 2016) especially in situations when chickens have access to free-range. This voluntary picking up of insects as feeds indicates that poultry are evolutionarily adapted to insects as a natural part of their diets.

When comparing all ATTD estimated using the 3 methods, the TC method had greater values than the 2 index methods (Ti and Cr) expect for the ATTD of N. This agrees with previous studies by Adeola et al, (1986) and Wang and Adeola (2018). The underlying principle for TC method is that the total input (FI) and total output (fecal and urinary output) should be totally accounted for. However, this is practically hard to guarantee while the underlying assumption for index method hinges on the total recovery of the index compound although, this is also hard to ascertain. This repeated observation suggests that the TC method might be more complete in respect to its substance of recovery than index methods. Hence, the TC method was used as a standard to measure relativity of the recovery. The observation for the ATTD of N followed a pattern that was inconsistent with those observed for DM and energy, such that the TC method had a pattern for linear decrease while the Ti and Cr methods were not different. The reason for this is quite unclear.

The result of the present study showed a higher numerical value for the recovery of Chromium over Titanium. This is contrary to the findings of Wang and Adeola (2018) who

reported that between the two index marker types, Titanium gave a higher recovery than Chromium. The explanation for the higher recovery of one marker over the other is unclear but it might be linked to the possible interaction between index marker in a biological system especially when TiO_2 and Cr_2O_3 are mixed in the same diet (Myers et al., 2004). Compared to the report of Wang and Adeola (2018), it seems the directionality of this interaction cannot be ascertained to have consistently favored a marker type due to the various factors in a biological system that could tilt the result either way. One of such factors is the characteristic squishy lining of the intestinal lumen which could be envisaged to aid the retention of the index marker. Although, irrespective of the marker type, using the recovered index concentration to calculate digestibility gave a similar estimate for the parameters. This facilitated a conclusion that the choice of marker type does not largely impact digestibility results. With an approximate range of 100 kcal/kg, the DE values for BSFLM from the 3 methods were not statistically different. The regression-derived DE estimate with TC, Ti, and Cr were 5,010, 4,907, and 4,927 kcal/kg, respectively. The ME derived using the TC method was 4,711 kcal/kg.

In conclusion, broiler chickens and pigs derived energy value which ranges from 77 to 91% on a dry matter basis. Further research that bridges the knowledge gap between the prebiotic effect of BSFLM chitin content and optimal growth performance response in broiler chickens or pigs fed diets containing BSFLM at various growth phases are necessary.

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Table 4-1. Ingredient composition, calculated, and analyzed composition of experimental diets used in Exp. 1 and 2.

	Exp. 1			Exp. 2		
		BSFLM, g/kg			BSFLM, g/kg	
Ingredient g/kg	RD[1]	100	200	RD[1]	100	200
Corn	585.80	523.10	460.30	622.90	553.22	483.55
Soybean meal	310.00	277.90	245.80	245.00	219.25	193.50
BSFLM	0.00	100.00	200.00	0.00	100.00	200.00
Soybean oil	-	-	-	43.50	38.93	34.36
Dry fat	50.00	44.82	39.65	-	-	-
Ground limestone	4.00	4.00	4.00	12.00	12.00	12.00
Monocalcium phosphate	14.00	14.00	14.00	13.00	13.00	13.00
Salt	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine HCl	1.50	1.50	1.50	4.20	4.20	4.20
DL-Methionine	2.00	2.00	2.00	0.80	0.80	0.80
L-Threonine	0.7	0.7	0.7	1.20	1.20	1.20
L-Tryptophan	-	-	-	0.10	0.10	0.10
Vitamin premix[2]	-	-	-	2.00	2.00	2.00
Mineral premix[3]	-	-	-	0.80	0.80	0.80
Selenium premix[4]	-	-	-	0.50	0.50	0.50
Vitamin-mineral premix[5]	3.00	3.00	3.00	-	-	-
Titanium dioxide premix[6]	25.00	25.00	25.00	25.00	25.00	25.00
Chromic oxide premix[7]	-	-	-	25.00	25.00	25.00
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated nutrient, g/kg						
Crude protein (N × 6.25)	201	235	270	177	212	247
Ether extract	67	77	87	69	79	88
Calcium	7.0	7.0	8.0	7.0	9.0	10.0
Non-phytate P	4.3	5.0	6.0	4.0	5.0	5.0
Analyzed nutrient						

Table 4-1 continued

Gross energy, kcal/kg DM	4,564	4,671	4,793	4,642	4,770	4,827
Crude protein (N × 6.25), g/kg	204	259	288	179	223	255

[1]RD- reference diet for experiment

[2]Provided the following quantities per kg of complete diet: vitamin A, 5,280 IU; vitamin D_3, 528 IU; vitamin E, 35.2 IU; menadione, 1.8 mg; riboflavin, 7.0 mg; $_D$-pantothenic acid, 17.6 mg; niacin, 26.4 mg; vitamin B_{12}, 0.03 mg.

[3]Provided the following quantities per kg of complete diet: I, 0.29 mg; Mn, 13.7 mg; Cu, 7.23 mg; Fe, 155 mg; Zn, 119 mg.

[4]Provided 0.3 mg Se/kg of complete diet.

[5]Provided the following quantities per kg of complete diet: vitamin A, 10,968 IU; vitamin D3, 5,286 IU; vitamin E, 22.0 IU; menadione, 8.76 mg; riboflavin, 11.0 mg; D-pantothenic acid, 22.0 mg; niacin, 88.2 mg; choline chloride, 1,542 mg; vitamin B12, 0.03 mg; biotin, 0.11 mg; thiamine mononitrate, 4.40 mg; folic acid, 1.98 mg; pyridoxine hydrochloride, 6.60 mg; I, 2.22 mg; Mn, 132 mg; Cu, 8.88 mg; Fe, 88.2 mg; Zn, 88.2 mg; Se, 0.60 mg.

[6]5 g Titanium dioxide plus 20 g corn.

[7]5 g chromium oxide plus 20 g corn.

Table 4-2. Proximate composition of the black soldier fly larvae meal (BSFLM)

Composition[1], (%)	BSFLM
Dry matter	93.90
Gross energy, kcal/kg	5,146
Nitrogen-free extracts[2]	8.29
Crude protein	55.11
Crude fiber	6.85
Crude fat	14.82
Ash	7.83

[1]Analyzed.
[2]Calculated: 100 − (water + crude protein + crude fiber + crude fat + ash).

Table 4-3. Growth performance, ileal digestibility and metabolizability of DM, nitrogen, and gross energy of chickens fed diets containing black soldier fly larvae meal (BSFLM) from d 16 to 21 post hatching (Exp. 1).

		BSFLM, g/kg			*P*-value	
Item[2]	RD[1]	100	200	SEM	Linear	Quadratic
Growth performance						
Initial BW, g	548	548	548	0.6	-	-
Final BW, g	821	863	821	17.2	0.992	0.066
BW gain, g	273	315	275	17.0	0.947	0.070
Feed intake, g	409	412	348	19.9	0.049	0.186
G:F, g/kg	666	765	790	18.5	< 0.001	0.129
Ileal digestibility, %						
DM	67.23	69.56	68.37	1.746	0.633	0.402
Energy	67.62	72.79	70.58	1.934	0.298	0.142
Ileal digestible energy, kcal/kg DM	3,086	3,400	3,383	90.4	0.036	0.157
Metabolizability, %						
DM	65.21	68.03	66.27	0.718	0.315	0.021
Nitrogen	48.04	58.51	51.42	2.477	0.352	0.012
Energy	70.09	73.18	72.25	0.661	0.036	0.026
Nitrogen-corrected Energy	66.88	68.39	67.71	0.484	0.242	0.086
ME, kcal/kg DM	3,199	3,419	3,463	30.6	< 0.001	0.035
ME_n, kcal/kg DM	3,070	3,219	3,268	23.2	< 0.001	0.099

[1]RD – reference diet in experiment 1.

[2]Each means represents 8 replicate cages with 8 chickens per cage; IDE = ileal digestible energy, ME = metabolizable energy, ME_n = nitrogen-corrected metabolizable energy.

Table 4-4. Digestibility and metabolizability of GE in experimental diets fed to pigs evaluated using total collection method (Exp. 2).

		BSFLM, g/kg			*P*-value	
Items[2]	RD[1]	100	200	SEM	Linear	Quadratic
Feed intake, g/d	767	746	780	25.9	0.740	0.406
GE intake, kcal/d	3,562	3,561	3,764	123.1	0.266	0.510
Feces output, g/d	94	95	107	3.8	0.033	0.279
GE in feces, kcal/kg DM	4,579	4,405	4,386	69.0	0.067	0.370
DE intake, kcal/d	3,133	3,142	3,297	112.0	0.317	0.604
Digestibility of GE, %	87.96	88.30	87.59	0.361	0.477	0.254
DE in diet, kcal/kg DM	4,084	4,212	4,228	17.0	<0.001	0.017
Urine output, g/d	1,520	1,464	1,360	140.0	0.433	0.891
GE in urine, kcal/kg	54	63	87	6.0	0.003	0.343
Urinary GE output, kcal/d	76	87	109	3.4	<0.001	0.214
ME intake, kcal/d	3,057	3,055	3,188	112.2	0.422	0.632
Metabolizability of GE, %	85.83	85.78	84.69	0.366	0.046	0.267
ME in diet, kcal/kg DM	3,985	4,092	4,088	17.0	0.001	0.020

[1]RD – reference diet in experiment 2.

[2]Each means represents 8 observations; DE = digestible energy, ME = metabolizable energy.

Table 4-5. Apparent total tract digestibility (ATTD) estimated with various methods and marker recovery in experimental diets fed to growing pigs (Exp. 2).

			BSFLM, g/kg			*P*-value	
Items[1], %	Method[2]	RD[3]	100	200	SEM	Linear	Quadratic
DM digestibility	TC	87.81	87.33	86.34	0.360	0.012	0.575
	Ti	85.22	84.56	83.70	0.323	0.005	0.800
	Cr	85.94	85.44	84.41	0.523	0.057	0.687
Digestibility of GE	TC	87.96	88.30	87.59	0.361	0.477	0.254
	Ti	85.41	85.75	85.17	0.338	0.625	0.281
	Cr	86.15	86.58	85.80	0.608	0.687	0.429
Digestibility of N	TC	86.79	84.56	82.09	0.869	0.002	0.914
	Ti	83.92	84.77	84.65	0.861	0.555	0.650
	Cr	84.75	85.65	85.27	1.010	0.721	0.613
DE in diet, kcal/kg DM	TC	4,084	4,212	4,228	17.0	<.0001	0.017
	Ti	3,965	4,091	4,111	15.9	<.0001	0.018
	Cr	3,999	4,130	4,141	29.0	0.004	0.117
Titanium recovery	Ti	82.95	82.44	84.09	2.209	0.721	0.695
Chromium recovery	Cr	87.09	87.52	88.33	3.132	0.783	0.962

[1]DE = digestible energy. Titanium or Chromium recovery was calculated as concentration of titanium or chromium in DM weight of total fecal output divided by the concentration of titanium or chromium in DM weight of total Feed intake.

[2]TC= total collection method; Ti = titanium dioxide index method; Cr = chromic oxide index method.

[3]RD – reference diet in experiment 2.

Table 4-6. Digestibility parameters expressed as a relative percentage to total collection method[1] (Exp. 2)

			Digestibility parameters, relative % to TC method		
BSFLM level, g/kg	[2]Method	No of replicates	DM	GE	Nitrogen
0	TC	8	100.00	100.00	100.00^{cd}
0	Ti	8	97.07	97.12	96.70^{e}
0	Cr	8	97.89	97.97	97.68^{de}
100	TC	8	100.00	100.00	100.00^{cd}
100	Ti	8	96.85	97.13	100.29^{cd}
100	Cr	8	97.84	98.06	101.32^{bc}
200	TC	8	100.00	100.00	100.00^{cd}
200	Ti	8	96.96	97.25	103.15^{ab}
200	Cr	8	97.78	97.96	103.88^{a}
0		24	98.32	98.36	98.12
100		24	98.23	98.40	100.54
200		24	98.25	98.40	102.34
	TC	24	100.00^{a}	100.00^{a}	100.00
	Ti	24	96.96^{b}	97.17^{b}	100.04
	Cr	24	97.83^{b}	98.00^{b}	100.96
[3]SEM			0.573	0.537	0.692
P value					
BSFLM level			0.970	0.993	< 0.001
Method level			< 0.001	< 0.001	0.279
BSFLM level*Method			0.999	0.999	< 0.001

[1]BSFLM = black soldier fly larvae meal. Each least squares mean represents 8 observations.
[2]TC= total collection method; Ti = titanium dioxide index method; Cr = chromic oxide index method.
[3]SEM = standard error of mean for simple effects.
[a,b,c,d,e]Means within a column without a common superscript differ at $P < 0.05$.

Table 4-7. Regression equations relating test ingredient-associated gross energy intake (kcal/kg) to test ingredient intake (g/kg DM) using black soldier fly larvae meal (BSFLM) in Exp. 1 and 2.

Item[2]		Regression equation[1]	R-square	SD
Exp. 1				
IDE		Y = 4,517 (676.7) × BSFLM + 22.17 (28.633)	0.669	87.3
ME		Y = 4,725 (308.1) × BSFLM + 8.27 (13.036)	0.914	39.7
ME_n		Y = 4,238 (240.9) × BSFLM + 4.46 (10.191)	0.933	31.1
Exp. 2				
	Method[3]			
DE	TC	Y = 5,010 (0.187) × BSFLM + 66.70 (93.736)	0.970	293.9
DE	Ti	Y = 4,907 (0.176) × BSFLM + 67.21 (88.389)	0.972	277.1
DE	Cr	Y = 4,927 (0.206) × BSFLM + 71.35 (102.925)	0.963	322.7
ME	TC	Y = 4,711 (0.188) × BSFLM + 66.46 (94.063)	0.966	294.9
	P values			
$DE_{TC}\ vs\ DE_{Ti}$	0 .690			
$DE_{TC}\ vs\ DE_{Cr}$	0.766			
$DE_{Ti}\ vs\ DE_{Cr}$	0.941			

[1] Values in parentheses are SE; Y is in kcal, Slope is in kcal/kg DM, Intercept is in kcal.

[2]IDE = ileal digestible energy; DE = digestible energy; ME = metabolizable Energy; ME_n = nitrogen-corrected metabolizable energy. The IDE, DE, ME, or MEn in BSFLM were estimated by the slope of the regression equation.

[3]TC= total collection method; Ti = titanium dioxide index method; Cr = chromic oxide index method.

CHAPTER 5. SUMMARY

5.1 Summary

The importance of adequate supply of dietary energy cannot be overemphasised as dietary energy content could modulate feed efficiency. All the studies conducted in this thesis further reinforced that as dietary energy increases, decreasing feed intake is observed, this is because animals will only eat to their energy satisfaction (Nyachoti et al., 2004; Jeffre et al., 2010). The effect of this feed intake adjustment is observed to impact other physiological functions such as growth and synthesis of new tissues (Kil et al., 2013). In order to prevent the shortcomings related to disproportionate energy concentration in diets, modern monogastric production needs to match dietary energy supply with energy requirements for maintenance and productive functions. This thesis work adds to the information pool regarding feed ingredient's energy values, specifically when the examined non-conventional ingredients are formulated into the diet of broiler chickens and pigs.

In Chapter 1, important factors to consider when formulating diets to meet the energy requirement of swine and poultry, especially when it relates to effective energy deposition and utilization were reviewed. Subsequently, the chapter highlighted some of the peculiarities associated with the use of fat, protein, and carbohydrates as energy sources. Most literature revealed that protein generates more heat increment than fat or carbohydrates, and that the lower heat increment of fat causes an extra caloric effect which is linked to an improved utilization of metabolizable energy calories. Furthermore, some lesser-known feed ingredient that could be used as alternative fat, fiber, or protein source were introduced.

In Chapter 2, the objective was to investigate utilization of energy in dry fat (DF) and stabilized rice bran (SRB) by broiler chickens. Two experiments were conducted. Three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing either DF at 50 or 100 g/kg replacement in Experiment (Exp.) 1 or SRB at 100 or 200 g/kg replacement (Exp. 2) of the energy-contributing ingredients in the RD. The experimental diets were fed from d 17 through d 22 post hatching. Results obtained from this study revealed that ether extract of DF could not be obtained, this was because the fatty acids in DF were not in a free form but were part of a calcium salt containing long chain fatty acids, therefore acid hydrolysis was performed. It was also inferred

that excessive inclusion DF could shift the Ca:P balance leading to deleterious effect on growth performance. For this reason and possible palatability tolerance level, an inclusion level of about 5% was recommended for DF in broiler chicken's diet. It was also observed that birds fed on diets with SRB inclusion had similar ileal digestible energy (IDE), metabolizable energy (ME) and nitrogen-corrected ME (MEn) with birds fed RD although a decline was observed in feed efficiency. From the results, we concluded that broiler chickens were able to utilize between 77 to 79% and 68 to 76% of the GE in DF and SRB, respectively. The respective regression-derived IDE, ME, and MEn estimates (kcal/kg DM) for DF were 6,047, 6,051, and 5,922. Respective estimates for SRB were 3,556, 3,437, and 3,193kcal/kg DM.

Chapter 3 included three experiments with the common objective of determining the energy value of hydrolyzed feather meal (HFM) and flash-dried poultry protein (FDPP) in broiler chickens and pigs. The HFM or FDPP were incorporated into a reference diet either at 3 levels (0, 75, or 150 g/kg) in Exp. 1 and 2 or 2 levels (0, 150 g/kg) in Exp. 3 by replacing the energy-yielding ingredients. The results from these experiments showed that HFM has a higher constituent of crude protein than FDPP, but its ash content is approximately 10 percentage points lower than that of FDPP. Energy values derived for HFM in chickens and pigs in this study were observed to be higher than previously reported in literatures (Pesti et al., 1989; Dale, 1992; and NRC, 2012). This suggested that energy concentration in HFM could be said to have been progressively utilized with better efficiency over the last decades and this might be due to improvement in processing techniques and management of poultry by-product. Also, results showed that broiler chickens utilize higher percentage of FDPP than pigs at the ME level. The regression-derived IDE, ME, and MEn concentrations in HFM were 4,509, 4,250, and 3,745 kcal/kg while those in FDPP were 3,221, 4,710, and 4,081 kcal/kg, respectively. In pigs, the DE and ME derived for HFM and FDPP were not different. Evaluated DE and ME concentration in HFM for pigs were 4,783 and 4,405 kcal/kg, respectively while those for FDPP were 4,553 and 4,320 kcal/kg, respectively.

The main objective of Chapter 4 was to determine the IDE, ME, and MEn of BSFLM in broiler chickens and its DE and ME in pigs. Additionally, the chapter reported the DE of BSFLM derived using 3 methods which included total collection (TC), titanium index (Ti), and chromium index (Cr) methods in pigs. In Exp 1 and 2, three diets were prepared: a corn-soybean meal reference diet (RD) and two test diets containing BSFLM at either 100 or 200 g/kg replacement of the energy-contributing ingredients in the RD. Results suggest a diminishing utilization of the test

ingredient at a point beyond 100 g/kg of inclusion, and that broiler chickens and pigs have similar utilization of the test ingredient especially at the ME level. Also, the comparison of the DE in BSFLM using three methods indicated no difference although the recovery of chromium index marker was numerically higher than that of titanium. It was concluded that the choice of marker type does not largely impact digestibility results. Results from this thesis could help broiler chicken and swine farmers expand the scope of their feed ingredient as this thesis provides information that aids with ease of formulating these non-conventional feed ingredients into diets. Further research may be needed to properly investigate the growth performance of chickens and pigs feed on FDPP and BSFLM at various growth phases. This could better promote the credibility of these non-conventional feed ingredients to farmers. Further research that bridges the knowledge gap between the prebiotic effect of BSFLM chitin content and optimal growth performance response in broiler chickens or pigs is also needed to guide against the possible deleterious effect of over inclusion of chitin as a result of its significant presence in BSFLM.

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