METHODOLOGICAL CONSIDERATIONS IN METABOLIZABLE ENERGY ASSAY OF FEEDSTUFFS FOR BROILER CHICKENS

by

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(Under the Direction of OLUYINKA ABIONA OLUKOSI)

ABSTRACT

There has been a gap in the literature regarding the accuracy of the methods used to calculate metabolizable energy (ME) values for feedstuffs. Feedstuff ME values vary from other reported ME values across studies. The differences in ME values of feedstuffs can be influenced by either bird-related, diet-related, and methodology-associated factors. However, the current methodologies do not consider the influence that these factors have on determining the ME values of feedstuffs. Understanding the factors that can influence the ME of feedstuffs can help reduce the variation in feedstuffs ME values. This study aimed to 1) Evaluate the influence of reference diet on ME of soybean meal and canola meal using difference vs. regression assay methods, and 2) Evaluate the influence of adaptation length on ME of corn and barley when supplemented with or without carbohydrase using total collection vs. index assay methods.

INDEX WORDS: Metabolizable energy, reference diet, adaptation length, assay method

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DEDICATION

I dedicate this dissertation to my dear mother, Narasamma Veluri, my father, Kesavaiah veluri, my sister, Harini Veluri. Thank you for being there with me all the time and for your support and unconditional love.

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CHAPTER 1

INTRODUCTION

Feed costs represent 70% of total production costs in broilers. Among the feed costs, energy costs represent the greatest proportion; accordingly, satisfying the energy requirements of broilers is essential as they eat to satisfy their energy requirements. Available energy from feedstuffs in broilers is represented as apparent metabolizable energy (AME), and when corrected for nitrogen retention, it is AMEn. Feed formulations in broilers is based on either AME or AMEn. Determining the most accurate AME and AMEn values for feedstuffs and using these values in feed formulations can reduce the risk of 1) environmental pollution caused by energy, nitrogen, and phosphorus emissions from broilers and 2) feed costs

The available methods to determine the AME and AMEn values may not produce accurate values. Drawbacks related to these methodologies have been discussed in previous reviews on metabolizable energy (Wu et al., 2020). Most of the issues associated with these methodologies are due to assumptions made during AME calculation. One of these assumptions is the additivity of feedstuffs, meaning that independent of other feed ingredients in the diet the AME and AMEn of the feedstuff remains constant. These assumptions are proven to be wrong in some of the previous studies. As the metabolizable energy of feedstuffs in a mixed diet depends on several other factors like bird-related, diet-related and methodology.

Some of the errors associated with these methodologies can be solved by understanding the factors affecting the metabolizable energy of feedstuffs in a mixed diet. In this current research,

we studied the influence of some of the factors affecting AME and AMEn of feedstuffs while determining. The two main objectives of this study are:

Objective 1: To determine the influence of reference diet type and assay method; difference vs. regression on AME and AMEn of soybean meal and canola meal.

Objective 2: To determine the influence of adaptation length and assay method; total collection vs. index method on AME and AMEn of corn and barley when supplemented with or without carbohydrase.

CHAPTER 2

LITERATURE REVIEW

ENERGY

Energy is defined as the capacity to do work and is measured either in calories or joule (Moore 1977; Atwater 1894). The total energy in a feed ingredient is measured as gross energy. Gross energy is defined as energy released as heat when a substance is completely oxidized to carbon dioxide and water. The gross energy of a feed can be determined by its chemical composition, such as the concentration of carbohydrates, proteins, and fats. However, gross energy does not represent all of the energy available to a bird. Digestible energy, metabolizable energy, and net energy are often used to represent available energy from the feed (NRC 1994). Usage of these terms depends on the species of interest. Total digestible nutrients (TDN) or net energy system is used in large ruminants like cattle and sheep. The swine industry is moving towards using net energy systems, but still digestible energy is commonly used. Apparent metabolizable energy is the most widely used term for available energy for poultry feedstuffs.

ENERGY PARTITIONING IN POULTRY

Digestible energy

Digestible energy is defined as gross energy intake minus the gross energy of excreta. Digestible energy is apparent because some of the energy in excreta comes from endogenous losses which

are not feed related. Digestible energy is not commonly used in poultry because of the difficulty in collecting excreta and urine separately (Sales and Janssens 2003). When used in poultry, digestible energy is defined as gross energy in diet subtracted from gross energy in ileal digesta along with endogenous energy losses (Yang et al. 2020). Therefore, digestible energy is referred to as apparent or ileal digestible energy (González-Ortiz, Olukosi, and Bedford 2016; Leslie, Moran, and Bedford 2007).

Metabolizable energy

The most commonly used and preferred estimates for available energy of feedstuffs in the poultry industry is metabolizable energy (Sibbald 1980). Metabolizable energy is defined as gross energy in feed minus gross energy in excreta and urine (NRC 1994).

Apparent metabolizable energy

Some endogenous losses are present in excreta and urine, so it is more appropriate to use apparent metabolizable energy (AME) rather than metabolizable energy. A correction for nitrogen retained in the body is made to convert the metabolizable energy values to a basis of nitrogen equilibrium for comparative purposes (Muztar and Slinger, 1981; Dale and Fuller, 1982; Dale and Fuller, 1984). There are significant differences in growth rates between birds undergoing protein accretion and fat deposition in young and old birds respectively, so correcting for nitrogen would be beneficial. A correction factor of 8.22, which is the energy value of uric acid in kcal/kg, is subtracted from AME to give nitrogen-corrected apparent metabolizable energy (AMEn)(Titus et al., 1959; Dale and Fuller, 1984). The nitrogen correction factor varies with species of interest and stage of production.

Standardized metabolizable energy

Endogenous losses comprise the fecal metabolic energy and endogenous urinary energy. Metabolic fecal energy includes digestive juices, bile, and abraded intestinal mucosa. In contrast, endogenous urinary energy constitutes energy from degraded tissues and products of catabolites (Sibbald 1975). These endogenous losses are specific, depending on feedstuff or diet, or non-specific which depend on dry matter intake and is independent of dietary conditions (Stein et al. 2007; Schulze et al. 1995). Standardized metabolizable energy takes into account non-specific endogenous energy losses (Jansman et al. 2002).

True metabolizable energy

True metabolizable energy (TME) takes into account both specific and non-specific endogenous energy losses. Several studies have proposed methods to determine TME, but these are still questionable. Sibbald et al., (1975) proposed a method for TME where one group of birds are force-fed the test feedstuffs, while another group of birds is starved. Excreta is collected after 24h from both groups of birds. In the original method, endogenous energy losses are determined from the excreta of starved birds (Sibbald 1975). Additional changes were later made by Sibbald et al., (1980) to this method by determining the endogenous losses from the same bird instead of using two groups of birds based on the reasoning that the endogenous energy losses and passage rate of feed will be different from bird to bird. Endogenous energy losses depend on the level of feed intake. If feed intake is high, then endogenous losses will be increased compared to low feed intake (Sibbald 1975). Villamide et al., (1998) determined that 48 h excreta collection is a more ideal period for determining TME. Like AME, a correction for nitrogen is made for TME called nitrogen

corrected TME (TMEn). Lotfi et al., (2020) developed an artificial neural network model using an Excel program to determine TMEn of feedstuffs based on chemical composition.

Net energy

As defined by Sibbald (1982), net energy is true metabolizable energy minus heat increment, which is the ultimate energy available to the bird for maintenance and production. Net energy can be determined by comparative slaughter technique or by indirect calorimetry. In the comparative slaughter technique, energy retained is determined primarily by the difference in carcass composition at the beginning and at the end of the study. Heat production is determined by the difference between energy retention and energy intake (Noblet and Van Milgen 2013). This method is very laborious but can be applied relatively easily in poultry compared to other large animal species (Blaxter 1966; Oluyinka and Adeola 2008). Unlike comparative slaughter technique, indirect calorimetry measures heat production by using respiration chambers to determine oxygen consumption and carbon dioxide production so that that heat production can be measured indirectly. Nehring and Haenlein, (1973) proposed another system to calculate net energy based on digestibility coefficients of crude fiber, crude protein, nitrogen-free extract, and crude fat. But this method is not commonly used and accepted because of inconsistency in digestibility coefficients (Sibbald 1982). Net energy system is the most commonly used in the swine industry (Jean Noblet 2015).

METHODS FOR CALCULATING METABOLIZABLE ENERGY

There are two methods commonly employed to determine the metabolizable energy of diets in poultry, namely total collection and index methods.

Total collection method

Total collection method involves collecting excreta quantitively for a period of 3 or 4 days and feed intake associated with the collection period. The collection period varies with the species of interest. Apparent metabolizable energy is determined using the formula

$$AME = GE_I - \left[GE_O \times \left(\frac{Excreta\ out\ put}{Feed\ intake}\right)\right]$$

AME is apparent metabolizable energy in kcal,

GE_I is gross energy intake in kcal,

GE_O is gross energy output in excreta in kcal, and

Excreta output in kg

Feed in take in kg.

This method is laborious and involves very meticulous data collection on feed intake and excreta output. But when compared to other large animal species total collection method can be employed relatively easily in poultry.

Index method

Index method of determination of metabolizable energy for diets was proposed by Han et al., (1976). An indigestible marker is added to feed and is recovered from the excreta. Concentrations of marker is measured in diet and excreta, so AME can be calculated by using the equation:

AME= $GE_I - \left[GE_O \times \left(\frac{C_I}{C_O}\right)\right]$

Where C_i is the concentration of marker in diet,

C_o is the concentration of marker in excreta,

GE_I is the concentration of gross energy in the diet in kcal/kg and

GE_O is the concentration of gross energy in excreta in kcal/kg.

Some of the other most commonly used markers in the swine and poultry industry are titanium dioxide, ferric oxide, carmine red, indigo carmine, chromic oxide and acid insoluble ash (AIA), an internal marker in the feed (Short et al. 1996; Schurch, Lloyd, and Crampton 1950; Payerle et al. 2015; Saldarriaga, Posada, and Trujillo 2017). Among all the markers, maximum recovery is observed for AIA followed by chromium oxide and titanium dioxide (Kavanagh et al. 2001). Han et al., (1976) reported that the AME of feedstuffs determined by both total collection and index method using chromic oxide gives an accurate estimate of AME of feedstuffs. Few studies show that the total collection method gives a more reliable AME estimate than the index method (Dourado et al. 2010; Tillman and Waldroup 1988). Due to the difficulties in the total collection method, the index method is still the most commonly used method for determining the AME of diets (Sales and Janssens 2003).

METHODS TO DETERMINE INGREDIENT METABOLIZABLE ENERGY

Total collection and index methods mostly give information on AME of diets but sometimes are used to calculate AME of feedstuffs when birds are fed a sole feedstuff. Over the years, several improvements have been made in the methods used to calculate individual feedstuffs' AME.

Direct feeding

Direct feeding is very straight forward. In this case, the test feed ingredient is the sole source of energy fed to the bird, either by tube feeding or ad libitum feeding. In this case, the AME of diet will be the AME of the test feedstuff (Sibbald 1976a).

Difference methods

Standard ingredient substitution

In this method, a standard ingredient of known metabolizable energy is fed to birds along with the test feedstuff. The AME of test feedstuff is determined by the difference in AME of diet and AME of standard feed ingredient. Glucose, sucrose, and corn starch are the most commonly used standard ingredients (Hill et al. 1960; Applegate et al. 2009). The main constraints of this method is that these diets are not nutritionally balanced, so AME determined by this method may not truly represent AME of feedstuffs that can be applied during feed formulations.

Basal substitution

In order to overcome the drawbacks of using nutritionally unbalanced diets while determining AME of feedstuffs, McIntosh et al., (1962) proposed a method where a nutritionally balanced diet called a reference diet is used. Test feedstuffs are added to this reference diet to make the test diets. Index or total collection method are used to determine AME of both test diet and reference diet. Test feedstuff AME is determined by the fractional contribution of reference diet and test feedstuff in the test diet. Although some studies use the fractional contribution of test feedstuff in reference diet, other studies use the fractional contribution of the energy of test feedstuff in the test diet (Nalle et al., 2011; Adeola et al., 2018; Olukosi, 2020).

Feedstuff AME can be determined by using the formula;

$$AME_{Tf} = \frac{AME_{Td} - (AME_{Rd} \times P_{Rd})}{P_{Tf}}$$

Where: AME_{Tf} = AME or AMEn (MJ/kg) of test feedstuff, AME_{Td} = AME or AMEn (MJ/kg) of test diet, AME_{Rd} = AME or AMEn (MJ/kg) of the reference diet, P_{Rd} = Proportional contribution

of the energy of the reference diet in the test diet and P_{Tf} = Proportional contribution of the energy of test feedstuff in the test diet.

Regression method:

A simple modification of basal substitution is multiple level basal substitution. Instead of adding test feedstuff at a single inclusion level, graded levels of test feedstuffs are added into the basal diet (Potter et al. 1960; Villamide et al. 1997). Calculation of AME of test feedstuff is similar to basal substitution method. AME of test feedstuff at each inclusion level is determined and regressed against the level of inclusion in the basal diet and extrapolated to 100% inclusion level.

FACTORS AFFECTING THE METABOLIZABLE ENERGY OF FEEDSTUFFS IN POULTRY

Several changes have been made in the methods used to determine metabolizable energy of feedstuffs in broilers. However, there is still some inconsistency in reported AME and AMEn values of feedstuffs. Several factors can influence the utilization of energy from feedstuffs in a mixed diet across the gastrointestinal tract. These factors are either bird-related, diet-related or methodology related.

Bird related factors

Age

Enzyme activity and richness of microbiota in the gastrointestinal tract determines metabolizable energy of feedstuffs, which further depends on bird's age. The AME of feedstuffs determined for younger birds may not be same for older birds. Metabolizable energy of feedstuffs

increases with age of birds after 10-days of age (O A Olukosi, Cowieson, and Adeola 2007)(Zhou et al. 2009). This in part can be explained by increase in digestive capacity of birds with age (Scott 1996). Endogenous enzyme activity of broilers increases with age (Noy and Sklan 1995; Leslie, Moran, and Bedford 2007). Gonzalez et al., (2000) found that broilers at the 9-days age of gave lower AME of flax seed than when determined at six weeks of age. Younger birds are more vulnerable to ANFs in flax seed compared to older birds. Interestingly, Yang et al., (2020) showed that the AME of corn and wheat based diets was greater when determined at 7 days of age than when determined at 14 days of age in broilers, which is due to severe endogenous energy losses in younger birds. It is possible that AME of diets when determined at less than 10-days of age is depends on feedstuff on interest.

Non-starch polysaccharides that are not digested in the upper gastrointestinal tract will be fermented by bacteria residing in the lower gastrointestinal tract. So, development of cecal microbiota compared to microbiota at other sections of gastrointestinal tract plays a major role in improving feed efficiency (Stanley et al. 2012). These bacteria include *Firmicutes* (Dumonceaux et al. 2006) and *Bacteroides* (Rios-Covian et al. 2016). Colonization of bacterial species in the gut varies with birds' age. The development of bacterial species in the gut starts as early as 24 hours of post-hatch (Wielen et al. 2002; Lev and Briggs 1956) and after 21 days, a stable and diverse microbial population is established (Lu et al. 2003; Richards et al. 2019). The utilization of energy from non-starch polysaccharides is more efficient after 21 days. The lower AME values of feedstuffs when determined before 21 days of age can be explained by the unavailability of NSP fermenting bacteria. Yang et al., (2020) showed that AME of corn and wheat improved after 21 days of age.

Role of genetics

Genetic background of the bird plays a major role in the development and type of microbiota established in earlier stages of growth (Qi et al. 2019). Healthy gut microbiota promotes enhanced absorption of nutrients in the feed by increasing the availability of nutrients from NSPs as explained in earlier paragraph (Apajalahti 2001; Gabriel et al. 2011; Zhao et al. 2013). After hatch, Ross and Hubbard breeds were mostly colonized by *Enterobacteriaceae* while Cobb with *Enterococcaceae* and *Clostridiaceae* (Richards et al. 2019). High weight lines have a more stable *Lactobacillus* community in their gut compared to low weight lines (Zhao et al., 2013). Compared to low weight lines, high weight lines have better nutrient utilization capacity and lower digesta pH in the jejunum and ceca (Dono, Sparks, and Olukosi 2014). Lower digesta pH eliminates pathogenic bacteria consequently promoting the growth of beneficial microbiota in the gut (Ferket 2004). This could be the reason for more stable gut microbial community in high weight lines compared to low weight lines. Layers have more abundant and stable microbiota which is responsible for high metabolic rates compared to broilers (Qi et al. 2019).

Health status

Bacteria, protozoan, worm infection and stress affect nutrient utilization of birds by reducing nutrient intake, absorption, or both. This literature will focus on the impact of stress on AME because stress can predispose birds to infection. Temperature, humidity, and feed composition are key stress factors that can affect nutrient utilization. Broilers are sensitive to high temperatures, and they regulate their body temperature by modulating their feed intake. Higher ambient temperatures reduces bird's feed intake, which in turn reduces metabolic heat from feed

digestion (Austic 1985; Howlider and Rose 1987). Conversely, lower ambient temperatures increase bird's feed intake and increases metabolic heat production (Yahav et al. 1998; Shinder et al. 2002). Bonnet et al., (1997) showed that AME of corn-wheat-soybean meal-based diet decreased with an increase in temperature from 22 to 32ºC. Heat stress also affects the relative abundance of *Bacteroidetes* and *Lactobacillus* in broilers at seven days of age (Shi et al. 2019).

Diet-related factors

Particle size or form of feed:

Particle size of the feed; coarse or finely ground, form of feed; pellet, crumble, or mash also affect AME. At 16 days of age, feeding fine maize particles improved AME compared to coarse feed in broilers (Kilburn and Edwards Jr 2001). Coarse or medium particle size does not affect AMEn of boilers at 21 days of age (Amerah et al. 2007). Feeding coarsely ground feed particles increases starch digestion by increasing the grinding action of gizzard, making them more suspectable to enzymes and microbial digestion (Rogel, Balnave, et al. 1987). Although increasing particle size of feed decreases AME of feed, it improves utilization of phosphorus and calcium, as large particle size lower passage rate (Kilburn and Edwards Jr 2001). The influence of feed form on AME depends on the age of the bird and feedstuff. Feeding pelleted feed to broilers at 21 days of age reduces AMEn and performance of birds compared to feeding a mash fed (Amerah et al. 2007; Jafarnejad et al. 2010). On the other hand, pelleted feeds produce higher AME values compared with crumbles, which in turn have higher AME than mash for roosters at 79 weeks age (Gonzalez et al., 2000).

Abdollahi et al., (2013a) demonstrated that pelleting improves the digestibility of nutrients in maize based diets while Barua et al., (2021) demonstrated that digestibility of starch in sorghum, barley, and wheat is higher in mash feed than pelleted feed. Steps in pelleting such as conditioning temperature also determine feedstuffs' nutrient digestibility (Abdollahi, Ravindran, and Svihus 2013b). The effect of particle size of feed on AME depends on the level of inclusion of coarse or fine fiber particles (Jiménez-Moreno et al. 2010). Pelleting with fine particle size has a negative effect on nutrient digestibility (Kilburn and Edwards Jr 2001). Zang et al., (2009) showed that in birds less than 21 days of age, both feed forms and particle size affect AME, but after 21 days of age, only the feed form has an impact on AME. In conclusion, the influence of the form or particle size of feed depends on feedstuff of interest, age, and uniformity of feed. Feeding fine particle size of feed in mash form at earlier stages of development and coarse particle size of feed in pellet form will be beneficial in nutrient utilization.

Composition of the diet

The poultry diet is formulated to meet the requirements for energy, protein or amino acids, fats, minerals, and vitamins. Cereal grains and oil seed meals are added to meet energy and protein requirements respectively. However, the quantity and quality of carbohydrate and proteins present differs among the cereal grains and oil seed meals, which in will eventually influence AME of diets and feedstuffs.

Carbohydrate:

Carbohydrates are the main sources of energy in feed. Chemical composition of carbohydrates in feedstuffs have an influence on energy, protein or fat utilization in mixed diets.

Increasing the carbohydrate content in the diet, increases pancreatic lipase activity, leading to better utilization of fats in diets (Maiorka et al. 2004). Increase in fiber content, a type of carbohydrate, in the diet decreases the digestibility of the fat and protein portion of the diet (Baer et al. 1997; Adams et al. 2018). This effect also depends on the type of fiber in feed; as to whether the fiber is soluble or insoluble (Lattimer et al., 2010; Osman et al., 2014). Insoluble fiber, due to its high cation exchange capacity, leads to increased lipid or fat excretion (Furda, 1990; Chau et al., 2004; Chau et al., 2005). Another theory is that soluble fiber in the diet can decrease the activity of enzymes that act on fiber (Tsujita et al. 2007). Bacteria in the lower gastrointestinal tract ferment the soluble fiber to short-chain fatty acids, which decreases pH in the gut, converting ammonia into ammonium. This ammonium cannot be absorbed by intestinal mucosa leading to excretion. Consequently, an increase in nitrogen excretion or a decrease in protein utilization is observed with an increase in soluble fiber intake (Osman et al. 2014). There is no consensus in the current literature on how soluble or insoluble fiber affects the digestibility of feed, fat, or protein in mixed diets.

Protein

The increase or decrease in crude protein content of the diet also affects energy availability from the feed (Villamide et al., 1998). Increasing the dietary crude protein content above 23% has a negative effect on available energy (Zeng et al. 2015; Nieto et al. 2002). Broilers cannot store excess nitrogen in the body and needs to be excreted. Nitrogen excretion in broilers is energy expensive and excreted as uric acid. So, significant amount of energy is exploited in removing excess nitrogen from the body, consequently decreasing the available energy from feeds and feedstuffs. This can be of practical importance when determining metabolizable energy of protein

feedstuffs, as the test diets generally have higher protein content. As suggested by Wu et al., (2020) an inclusion level of 15% for protein meals is beneficial when determining AME and AMEn of protein feedstuffs by basal substitution.

Chalova et al., (2016) stated that 50% of nitrogen emissions that affect quality of air, soil and water comes from broiler excreta. Continuous efforts are being made on strategies to reduce nitrogen excretion into the environment. One of the strategies is reducing the crude protein content of diets so as to reduce nitrogen excretion into environment. However, reducing CP causes an imbalance in amino acid composition. Feed intake is reduced when the diet has an imbalanced protein or amino acid composition compared to a diet that is balanced with all the essential amino acids (Swatson et al. 2002). This can be overcome by adding synthetic amino acid to diets and proven to show beneficial effects by increasing the expression of genes related to protein metabolism. (Sigolo et al. 2017; Van Harn, Dijkslag, and Van Krimpen 2019; Duan et al. 2016).

Antinutritional factors

Antinutritional factors (ANF) are substances that, when present in animal feed, either by themselves or their metabolites, reduce the availability of one or more nutrients, or energy. Most feedstuffs have one or more ANF present in them, but this literature review highlights the ANF in most commonly used feedstuffs in broiler chickens.

Non-starch polysaccharides:

Total dietary fiber in feed is composed of non-starch polysaccharides, lignin, and polyphenols. Non-starch polysaccharides (NSPs) are either soluble or insoluble in nature. Both soluble and insoluble NSPs act as antinutritional factors, but their mechanism is different. An

increase in soluble NSPs in feedstuffs leads to reduced AME (Annison 1991). Soluble NSPs increase intestinal viscosity, reduce feed passage rate, feed intake, and availability of nutrients to digestive enzymes, and finally leads to reduced absorption of nutrients in the gastrointestinal tract (Smits and Annison 1996; Edwards, Johnson, and Read 1988). Unlike soluble NSPs, insoluble NSPs improve passage rate and gut motility, which helps in better absorption of starch (Mollah and Annison 1981; Amerah, Ravindran, and Lentle 2009; Hetland, Svihus, and Krogdahl 2003).

Amylase inhibitors

Amylase inhibitors present in wheat and oat hulls act against pancreatic amylase, decreasing starch digestibility. Pepsin in gizzard can deactivate amylase inhibitors in oats. In wheat, pelleting can make these amylase inhibitors more susceptible to pepsin and prevent amylase inhibitor activity (Rogel, Annison, et al. 1987; Abdollahi, Ravindran, and Svihus 2013b).

Tannins

Tannins are polyphenolic compounds present in canola meal and sorghum (Durkee 1971). Tannins negatively affect protein digestion by forming a complex with protein-digesting enzymes (Nelson et al. 1975; Nyachoti, Atkinson, and Leeson 1996; Torres et al. 2013).

Glucosinolates

Glucosinolates are the ANFs present in canola meal and rapeseed meal. Concentration of glucosinolates in feed determines ANF activity and effect on bird performance (Tripathi and Mishra 2007). Glucosinolate content of higher than 7.7µmol/ gm of diet birds will have significant

adverse effects on growth performance and digestibility (Mawson, Heaney, Zdunczyk, and Kozłowska 1994).

Phytic acid

Phosphorus in feedstuffs in present as phytic acid or phytin called as phytate P and non-phytate P. Phosphorus in phytic form cannot be utilized by poultry as it lack enzymes that can act on phytic acid to release P. Phytic acid is considered as potent ANF in poultry as it reduces performance of broilers by impairing the digestibility of energy, protein and other minerals (Woyengo and Nyachoti 2013; Ravindran et al. 2006). Phytic acid binds to amylase, sucrase and maltase consequently reducing their activity and availability for carbohydrate digestion (Liu et al. 2008). Phosphate linkages in phytate binds to starch making starch unavailable for absorption (Thompson 1986). Phytate reduces protein and amino acid digestibility by forming insoluble complex with protein and by increasing the endogenous loss of amino acids (Ravindran et al. 1999; Cowieson, Acamovic, and Bedford 2004; Rajendran and Prakash 1993). Negatively charged phytic acid binds to positively charged minerals and reduced the absorption of minerals (Michael R Bedford 2000). It was also shown that phytic acid by reducing the pepsin activity and by altering the pH of jejunal digesta reduces Ca, Mg, Fe, Na and S absorption and increase (Woyengo et al. 2010; Cowieson, Acamovic, and Bedford 2004).

Feed additives

Feed-additives improve the performance and health of animals by increasing the availability of nutrients and by promoting growth of beneficial bacteria in the gut. The most commonly used feed additives in the poultry industry are exogenous enzymes, prebiotics,

probiotics and organic acids. Chickens lack enzymes that can act on ß-linkages of NSPs in feedstuffs. Therefore, exogenous enzymes such as NSPases act on ß-linkages of soluble NSPs and decrease the digesta viscosity and hence improve nutrient utilization (Bedford 2006). Soluble NSPs in wheat such as arabinoxylans decrease AME by increasing digesta viscosity (Yegani and Korver 2012; Choct and Annison 1992). The addition of xylanase to a wheat-based diet significantly improved AME by decreasing digesta viscosity and increasing the surface area of absorption (Wu et al. 2004). ß-glucans are soluble NSPs in barley. Similar to ANFs such as arabinoxylans, ß-glucans increase digesta viscosity (White et al., 1983). Oats and barley are feedstuffs commonly used in the poultry industry which contain a significant amount of ß-glucans as ANFs (Jiménez-Moreno et al. 2013). The addition of glucanase to a barley, and oats-based diets improve the AME of barley and oats (Fuente et al. 1998; MacLeod et al. 2008). The response of broilers to glucanase depends on the age of birds. Older birds can generally counterbalance the ANF activity of ß-glucans better than younger birds (Rotter et al. 1990). Supplementing a combination of xylanase and glucanase in a wheat-barley-based diet also improved AME (Mathlouthi et al., 2003).

Proteases are another class of enzymes used to improve the utilization of protein or amino acids in feedstuffs. Adding protease and xylanase improved AME and apparent ileal digestibility of nitrogen and amino acids in protein feedstuffs (Romero et al. 2013; Cowieson and Ravindran 2008). The combination of enzymes used and broilers' age also affect the AME of diets (Kocher et al. 2003; O A Olukosi, Cowieson, and Adeola 2007).

Phytase addition to diet increases the availability of phosphorus for absorption by releasing P from phytate (Singh et al. 2003). Addition of phytase to broiler diets improves energy, protein and mineral utilization (O A Olukosi, Cowieson, and Adeola 2010; Macleod 1997; Rutherfurd et

al. 2012; Ravindran et al. 2006). Decrease in activity of amylase, sucrase and maltase by phytate in the diet is improved by adding phytase to the diet which consequently improves energy utilization and AME (Oluyinka A Olukosi, Cowieson, and Adeola 2008)(Liu et al. 2008). Phytase along with xylanase, protease and amylase has proven to be more beneficial in terms of growth performance, and nutrient utilization (O A Olukosi, Cowieson, and Adeola 2007; Cowieson and Adeola 2005; Józefiak et al. 2010). Olukosi et al., (2008) showed that phytase and carbohydrase supplementation to energy and P deficit diets improved net energy for production and energy retained as protein and is comparable to diet with sufficient energy and P. However, improvement in AME and AMEn by phytase or a combination of phytase with carbohydrase is not consistent in some feedstuffs and across studies (Ravindran, Cowieson, and Selle 2008; Woyengo and Nyachoti 2011; Rutherfurd et al. 2012; Ravindran et al. 1999). So, an improvement in AME with phytase supplementation seems to be feedstuff specific. Chemical composition of the diet specifically type of fiber and inherent endogenous phytase activity in feedstuffs plays major role.

Enzyme supplementation is more beneficial when included in the mash diet compared to pellet (Bustany 1996). Pelleting disrupts the cell wall to some extent, so a significant effect of enzyme action may not be seen in a pelleted diet than that seen in mash diets (Pettersson, Graham, and Åman 1991; Ghobadi and Karimi 2012).

Methodology aspects

Composition of reference diet

As described earlier, a reference diet is used for determining the AME of test feedstuffs. Reference diet is formulated using main energy ingredients like corn, wheat, barley, and protein

feedstuffs such as soybean meal, canola meal, meat, and bone meal. Difference and regression methods are based on the assumption of additivity of feedstuffs. The metabolizable energy of test feedstuffs remains constant and is independent of the other feedstuffs in test diets. This assumption is often tested, and the AME of feedstuffs may not be additive and depend on the composition of reference diet. AME and AMEn of corn distillers dried grains with soluble (CDDGS) was greater when a semi-purified based diet is used as reference diet compared to a corn-SBM-based reference diet (Adeola et al., 2009). A similar kind of research was conducted by Olukosi et al., (2010) to determine the AME of meat and bone meal (MBM) using a corn-SBM based reference diet. Graded levels of wheat were added to the reference diet, along with MBM. Adding wheat to the reference diet reduced the AME of MBM by 5%. This reduction in AME of MBM after adding wheat to corn-SBM reference diet might be due to the antinutritive activity of arabinoxylans in wheat. Arabinoxylans increase digesta viscosity, absorption of nutrients, and may thus reduce the AME of MBM. These two studies conducted using different methods raise an essential concern for considering the effect of the composition of reference diet used when determining AME of feedstuffs by basal substitution method.

Adaptation period

A specific period of time is required for birds to adopt to the physicochemical properties of the relatively new test diet so that energy utilization from the feedstuff is optimum. The period taken by birds to produce optimum AME for test feedstuff is defined as adaptation length. The adaptation period used in the poultry industry is usually 12, 7, and 4 days, although this varies depending on the species and feedstuff of interest (Oluyinka A Olukosi, Adedokun, and Agboola 2017). Sibbald and Slinger, (1963) were the first to study the effect of adaptation length, referred

to acclimatization to poultry feed. They suggested that younger birds require longer adaptation periods than mature birds. A minimum of 4 days of adaptation length is sufficient for corn, wheat, maize, and barley in broilers, as feeding for 4, 7, 12, and 14 days produced similar AME values (Dunaway and Adedokun 2019; Oluyinka A Olukosi, Adedokun, and Agboola 2017; O A Olukosi 2020). However, the AME of maize and barley in turkeys vary with the adaptation length used. Hence, an optimum adaptation length for these feedstuffs needs to be specified in turkeys (Olukosi et al., 2017). The AME of soybean meal was greater when determined at four days of adaptation length than when determined at seven days of adaptation length (O A Olukosi 2020). In general, the AME of diets increases with age up to 14 days of age and remains constant after that (Batal and Parsons, 2002). Differences in reported AME values for birds at different growth stages are due to differences in the digestive tract development. In older birds, the digestive tract is more developed, with a more diverse microbiome count in the intestinal caeca compared to younger birds (Barnes et al. 1972). A well-developed gut increases the digestibility of diets by increasing utilization and absorption of nutrients (Apajalahti J. et al., 2016). Therefore, there is a need to optimize the adaptation length needed for feedstuffs to determine the most accurate AME values. Studying the effect of adaptation length on AME of feedstuffs, when supplemented with enzymes, will give most relevant AME values for feedstuffs that can be used in feed formulations.

Difference vs Regression methods

Difference and regression methods are the most commonly used methods to determine the AME of feedstuffs. But the reliability and repeatability of AME values produced by difference or regression methods are still questionable. In broilers, the effect of the method depends on the type of cereal grains and protein feedstuffs. Regression and difference methods produced similar AME

values for sorghum, wheat, and maize (Bolarinwa and Adeola 2016; O A Olukosi 2020). Regression method produced greater AME values for corn and barley than determined by using difference method (Lopez and Leeson 2008; O A Olukosi 2020). The AME of soybean meal increased with an increase in substitution level when determined by the difference method. However, the regression method also produced similar AME values for combinations of different substitution levels, (O A Olukosi 2020; Lopez and Leeson 2008). When determining the AME of fat sources, difference and regression methods produced similar AME estimates (Su et al. 2015). With the available research and data on regression and difference methods, it's hard to define which method is the most reliable. The regression method produced lower standard error of mean, (SEM) values, compared to the difference method ((Tillman and Waldroup, 1988; Lopez and Leeson, 2008; Olukosi, 2020). Based on SEM values therefore, the regression method appears to be more reliable than the difference method. Careful selection of substitution levels should be made while determining the AME of various feedstuffs by regression method. Higher inclusion levels of up to 50% can be used for cereal-based diets. An imbalance in energy or protein metabolism occurs at higher inclusion levels of protein feedstuffs in reference diets giving erroneous AME values. Selection of substitution level of protein feedstuffs into reference diets should be based on type of ANFs and crude protein content of the feedstuffs.

CONCLUSION

Providing a diet that meets energy requirements of birds is one of the most important consideration in poultry industry. Available energy from feedstuffs is represented as apparent metabolizable energy in broilers. Determining the most accurate AME values for feedstuffs and using these values in feed formulations is important in terms of reducing energy excretion into the

environment and for a cost-effective feed formulation. As AME values produced by feeding the feedstuff alone will not represent true to its best researchers are left with mixing of test feedstuff in a mixed diet. However, assumptions made during AME determination of feedstuffs in a mixed diet are proven to be wrong in the current literature. A method that can determine the accurate AME of feedstuffs in a mixed diet is still an unanswered question, as there are several factors that can affect AME of feedstuff in a mixed diet. A thorough knowledge on how these factors that can affect AME will erase some errors in AME values produced. Among the factors that affect AME are adaptation length, composition of the reference diet and method used are factors that have the potential to cause a variation in AME values across studies. The current research will focus on these factors in commonly used protein feedstuffs like soybean meal and canola meal and energy yielding ingredients like corn and barley.

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CHAPTER 3

METABOLIZABLE ENERGY OF SOYBEAN MEAL AND CANOLA MEAL AS INFLUENCED BY THE REFERENCE DIET USED AND METHOD[1]

[1] Shravani Veluri, Oluyinka A Olukosi, *Animals* 2020, *10*(11), 2132

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ABSTRACT

A 21-day experiment was conducted to study the effect of reference diet type and calculation method on determined apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) of soybean meal (SBM) and canola meal (CM). Two-hundred forty male broilers were allocated to 10 treatments with eight replicates per treatment and three birds per replicate. Treatments included corn-SBM and corn-CM reference diets. To each reference diet, 300 or 450 g/kg of SBM or CM were added to make a total of eight test diets. The diets were fed to birds for 7 days from day 14 and excreta were collected on days 20 and 21. Difference and regression methods were used to calculate the AME and AMEn of SBM in corn-SBM or corn-CM reference diets and the same was done for calculation of AME and AMEn of CM in corn-SBM or corn-CM reference diets. For AME calculated by the difference method, data were analyzed as 2×2×2 factorial arrangement. There was no significant interaction for AME values. AME of SBM and CM substituted at 300 g/kg in corn-CM reference diet gave greater AME values compared to inclusion in the corn-SBM reference diet ($P < 0.01$) and at 450 g/kg inclusion level ($P < 0.01$). There was significant ($P < 0.05$) 3-way interaction (reference diet, inclusion level, and protein feedstuffs) for AMEn. The AMEn of SBM increased with an increase in inclusion level in the corn-CM reference diet but not in the corn-SBM reference diet. In contrast, AMEn of CM decreased ($P < 0.05$) with increased inclusion level of CM in the corn-SBM reference diet not in corn-CM reference diet. Data of AME and AMEn calculated using the regression method was analyzed as 2×2 factorial arrangement. AME and AMEn of the test feedstuffs were greater when ($P < 0.01$) corn-CM reference diet compared with corn-SBM reference diet was used. For

comparison of the calculation using difference or regression methods, the data were analyzed as 2×2×2 (calculation method, protein feedstuff and reference diet). There was no significant 3-way interaction, but feedstuff × method interaction was significant for AME and explained by AME of SBM not affected by calculation method, whereas AME of CM was generally lower when determined using the regression method ($P < 0.05$). In conclusion, the reference diet used in AME and AMEn assays for protein feedstuffs should be taken into consideration especially in comparing studies. This is because the difference and the regression methods did not give similar AMEn values for protein feedstuffs assayed in the current experiment.

INTRODUCTION

Feed represents 70 percent of the total production cost in broiler production, of which the energy represents the greatest proportion. Available energy from feedstuffs in broilers is determined using apparent metabolizable energy (AME) and as nitrogen corrected apparent metabolizable energy (AMEn). Accurate determination of AME value of feedstuffs using these values in feed formulations is considered as one of the important criteria required to improve feed efficiency and reduce feed costs (Oluyinka A Olukosi, Adedokun, and Agboola 2017; Adeola et al. 2018). However, there is wide variability in the reported AME and AMEn values for most feedstuffs making interpretation of the values and usage in feed formulation very challenging. Variation in reported AME values are often due to genetic factors, growing conditions, age, dietary factors and methods used while determining these AME values (Wu et al., 2020; Sibbald, Summers, and Slinger 1960; Mtei et al. 2019). Over the years several changes have been made in the experimental design and assumptions made during AME calculation (Hill et al. 1960; Sibbald

1976a; 1976b; Pesti and Edwards Jr 1983; Adeola and Ileleji 2009; O A Olukosi, Cowieson, and Adeola 2010). In addition, there are various methods used to determine the AME and AMEn of feedstuffs, among all the methods basal diet substitution and multiple linear regression method

In basal diet substitution or multiple linear regression methods, the key is to formulate a reference diet which is a complete and nutritionally balanced diet. Test feedstuffs are included in the basal diets at different levels to make test diets so that AME can be determined either by difference or regression method (Hill and Anderson 1958; O A Olukosi and Adeola 2009). Recent studies show that the AME of test feedstuffs depends on the composition of the reference diet used. As the composition of the reference diet changes, so does the AME of test feedstuff. For example, in a study reported by Olukosi et al., (2010) adding graded levels of wheat to the reference diet reduced the AME of meat and bone meal by 5%.

Soybean meal (SBM) and canola meal (CM) are the two most commonly-used plant protein feedstuffs for broilers. Because these protein feedstuffs constitute almost 30 percent of the broiler diets it is essential to characterize the various factors that might affect their AME and AMEn values especially regarding the calculation method or basal diets used in the assay. This is especially so because incorporation of additional SBM or CM, for example, dramatically changes the protein content of the test diets, and this may be dependent on type of feedstuffs used in the basal diet. The objective of the current study was to determine the effect of the reference diet type (CM or SBM based), assay method and inclusion levels of the test feedstuff in the assay diet on the AME and AMEn of SBM and CM.

MATERIALS AND METHODS

Birds and diets

This study was approved by the Institutional Animal Care and Use Committee at the University of Georgia (AUP: A2018 08-026-Y1-A0), Athens, GA. A total of 240 male broilers at zero-day old were used for the study. Birds received a standard pre-experimental diet based on wheat-SBM from day 0 to 14. On day 14, birds were allocated to 10 treatments with 8 replicates per treatment and 3 birds per replicate. The 10 treatments comprised corn-SBM and corn-CM reference diets (Table 3.1), and for each reference diet, four additional test diets were made consisting of two substitution levels (300 or 450 g/kg) of each of SBM or CM (Table 3.2). Titanium dioxide (TiO2) was added as an indigestible marker into each diet to determine nutrient utilization by the index method. The experimental diets were fed from days 14 to 21. Birds were given *ad-libitum* access to feed and water. Body weight and feed intake data were collected on days 14 and 21. Excreta were collected on days 20-21.

Chemical analysis

Diets and excreta samples were ground through a 0.5-mm screen and analyzed for dry matter, gross energy, nitrogen, and TiO2 concentration. For dry matter determination, samples were dried at 105ºC in a drying oven for 24 h (AOAC Method 934.01; AOAC, 1990). Gross energy was determined using an isoperibol bomb calorimeter (Model 6200, Parr Instruments, Moline, IL) using benzoic acid as a calibration standard. Total nitrogen content was determined by the

combustion method (Method 968.06; AOAC, 1990). TiO2 concentration was measured using the method proposed by Short et al. (1996). Crude fat content of reference diets and test feedstuffs, SBM and CM was determined gravimetrically using extraction by petroleum ether in an Ankom^{TM} XT 15 extraction system.

CALCULATIONS

The metabolizable energy of diets was measured using the index method. Apparent metabolizable energy (AME) was calculated subsequently by using the formula:

$AME = (GE_I\text{-}G_O\ [C_I/C_O\ ])$

GE_I = Gross energy of the diet

GE_O = Gross energy of the excreta

C_I = Concentration of titanium in the diet

C_O = Concentration of titanium in the excreta

Nitrogen-corrected apparent metabolizable energy (AMEn) was calculated by using 8.22 kcal/g as a correction factor.

$AMEn = AME\text{-}[(8.22 \times(N_I\text{-}N_O))/FI]$

N_I = Nitrogen concentration in the diet

N_O = Nitrogen concentration in the excreta

FI = Feed intake

The AME and AMEn of test feedstuffs (SBM and CM), using either of the reference diet types (corn-SBM or corn-CM) were calculated using both the difference and the regression methods. For the difference method, the AME or AMEn of test feedstuffs were calculated after correcting

the contribution of test feedstuff in the test diet for the fractional contribution of energy by the test feedstuffs into the test diets.

AME or AMEn of test feedstuffs were calculated using the formula:

$AME_{TF} = AME_{TD}-(AME_{RD}\times P_{RD}))/P_{TF}$

AME_{TF} = AME of test feedstuff

AME_{TD} = AME of test diet

AME_{RD} = AME of the reference diet

P_{RD} = Proportional contribution of the energy of the reference diet in the test diet

P_{TF} = Proportional contribution of the energy of test feedstuff in the test diet

For calculation of AME and AMEn of SBM and CM by the regression method, SBM- or CM-associated AME or AMEn intake in MJ were regressed against SBM or CM intake in kilograms. The AME or AMEn values of the test feedstuffs were the slope of the regression equations. Consequently, there were eight AME or AMEn values for each feedstuff corresponding to the number of replicates per treatment.

STATISTICAL ANALYSIS

All of the statistical analyses were conducted using JMP (JMP pro version 15.) The data for AME and AMEn of test feedstuffs calculated by the difference method were analyzed as a $2 \times 2 \times 2$ factorial to establish the effect of reference diet type (corn-SBM or corn-CM), protein feedstuff (SBM or CM) and test feedstuff substitution levels (300 or 450 g/kg). The data for AME and AMEn of test feedstuffs calculated by the regression method were analyzed as a 2×2 factorial to show the effect of reference diet type (corn-SBM or corn-CM) and protein feedstuff (SBM and CM). The AME and AMEn data calculated using regression and difference methods were

compared using a 2 × 2 × 2 factorial arrangement for delineation of the effect of reference diet (corn-SBM or corn-CM), protein feedstuff (SBM or CM), and method (Difference or Regression). The main effect means were discussed when there are no significant interactions. Simple effects are discussed when two- or three-way interactions, as appropriate, are significant. Significantly different means ($P \leq 0.05$) were separated using Turkey's HSD.

RESULTS

Crude fat analysis of reference diets, SBM and CM

The analyzed crude fat content of corn-SBM and corn-CM reference diets in dry matter basis is 4.38% and 5.63% and crude fat content for SBM and CM feedstuffs used for this study are 1.8% and 2.6%, respectively.

AME and AMEn of protein feedstuffs determined using the difference method

There was no significant two- or three-way interaction for AME (Table 3.3). The AME of test feedstuffs, when determined using the corn-CM reference diet, was greater ($P < 0.01$) than when determined using the corn-SBM reference diet (Figure 3.1). In addition, AME of CM was greater than AME of SBM, and the AME was greater ($P < 0.01$) when test feedstuffs were substituted at 300 g/kg compared to substitution at 450 g/kg (Figure 3.2). There was a significant ($P < 0.05$) reference diet × feedstuff × substitution level interaction for AMEn calculated by the difference method (Figure 3.3). The AMEn of SBM was not influenced by an increasing inclusion level of SBM in the corn-SBM reference diet whereas AMEn of SBM increased ($P < 0.05$) as the inclusion level of SBM increased in corn-CM reference diet. On the other hand, the AMEn of CM decreased

($P < 0.05$) with increased inclusion level of CM in the corn-SBM reference diet, whereas there was no effect of CM inclusion level when AMEn was determined in the corn-CM reference diet.

AME and AMEn of protein feedstuffs determined using the regression method

There was no significant reference diet × feedstuff interaction for AME or AMEn determined by the regression method (Table 3.4). The AME and AMEn of test feedstuffs, when determined using the corn-CM reference diet, was greater ($P < 0.01$) than when determined using the corn-SBM reference diet, and AME of SBM was greater ($P = 0.05$) than CM. There was no significant effect of protein feedstuff on AMEn.

Effect of the method on AME and AMEn of protein feedstuffs

There was no significant three-way interaction between factors for AME and AMEn, but there was significant protein feedstuff × method interaction ($P < 0.01$) on AME (Table 3.5). There was no significant difference between the AME of SBM calculated by the difference and the regression methods, but the AME of CM calculated by the difference method was greater ($P < 0.01$) than the regression method. The AME and AMEn of protein feedstuffs, when determined using the corn-CM reference diet was greater ($P < 0.01$) than when determined using the corn-SBM reference diet. Overall, the AME and AMEn of SBM were not significantly different.

DISCUSSION

Metabolizable energy is the most commonly used expression for evaluating energy availability from feed and feedstuffs for poultry. In determining the AME of feedstuffs, a nutritionally balanced diet is used as reference diet into which test feedstuffs are incorporated at

different levels and AME is determined either by difference or regression method, among other indirect methodologies. Two important questions are these: What if the composition of the reference diet changes, does it still give the similar AME and AMEn values for the test feedstuffs? Will the changes be influenced by the mathematical treatment of the assay data? The objective of the current experiment was to test the effect of composition of reference diet on determined AME and AMEn of SBM and CM. In order to achieve this, SBM or CM were incorporated at 300 or 450 g/kg into either of two reference diets (corn-SBM and corn-CM reference diets). This allowed AME and AMEn of both SBM and CM to be determined in both corn-SBM as well as corn-CM reference diets. The major differences between the two reference diets were in their primary protein feedstuff (SBM in corn-SBM reference diet and CM in corn-CM reference diet) and marginal difference in proportion of energy-yielding feedstuffs (953 and 946 g/kg in corn-SBM and corn-CM reference diets, respectively). The two reference diets were formulated both to be similar in energy and nutrient contents and to meet nutritional requirement for Cobb 500 broiler chickens.

The AME for SBM and CM was greater when included in corn-CM reference diet compared to inclusion in corn-SBM reference diet. The different values of metabolizable energy observed for the same feedstuff when included in two reference diets was likely due to interaction with test feedstuff and its metabolizable energy (O A Olukosi, Cowieson, and Adeola 2010) (Oluyinka A Olukosi and Adeola 2010). When test feedstuffs, SBM and CM, were added into the reference diets used in the current experiment, the CP content of test diets with corn-SBM as reference diet was greater than CP content of the diets with corn-CM as reference diets. Adding the test feedstuffs (CM or SBM) at the same inclusion levels into the two reference diets resulted in lower dietary protein level in corn-CM reference diet compared to corn-SBM reference diet.

The dramatically higher dietary CP content observed in test diets with corn-SBM reference diets compared to corn-CM reference diets has implication on energy utilization. Nieto et al., (2002) suggested that CP content of diets up to 23% can be well utilized by broilers without having negative effects on the metabolizable energy, but higher CP content leads to excretion of the excess N with significant energy expenditure. Clearly, energy excretion will be higher for diets with higher CP content than usual 20-23% CP, which will translate to lower energy availability and consequently lower AME. In agreement with this, nitrogen and energy excretion from diets with corn-SBM as reference diet were greater when compared to diets with corn-CM as reference diet in the current study. Therefore, this partly explained why the AME of test feedstuffs included in corn-SBM reference diet was less than AME of test feedstuffs with corn-CM as reference diet.

With particular reference to the difference method, the AME of SBM and CM decreased with increase in their inclusion levels in assay diets. This is expected because in test feedstuffs with high CP level, variation in inclusion level of the test feedstuff can dramatically alter the dietary protein level with concomitant influence on dietary AME (May and Bell 1971; Olson et al. 1961; Lopez and Leeson 2008). Diets with SBM or CM substituted at 300 g/kg into the reference diet had lower CP content than the diets with substituted SBM or CM at 450 g/kg. This increase in dietary CP, as mentioned earlier, resulted in higher nitrogen and energy excretion ultimately leading to lower AME of the test feedstuff. A similar effect of reduction in AME with increase in inclusion level of SBM from 20 to 30 % was reported by Lopez., et al, (2008) where AME of SBM included at 20% was greater AME compared to 30% inclusion in the reference diets. This decrease in AME with an increase in inclusion level for CM was also observed in some other studies when CM inclusion increased from 10 to 20 % (Gopinger et al. 2014; Zhong and Adeola 2019). In the current study, it was possible to study the effect of increasing the test feedstuffs in

both reference diet types. The decrease in AME with increase in inclusion level of high-protein feedstuffs was independent of reference diet used or test feedstuff being evaluated.

Another possible factor that may confer differences on AME of feedstuffs determined in CM or SBM reference diets is the anti-nutritional factors (ANF) in the feedstuffs and their interaction with test feedstuffs. For example, it is anticipated that high fiber and glucosinolates in CM, compared with SBM, may influence feed intake (Toghyani et al. 2014; Mawson, Heaney, Zdunczyk, and Kozlowska 1994; Khajali and Slominski 2012) and consequently reduce AME of test feedstuffs. In the current study, there was no effect of reference diet type on feed intake. Therefore, it appears that this factor was not influential in the current experiment. In addition, Olukosi et al., (2017) indicated that there was no correlation between AME and glucosinolate content of meals of modern breeds of canola.

The other possible reason for the observed greater metabolizable energy value when corn-CM is used as reference diet is due to ether extract content of the test feedstuff. The CM used for this study had gross energy of 4,707 kcal/kg, this greater amount of gross energy in CM than usual can be linked to higher ether extract content (Oluyinka A Olukosi et al. 2017). The ether extract in the test CM, and in feedstuffs generally, can improve AME and AMEn of a diet by reducing the passage rate of the digesta which can lead to increased energy availability (Urriola and Stein 2010; Zhong and Adeola 2019; Woyengo, Kiarie, and Nyachoti 2010). This also partly explains the observed greater AME and AMEn values of SBM and CM when determined in the corn-CM reference diet compared to the corn-SBM reference diet.

The AMEn of SBM and CM was influenced by both the reference diet used and level of inclusion of test feedstuffs into the reference diets. Applying nitrogen correction to feedstuffs AME gives an estimate of comparable available energy from feedstuffs with different CP content

(Leeson et al. 1977). So, it was expected that even if the test diets differ in their CP content and give different AME values for same feedstuffs at different inclusion levels, correcting for N may take care of the energy retained as protein. The observation from the current study indicate that this may not be the case when reference diet has the same protein feedstuff and test protein feedstuff (i.e., SBM being added to e.g. corn-SBM, rather than corn-CM reference diet).

For the AMEn of SBM, the inclusion level of the test feedstuff had no effect on assayed AMEn when included in corn-SBM reference diet. On the other hand, when included in corn-CM reference, assayed AMEn of SBM increased with increase in inclusion level. In contrast, assayed AMEn of CM was not influenced by inclusion level in corn-CM reference diet, whereas AMEn of CM decreased with an increase in inclusion level of CM in corn-SBM reference diet. The effect of inclusion level on nitrogen retention has been reportedly due to the combined effect of quality of test protein feedstuff, age of birds, and inclusion levels used (Lopez and Leeson 2008; Leeson et al. 1977). Unlike AME, AMEn of test feedstuffs specifically for protein feedstuffs is more sensitive to quality of the reference diet used and level of inclusion of the test protein feedstuff into these reference diets.

The mathematical treatment of AME and AMEn data was also investigated in the current study. Difference and regression methods are two commonly used methods for calculation of metabolizable energy of feedstuffs but only few studies actually did a comparison of AME and AMEn of feedstuffs when determined by both methods (Lopez and Leeson, 2008). Metabolizable energy as determined by difference method assumes that the test feedstuff and the feedstuffs in reference diet do not interact with each other and will be additive. So, AME and AMEn values for test feedstuffs irrespective of the composition of the reference diet used. It is recognized that there are many still undefined issues AME assays (Wu et al., 2020; Mtei et al. 2019). The focus in the

current study was on comparing AME and AMEn of SBM and CM when determined both by difference and regression methods. AME of CM was greater when determined using the difference method compared to the regression method. In contrast AMEn of SBM and CM was greater when determined using the regression method compared to difference method. That observation may be related to the effect of nitrogen correction in the test diets with excess nitrogen content. The take-away from the results of the current study was that regression method gave a more consistent value for AME and AMEn compared with the difference method. This can be seen from the comparatively lower standard error values for AME and AMEn determined by the regression method compared with the difference method. A similar observation was made earlier (Tillman and Waldroup, 1988) but the current experiment was particularly relevant in these regards because of cross-evaluation of AME and AMEn of the protein feedstuffs in two different reference diets.

The conclusion from the observations in the current experiment was that AME of SBM depended on the reference diet and inclusion level used in the assay, whereas AMEn depended on the method used in calculation but not on reference diet and inclusion level. On the other hand, both AME and AMEn of CM were influenced by the reference diet, inclusion level, and calculation method. Consequently, the reference diet type is an important consideration when making comparison across studies.

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Table 3.1: Ingredients and composition of the basal diets

Ingredients g/kg	Corn-SBM	Corn-CM
Corn	603.4	471.4
Soybean meal	320	0
Canola meal	0	420
Soybean oil	30	55
DCP	16	19.5
Limestone	9	8.5
Titanium dioxide	5	5
Vitamin premix[1]	5	5
Trace minerals premix [2]	5	5
Methionine	1.5	1
Lysine	1.5	4.5
Threonine	0.5	2.0
Salt NaCl	3.1	3.1
Total	1000	1000
Calculated nutrient content		
Protein, g/kg	198.9	195.0
ME, kcal/kg	3060	2936
Ca, g/kg	8.6	8.7
P, g/kg	6.5	5.2
Ca:P	1.34	1.24
Non-phytate P, g/kg	4.2	4.2

[1] Vitamin A, 5484 IU; vitamin D3, 2643 ICU; vitamin E, 11 IU; menadione sodium bisulfate, 4.38 mg; riboflavin, 5.49 mg, d-panthothenic acid, 11 mg; niancin, 44.1 mg, choline chloride,

771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg

[2] Iodine, 1.11 mg; manganese, 66.06 mg; copper, 4.44 mg; iron, 44.1 mg; zinc, 44.1 mg; selenium, 300 µg.

Table 3.2: Experimental diets

Diet No	Test feedstuff	Basal diet used	Test feedstuff inclusion (g/kg) in the basal diet	Analyzed crude protein of the diets, g/kg DM
1		Corn-SBM	0	228.9
2	Soybean meal	Corn-SBM	300	315.1
3	Soybean meal	Corn-SBM	450	353.2
4	Canola meal	Corn-SBM	300	288.0
5	Canola meal	Corn-SBM	450	307.2
6		Corn-CM	0	231.5
7	Soybean meal	Corn- CM	300	305.7
8	Soybean meal	Corn- CM	450	353.6
9	Canola meal	Corn- CM	300	287.9
10	Canola meal	Corn- CM	450	310.2

SBM – soybean meal

CM – canola meal

Table 3.3: Influence of reference diet type and substitution levels on AME and AMEn of SBM or CM determined by the difference method

Reference diet	Feedstuff	Substitution level, g/kg	AME, kcal/kg	AMEn, kcal/kg
The main effect means for reference diet type				
Corn-SBM			2842	2431
Corn-CM			2909	2443
Pooled SEM			14	17
Main effect means for the feedstuff type				
	Soybean meal		2851	2385
	Canola meal		2899	2486
Pooled SEM			14	17
Main effect means for substitution level				
		300	2913	2421
		450	2837	2450
Pooled SEM			14	17
Simple effect means				
Corn-SBM	Soybean meal	300	2830	2366^{cd}
		450	2794	2397^{cd}
	Canola meal	300	2901	2593^{a}
		450	2837	2366^{cd}
Corn-CM	Soybean meal	300	2942	2302^{d}
		450	2837	2478^{abc}
	Canola meal	300	2976	2426^{bcd}
		450	2880	2560^{ab}
Pooled SEM			26	33
P-values for the main effect and interactions				
Reference diet type (RD)			<0.001	0.614
Feedstuff			0.013	< 0.001
Substitution level (Level)			< 0.001	0.233
RD × Feedstuff			0.606	0.922
Feedstuff × Level			0.802	0.002
RD × Level			0.176	< 0.001
RD × Feedstuff × Level			0.590	0.024

n = 8 for the simple effects and n = 32 for the main effects; SEM – standard error of the means;

a-d Means in a column, and within a group, but with no common superscripts are significantly different.

Table 3.4: Influence of reference diet type on AME and AMEn of soybean meal or canola meal determined by the Regression method

Reference diet	Feedstuff	AME, kcal/kg	AMEn, kcal/kg
Main effect means for reference diet type			
Corn-SBM		2823	2,558
Corn-CM		2882	2,644
Pooled SEM		7	7
Main effect means for the feedstuff type			
	Soybean meal	2878	2,594
	Canola meal	2825	2,608
Pooled SEM		7	7
Simple effect means			
Corn-SBM	Soybean meal	2854	2,556
	Canola meal	2789	2,560
Corn-CM	Soybean meal	2899	2,633
	Canola meal	2863	2,656
Pooled SEM		10	10
P-values for the main effect and interactions			
Reference diet type (RD)		< 0.001	<0.001
Feedstuff		<0.001	0.190
RD × Feedstuff		0.081	0.331

n = 8 for the simple effects and n = 16 for the main effects; SEM – standard error of the means;

Table 3.5: Influence of Method on AME and AMEn of soybean meal or canola meal

Reference diet	Feedstuff	Method	AME, kcal/kg	AMEn, kcal/kg
Main effect means for reference diet type				
Corn-SBM			2837	2474
Corn-CM			2890	2567
Pooled SEM			10	10
Main effect means for feedstuff				
	Soybean meal		2858	2512
	Canola meal		2868	2526
Pooled SEM			10	10
Main effect means for feedstuff				
		Difference	2875	2435
		Regression	2851	2603
Pooled SEM			10	10
P-values for the main effect and interactions				
Corn-SBM	Soybean meal	Difference	2813	2380
	Canola meal		2890	2390
	Soybean meal	Regression	2854	2557
	Canola meal		2789	2560
Corn-CM	Soybean meal	Difference	2870	2478
	Canola meal		2928	2493
	Soybean meal	Regression	2899	2634
	Canola meal		2863	2658
Pooled SEM			17	19
P-values for the main effect and interactions				
Reference diet type (RD)			<0.001	<0.001
Feedstuff			0.517	0.370
Method			0.066	<0.001
RD × Feedstuff			0.797	0.656
Feedstuff × Method			< 0.001	0.952
RD× method			0.634	0.601

RD × Feedstuff × Method	0.312	0.783

n = 8 for the simple effects and n = 32 for the main effects; SEM – standard error of the means;

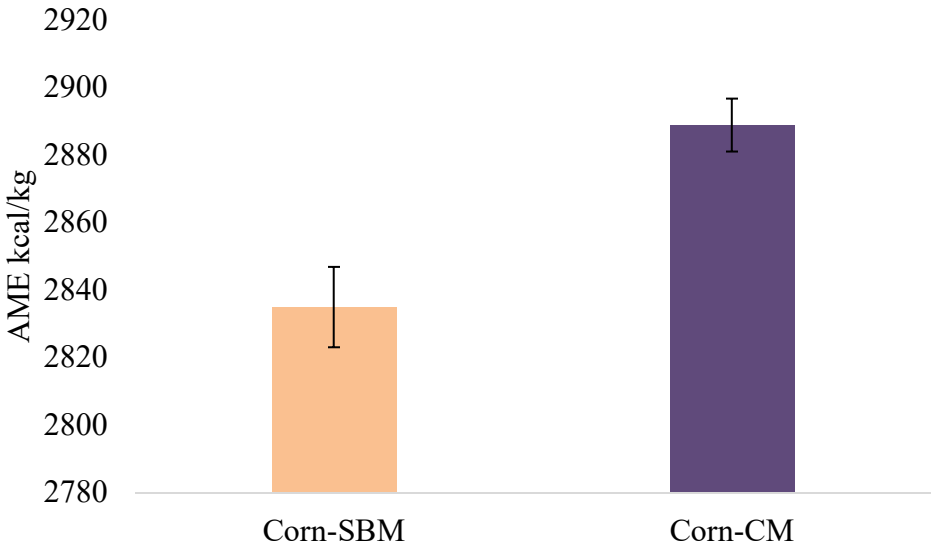

Figure 3.1: Influence of reference diet type on AME of SBM and CM determined by the difference method. Birds were randomly assigned to 10 treatments: corn-SBM (reference diet) corn-CM (reference diet), 8 additional test diets with SBM and CM as test feedstuffs included into each reference diet at 300 g/kg or 450 g/kg inclusion level, meaning inclusion level of test feedstuffs into reference diets at 30% and 45% inclusion levels respectively. Excreta collected on day 21 was used to determine AME of test feedstuffs SBM and CM at 30% and 45% inclusion levels by difference method. Factors, corn-SBM and corn-CM represents AME of test feedstuffs, SBM and CM determined using either corn-SBM or corn-CM as reference diets. Bars ($\pm$ SEM), without a common superscript differ significantly ($P < 0.05$). n = 32.

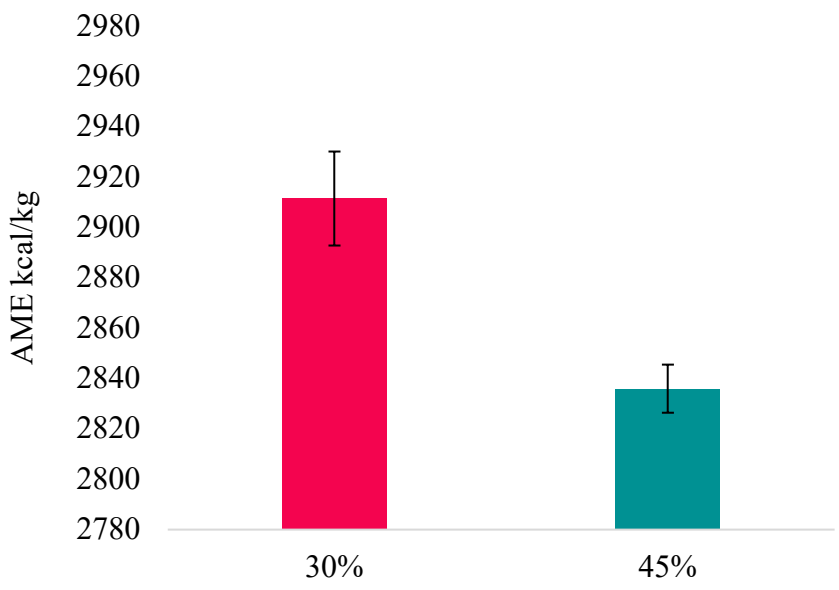

Figure 3.2: Influence of inclusion level on AME of feedstuffs determined by the difference method. Birds were randomly assigned to 10 treatments: corn-SBM (reference diet) corn-CM (reference diet), 8 additional test diets with SBM and CM as test feedstuffs included into each reference diet at 300 g/kg or 450 g/kg inclusion level, meaning inclusion level of test feedstuffs into reference diets at 30% and 45% inclusion levels respectively. Excreta collected on day 21 was used to determine AME of test feedstuffs SBM and CM at 30% and 45% inclusion levels by difference method. Factors, 30% and 45% represent AME of both SBM and CM determined at 30% and 45% inclusion level. Bars (± SEM), without a common superscript differ significantly ($P < 0.05$). n = 32.

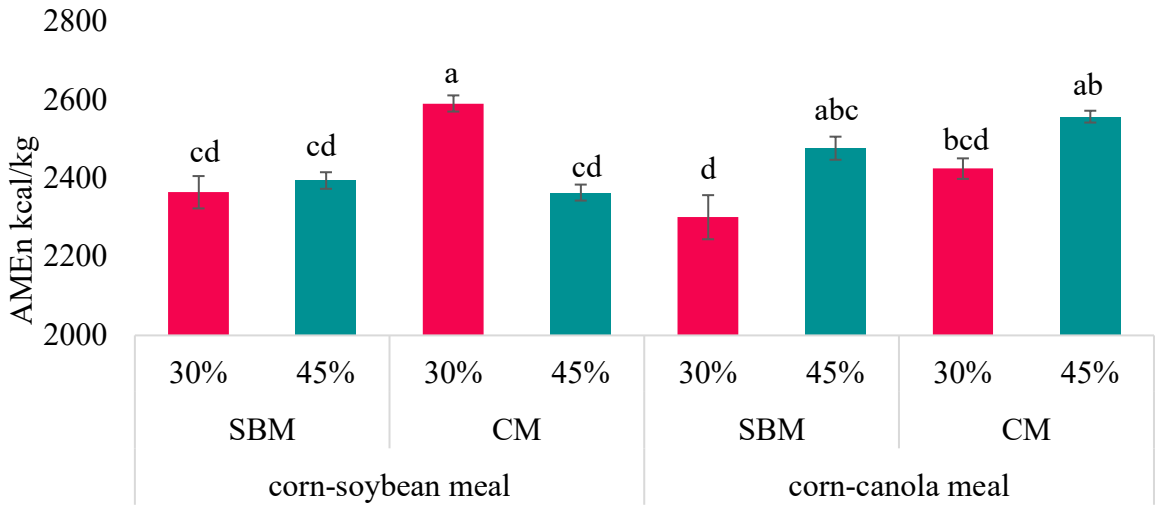

Figure 3.3: Influence of reference diet type and inclusion level on AMEn of SBM and CM determined by the difference method. Birds were randomly assigned to 10 treatments: corn-SBM (reference diet) corn-CM (reference diet), 8 additional test diets with SBM and CM as test feedstuffs included into each reference diet at 300 g/kg or 450 g/kg inclusion level, meaning inclusion level of test feedstuffs into reference diets at 30% and 45% inclusion levels respectively. Excreta collected on day 21 was used to determine AME of test feedstuffs SBM and CM at 30% and 45% inclusion levels by difference method. Factors, corn-SBM and corn-CM represents AME of test feedstuffs determined using either corn-SBM or corn-CM as reference diets. Factors, 30% and 45% are level of inclusion of test feedstuffs into reference diets at 300 g/kg and 450 g/kg inclusion levels. Bars ($\pm$ SEM), without a common superscript differ significantly ($P < 0.05$). n = 8.

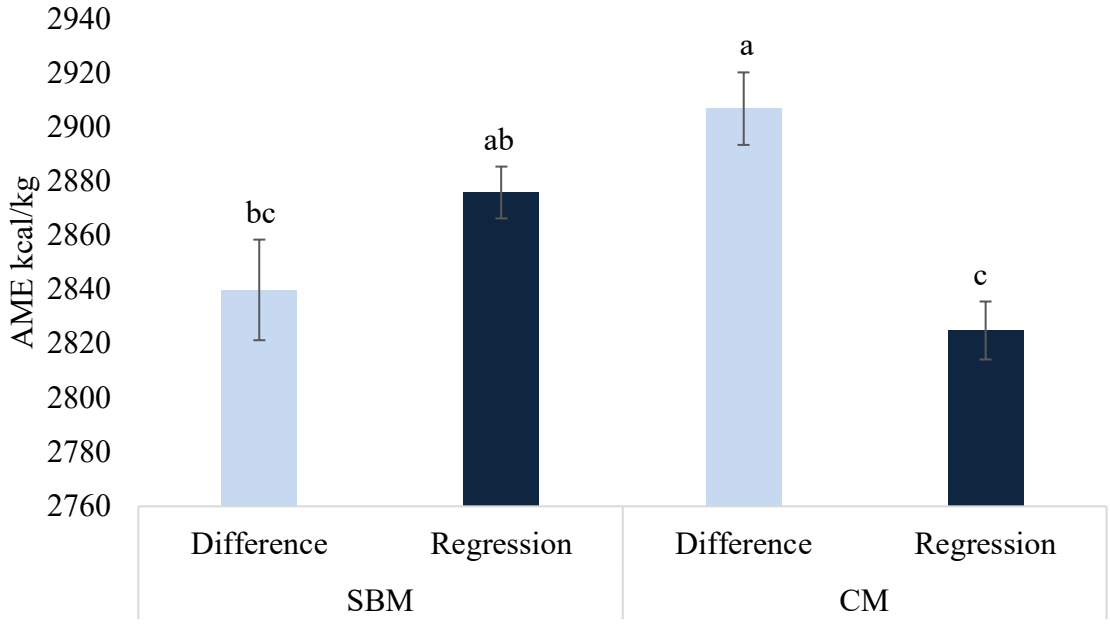

Figure 3.4: Influence of assay method on AME of SBM and CM (P < 0.05). Bars ± SEM means with no common superscripts differ significantly. Birds were randomly assigned to 10 treatments: corn-SBM (reference diet) corn-CM (reference diet), 8 additional test diets with SBM and CM as test feedstuffs included into each reference diet at 300 g/kg or 450 g/kg inclusion level, meaning inclusion level of test feedstuffs into reference diets at 30% and 45% inclusion levels respectively. Excreta collected on day 21 was used to determine AME of test feedstuffs SBM and CM at 30% and 45% inclusion levels by difference method and regression method. Bars (± SEM), without a common superscript differ significantly (P < 0.05). n = 16.

CHAPTER 4

INFLUENCE OF ADAPTATION LENGTH AND ASSAY METHOD ON METABOLIZABLE ENERGY ASSAYS OF CORN AND BARLEY SUPPLEMENTED WITH OR WITHOUT CARBOHYDRASE[2]

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ABSTRACT

This study evaluated the influence of adaptation length (AL) and assay methods; total collection (TCM), and index method (IM) on apparent metabolizable energy (AME and AMEn) of corn and barley when supplemented with carbohydrase. A total of 432 Cobb 500 broilers were used for the study in a 3 × 2 × 3 factorial with factors AL (10, 7, and 4-days), enzyme (with or without) and wheat-soybean meal reference diet (RD), and cereal grains (corn or barley) incorporated into RD at 300 g/kg. Birds on 10, 7, or 4 d AL received experimental diets starting from d 11, 14, and 17 of age, respectively. Total excreta was collected on d 20 and 21. Nutrient utilization assay was done by TCM or IM, and feedstuff AME and AMEn were calculated by the difference method. There was a significant ($P < 0.001$) three-way interaction among AL, enzyme, and cereal grains on AME and AMEn determined by the index method. Enzyme supplementation has no effect on AME and AMEn of corn at 7 and 4 d AL, and for barley at 10 and 7 d AL. Enzyme supplementation improved AME and AMEn corn at 10 d AL for barley at 4 d AL. There was no significant three-way interactionamong AL, Enzyme and cereal grains for AME and AMEn by TCM. Significant two-way interaction between factors, feedstuff and AL ($P =0.002$); feedstuff and Enzyme ($P < 0.001$) were observed for AME and AMEn. Barley AME and AMEn tends to increase with AL, whereas no effect of AL on corn. Enzyme supplementation has greatest effect on AME and AMEn of barley compared to corn. For AME, AL and enzyme interaction was significant ($P = 0.023$). Enzyme supplementation tends to increase AME with increase in AL when supplemented with enzymes but not for diets without enzymes. In conclusion, influence of AL and enzyme supplementation on metabolizable energy of feedstuffs is more pronounced in TCM compared to IM.

INTRODUCTION

Corn and barley are commonly used cereal grains in broilers diets. However, these cereal grains, as with most plant feedstuffs have antinutritive factors that may limit their use in broiler feeds. Arabinoxylans and β-glucans are the non-starch polysaccharides (NSPs) in corn and barley, respectively. Birds lack enzymes that act on β-linkages of NSPs and consequently, NSP are reported to increase the viscosity in gastrointestinal tract thus reducing the available energy from diets (Partridge and Bedford, 2001; Choct, 2006). Increased intestinal viscosity also promotes the growth of harmful bacteria in the gut (Kaldhusdal and Hofshagen, 1992). Supplementation of xylanase and glucanase in corn- and barley-based diets help in hydrolyzing these NSPs releasing xylo-oligosaccharides thus reducing the viscosity and increasing available energy from feedstuffs (Almirall et al., 1995). Bacteria in the cecum ferment xylo-oligosaccharides releasing volatile fatty acids (VFAs) which further enhances growth of beneficial bacteria and promote intestinal health (Mathlouthi et al., 2002; González-Ortiz et al., 2020).

When determining AME of feedstuffs, diets with test feedstuffs are fed to broilers for a certain period so that they can adopt to the relatively new feedstuffs and give optimum AME values. This period taken by broilers to produce optimum AME is known as adaptation length. Adaptation length of 7 or 4 days is usually applied in AME assay of feedstuffs. The influence of adaptation length on barley and wheat are studied extensively in previous literature (Olukosi et al., 2017; Dunaway and Adedokun, 2019; Olukosi, 2020). But the optimum length of adaptation is not clearly defined for feedstuffs specifically when supplemented with carbohydrase.

Total collection method or index method using indigestible markers are used to calculate AME of feedstuffs. Owing to the practical difficulties in total collection methods, digestibility studies in recent years are conducted mostly by indicator method by using chromic oxide or

titanium dioxide as a marker (Rutherfurd et al., 2002; Smeets et al., 2015). Dourado et al., (2010) showed that total collection method is more reliable than indicator method while another study show that indicator method gives similar estimate of AME, (Vogtmann et al., 1975). Sales and Janssens, (2003) observed that the index method, compared to total collection method produced lower AME for fibrous diets. When diets supplemented with enzymes, a detailed understanding of how total collection and index method influence AME of feedstuffs in mixed diets is essential.

Precision feeding of broilers reduces feed costs and nitrogen excretion into the environment (Olukosi and Adeola, 2010). In this instance, broilers are fed with diets that meets the nutrient requirements as close as possible, so nitrogen and energy excretion will be minimum. Precision feeding requires careful selection of AME values for feedstuffs and using these values in feed formulations. However, there is variability in reported AME values for most of the feedstuffs, as there are several factors that affect AME in digestibility studies. Adaptation length and method used (total collection or index) are two of the factors that can influence AME of feedstuffs. The objective of this study is to investigate the influence of adaptation length on metabolizable energy of corn and barley when supplemented with carbohydrase using total collection or index method.

MATERIAL AND METHODS

This experiment was approved by the Institutional Animal Care and Use Committee at the University of Georgia (AUP: A2018 08-026-Y1-A0). A total of 324-day old Cobb 500 male broilers at zero-day old were used to study the effect of adaptation length on AME of corn and barley with or without enzyme supplementation. Birds received a pre-experimental diet (Table 4.1) based on wheat and soybean meal until the introduction of the respective experimental diets based on adaptation length allocation. Treatments were arranged in a 3 × 3 × 2 factorial with

adaptation length (10, 7 or 4 d), feedstuff (corn or barley) and Enzyme (with or without) as factors (Table 4.2). Each treatment had 6 replicates with 3 birds per replicate. Reference diet was based on wheat-SBM, and into the reference diet 300 g/kg of corn or barley were added (Table 4.1). Each of the three basal diets (one reference and two test diets) was then divided into two batches, supplemented or not with combination of xylanase (0.1 g/kg) and glucanase (0.1 g/kg) to make a total of 6 diets. Birds allocated to 10-dadaptation length received experimental diets from starting from day 11, whereas birds allocated to 7 or 4-d of adaptation length received experimental diets starting from d 14 or 17, respectively. Total excreta voided were collected every 8h on day 20 and 21. Ceca contents were collected from all the birds in a cage on d 21 after euthanizing the birds by carbon dioxide asphyxiation. Ceca contents were stored at -20ºC until further analysis.

CHEMICAL ANALYSIS

Diets, excreta, corn and barley samples were ground to pass through 0.5 mm screen prior to chemical analysis. Diets and excreta samples were analyzed for TiO2 , dry matter, nitrogen and gross energy. Concentration of TiO2 was determined by using the method proposed by Short et al., (1996). Dry matter was determined by drying the samples in a drying oven at 100ºC for 24 hours. Total nitrogen content was determined by the combustion method (Method 968.06; AOAC, 1990). Isoperibol bomb calorimeter with benzoic acid as standard is used to estimate gross energy (Model 6200, Parr Instruments, Moline, IL). Short chain fatty acid content in ceca was analyzed as free fatty acids by gas chromatography using meta-phosphoric acid as an internal standard (Agilent Technologies, Santa Clara, CA).

CALCULATIONS

Total collection and index method was used to calculate apparent metabolizable energy (AME, kcal/kg) of diets.

The following formula was used to determine AME of diets by index method;

$AME = GE_I - [GE_O \times (C_I / C_O)]$;

The following formula was used to determine AME of diets by total collection method;

$AME = ((DMI \times GE_I) - (\text{Excreta output} \times GE_O))/DMI$

Where:

AME = Apparent metabolizable energy in kcal/kg

GE_I = Gross energy of the diet in kcal/kg

GE_O = Gross energy of the excreta in kcal/kg

C_I = Concentration of titanium (%) in the diet

C_O = Concentration of titanium (%) in the excreta

DMI = Dry matter intake in kg

Nitrogen correction was applied to AME by using 8.22 as a correction factor.

$AMEn = AME - 8.22 \times (N_I - N_O \times [C_I / C_O])$

The difference method was used to determine the AME and AMEn of test feedstuffs, corn and barley, by using the formula:

$AME_{TF} = (AME_{TD} - (AME_{RD} \times P_{RD}))/P_{TF}$

Where:

AME_{TF} = AME of test feedstuff in kcal/kg

AME_{TD} = AME of test diet in kcal/kg

AME_{RD} = AME of reference diet in kcal/kg

P_{RD} = Proportional contribution of energy of reference diet in test diet

P_{TF} = Proportional contribution of energy of test feedstuff in test diet

The AME and AMEn of enzyme-supplemented reference diet was used in calculating the AME and AMEn of test feedstuff in enzyme-supplemented test diets, and vice versa.

STATISTICAL ANALYSIS

Statistical analyses were conducted using JMP (JMP pro version 15). The data for AME and AMEn of corn and barley were analyzed as a 3 × 2 × 2 factorial to incorporate the effect of adaptation length (10, 7 and 4 days), feedstuff (corn or barley) and Enzyme (with or without). For the comparison of AME and AMEn determined using the two methods (total collection or index methods), the data were analyzed as 2 × 2 × 2 × 3 corresponding to methods, feedstuff, enzyme and adaptation length. When there are no significant interactions main effects means were discussed. Simple effect means are discussed where interactions were significant. Turkey's honest significant difference test was used to separate significantly different means.

RESULTS

Influence of adaptation length, enzyme supplementation and cereal type on AME and AMEn determined by difference using index method

There was significant three-way interaction ($P < 0.001$) between factors adaptation length, feedstuff and Enzyme for AME (Figure 4.1) and AMEn (Table 4.3 and Figure 4.2). Enzyme supplementation improved ($P < 0.001$) AME and AMEn of corn in birds receiving assay diets for 10 d of adaptation, but not 7 or 4 d of adaptation length. Enzyme supplementation had no effect

on AME and AMEn of barley at 10 and 7 d of adaptation length, however enzyme supplementation tends ($P < 0.001$) to improve AME and AMEn of barley at 4 d adaptation length.

Influence of adaptation length, enzyme supplementation and cereal type on AME and AMEn determined by difference using total collection method

There was no significant three-way interaction between factors adaptation length, Enzyme and feedstuff on AME (Figure 4.3) and AMEn (Table 4.4). There was significant feedstuff × adaptation length interaction for AME and AMEn (Figure 4.5)($P < 0.005$). Adaptation length had no effect on AME and AMEn of corn, but for barley AME and AMEn were greater ($P < 0.001$) when determined at 10 d of adaptation length compared to 7 and 4 d of adaptation. There was significant feedstuff × enzyme interaction for AME and AMEn (Figure 4.4) ($P < 0.001$). Enzyme supplementation improved AME and AMEn of both feedstuffs, however AME and AMEn of corn was greater than barley. There was significant adaptation length × enzyme interaction ($P < 0.05$) for AME but not AMEn. Enzyme supplementation improved AME with an increase in adaptation length. However, when not supplemented with enzymes, there was no linear effect of adaptation length on AME.

Influence of assay method, adaptation length, cereal type and Enzyme supplementation on AME and AMEn of the test feedstuffs

There were no significant four-way interaction between factors method, AL, feedstuff and enzyme supplementation on AME and AMEn (Table 4.5). There was significant method × enzyme × feedstuff interaction on AME (Figure 4.6) and AMEn (Figure 4.7) ($P < 0.01$). When determined by total collection method there was no significant difference between AME of corn when

supplemented or not with enzyme, however, enzyme supplementation improved AMEn when determined by total collection method, and both AME and AMEn when determined by index method. When determined by total collection method AME and AMEn of barley was greater when supplemented with enzyme compared to when not supplemented with enzyme. When determined by index method the AME and AMEn of barley was not different with or without enzyme supplementation. There was significant enzyme × adaptation length × feedstuff interaction on AME (Figure 4.8) and AMEn (Figure 4.9) ($P < 0.01$). When not supplemented with enzyme AME and AMEn of corn tends to decrease at 10 d adaptation length. When supplemented with enzymes AME and AMEn of corn was no different with 4, 7 and 10 d AL. Barley AME and AMEn tends to decease at 7 d AL when not supplemented with enzyme, however when supplemented with enzyme AME and AMEn of barley was not different when fed for 4, 7 and 10 d AL.

Influence of adaptation length, enzyme supplementation and experimental diet type, on ceca short chain fatty acid content

There was no significant main effect of adaptation length, nor two- or three-way interactions between the factors diet, adaptation length and enzyme on acetate, propionate, butyrate, and total SCFA content (Table 4.6). However, enzyme supplementation increased caeca content of acetate ($P = 0.039$) and total SCFA ($P = 0.041$) and tended ($P = 0.064$) to increase caeca butyrate content in ceca. Reference diet supplemented with corn and barley has greater acetate ($P = 0.003$), propionate ($P = 0.017$), butyrate ($P = 0.019$) and total SCFA ($P = 0.001$) content of ceca compared to reference diet.

DISCUSSION

Carbohydrase supplementation to diets increases AME of cereal-based feedstuffs by increasing energy utilization from fiber (Bedford and Morgan, 1996; Olukosi et al., 2007). Using these improved AME values of feedstuffs in feed formulations reduces the feed costs. However, the influence of adaptation length and method used (total collection or index method) while determining metabolizable energy of feedstuffs when supplemented with enzymes is not well document in current literature. Therefore, the main goal of the study is to investigate 1) the influence of adaptation length and 2) comparison of metabolizable energy values produced by total collection or index method for corn and barley when supplemented with xylanase and glucanase.

Wheat-SBM based diet is used as reference diet to determine AME and AMEn of corn and barley by difference method. Corn or barley was added at 300 g/kg into the wheat-SBM based reference diet. To determine the interaction effects of enzyme supplementation and adaptation length, additional treatments were prepared by adding xylanase and glucanase to the test diets and reference diets. Whilst the test diets with corn and barley have both xylanase and glucanase, it was expected that xylanase and glucanase was supposed to act on corn and barley and improve their AME consequently. However, Xylanase activity was not recovered in test and reference diets, but glucanase activity was observed in diets. So, a significant effect of xylanase supplementation on metabolizable energy of corn is not very well observed in this current study. But any improvement in metabolizable energy observed was due to glucanase activity. Enzyme supplementation to experimental diets improved acetate and total VFA production in ceca, it was expected and is in agreement with earlier studies (Wang et al., 2005; Józefiak et al., 2006).

The AME and AMEn of corn determined by index method at 7 or 4 d adaptation length was not different with or without enzyme supplementation. This was expected as xylanase activity

was not recovered in diets. However, AME and AMEn of corn decreased at 10 d of adaptation length when not supplemented with enzymes. Decrease in AME and AMEn of corn observed at 10 d of adaptation length was improved by adding glucanase to the diet. Leslie et al., (2007) showed that added glucanase to corn improved digestibility of corn by degradation of hemicellulose and increasing the availability of starch for digestion. So, the improvement in AME of corn observed is due to increase in starch digestion by action of glucanase on corn. An improvement in AME and AMEn of corn due to glucanase was not observed at 7 or 4 d of adaptation length, may be 10 days of adaptation is required to see effect of glucanase on AME and AMEn of corn.

Neither the significant effect of Enzyme nor the effect of adaptation length was observed for AME and AMEn of barley at 10 and 7 d adaptation length determined by index method. It was expected that glucanase supplementation improves AME and AMEn of barley. However, barley AME and AMEn was similar with or without enzyme supplementation at 10 and 7 d of adaptation length. Inclusion level of 300 g/kg into the reference diet might not be sufficient to produce significant effect on AME and AMEn of barley. Influence of enzyme supplementation on barley depends on its level of inclusion into reference diets (Rotter et al. 1990). Inclusion level of 300 g/kg may not be sufficient to see a significant effect of enzyme supplementation determined by index method. Fuente et al., (1995) showed that enzyme supplementation has no effect of AME of barley when supplemented at 300 g/kg inclusion level, significant effect of enzyme supplementation was observed with increase in inclusion level after 400g/kg inclusion of barley into reference diets. Barley AME and AMEn tends to decrease at 4 d of adaptation length. Olukosi, (2020) also observed a decrease in AME and AMEn of barley at 4 d of adaptation length. However,

4 d of adaptation length may be sufficient when supplemented with enzymes regardless of low inclusion level.

When determined by total collection method, adaptation length has no effect on AME and AMEn of corn when not supplemented with enzymes. These results are in agreement with index method except that a decrease in AME and AMEn of corn at 10 d adaptation length is not observed. However, in agreement with index method glucanase supplementation improved AME and AMEn of corn at 10 d of adaptation length. This consistent increase in AME and AMEn of corn by glucanase supplementation at 10 d of adaptation length by both index and total collection method shows strongly that 10 d of adaptation length is needed to observe glucanase effect on corn.

Barley AME and AMEn tends to increase with an increase in adaptation length from 7 to 10 d with or without enzyme supplementation. Significant improvement in AME and AMEn of barley with glucanase supplementation is observed by total collection method. Whereas index method failed to detect effect of glucanase at 10 and 7 d of adaptation length. Increase in AME with increase in adaptation length was expected for barley and observed in previous studies when not supplemented with enzymes (Olukosi, 2020). The same observation can be linked to when supplemented with enzymes.

Index and total collection method did not give similar results and trends for effect of enzyme and adaptation length. The difference in enzyme response and trends for AME and AMEn of feedstuffs might be due to 1) failure to accomplish flawless data collection required for total collection method 2) titanium recovery and 3) uneven flow rate of marker in the diet. Greater AME and AMEn values for feedstuffs observed in total collection method compared to index method was expected and is in agreement with other studies (Sales and Janssens, 2003; Smeets et al., 2015). Collecting all of the excreta is not possible most of the times, this might produce greater

digestibility values for feedstuffs by total collection method to index method. Various studies show that choice and concentration of marker in the diets affects AME values produced specifically upon supplementation with enzymes (Scott and Boldaji, 1997; Olukosi et al., 2012). This might be of special concern in studies related to fiber digestion and enzymes which modify passage rate of digesta. Accuracy and repeatability of digestibility values produced by titanium and total collection methods for feedstuffs supplemented with enzymes needs to be investigated.

In conclusion, the influence of adaptation length on AME and AMEn depends on the type of cereal grain used and method used to determine digestibility values when supplemented with glucanase. The trends observed for effect of adaptation length on AME and AMEn of corn and barley was more pronounced in total collection compared to index method.

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Table 4.1: Description of treatments

Treatment	Diet	Enzyme	Adaptation length, d
1	Reference		10
2	Reference +300 g/kg Corn		10
3	Reference +300 g/kg Barley		10
4	Reference	Xylanase + Glucanase	10
5	Reference +300 g/kg Corn	Xylanase + Glucanase	10
6	Reference +300 g/kg Barley	Xylanase + Glucanase	10
7	Reference		7
8	Reference +300 g/kg Corn		7
9	Reference +300 g/kg Barley		7
10	Reference	Xylanase + Glucanase	7
11	Reference +300 g/kg Corn	Xylanase + Glucanase	7
12	Reference +300 g/kg Barley	Xylanase + Glucanase	7
13	Reference		4
14	Reference +300 g/kg Corn		4
15	Reference +300 g/kg Barley		4
16	Reference	Xylanase + Glucanase	4
17	Reference +300 g/kg Corn	Xylanase + Glucanase	4
18	Reference +300 g/kg Barley	Xylanase + Glucanase	4

Table 4.2: Ingredient composition (g/kg) and calculated analysis of the diets

Feedstuff	Pre-experimental	Reference diet	Basal + 300 g/kg Corn	Basal + 300 g/kg Barley
Wheat	595.0	638.0	433.0	434.0
Soybean meal	310.0	267.0	181.0	181.0
Soybean oil	50.0	48.0	32.0	32.0
Corn	-	-	300.0	-
Barley	-	-	-	300.0
DL-Methionine	1.5	1.6	2.1	2.0
Lysine	1.5	2.0	5.4	4.5
Threonine	0.6	0.5	2.0	1.5
Dicalcium phosphate	20.0	18.5	18.2	17.5
Limestone	7.0	6.6	7.6	8.0
Sodium chloride	4.0	4.0	4.0	4.0
Titanium dioxide	0	5.0	5.0	5.0
Vitamin premix[1]	5.0	5.0	5.0	5.0
Trace mineral premix[2]	5.0	5.0	5.0	5.0
Total	1,000	1,000	1,000	1,000
Calculated nutrient content				
Protein, g/kg	21	19.6	15.6	16.8
ME, kcal	2,951	2,954	3,016	2,801
Ca, g/kg	9.0	8.4	8.4	8.4
Total P, g/kg	6.9	6.4	6.2	6.3
Non-phytate P, g/kg	4.5	4.2	4.2	4.2

[1] Vitamin A, 5484 IU; vitamin D3, 2643 ICU; vitamin E, 11 IU; menadione sodium bisulfate, 4.38 mg; riboflavin, 5.49 mg, d-panthothenic acid, 11 mg; niancin, 44.1 mg, choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg

[2] Iodine, 1.11 mg; manganese, 66.06 mg; copper, 4.44 mg; iron, 44.1 mg; zinc, 44.1 mg; selenium, 300 µg.

Table 4.3: Influence of adaptation length and enzyme supplementation on AME and AMEn of corn and barley determined by difference using the index method

Feedstuff	Adaptation length, d	Enzyme	AME (kcal/kg)	AMEn (kcal/kg)
The main effect means for feedstuff				
Corn			3,225	2,823
Barley			2,523	2,962
Pooled SEM			26	30
The main effect means for adaptation length				
	10		2,760	2,712
	7		2,900	2,855
	4		2,988	2,959
Pooled SEM			32	37
The main effect means for Enzyme				
		No	2,812	2,765
		Yes	2,951	2,917
Pooled SEM			26	30
Simple effects means				
Corn	10	No	2,783^{b}	2,726^{b}
	7	No	3,139^{a}	3,085^{a}
	4	No	3,458^{a}	3,435^{a}
	10	Yes	3,308^{a}	3,301^{a}
	7	Yes	3,459^{a}	3,434^{a}
	4	Yes	3,352^{a}	3,335^{a}
Barley	10	No	2,498^{bc}	2,461^{bc}
	7	No	2,452^{bc}	2,389^{bc}
	4	No	2,414^{c}	2,366^{c}
	10	Yes	2,548^{bc}	2,474^{bc}
	7	Yes	2,572^{bc}	2,534^{bc}
	4	Yes	2,661^{bc}	2,634^{bc}
Pooled SEM			65	73
P-value for the main effects and interactions				
Feedstuff			< 0.001	< 0.001
Adaptation length			< 0.005	< 0.005
Enzyme			< 0.001	< 0.001
Feedstuff × Adaptation length			< 0.005	< 0.016
Enzyme × Adaptation length			0.113	0.186
Feedstuff × Enzyme			0.217	0.179
Feedstuff × Adaptation length × Enzyme			< 0.001	<0.001

n = 36 for main effects of feedstuff and Enzyme; n = 24 for main effects of adaptation length; n = 6 for simple effects means; SEM- standard error of the means. a-c Means in a column, and within a group, but with no common superscripts are significantly different.

Table 4.4: Influence of adaptation length and enzyme supplementation on AME and AMEn of corn and barley determined by difference using the total collection method

Feedstuff	Adaptation length, d	Enzyme	AME (kcal/kg)	AMEn (kcal/kg)
The main effect means for feedstuff				
Corn			3,516	3,414
Barley			2,776	2,699
Pooled SEM			26	25
The main effect means for adaptation length				
Adaptation length				
	10		3,299	3,195
	7		3,064	2,990
	4		3,114	3,024
Pooled SEM			31	31
The main effect means for Enzyme				
Enzyme				
		No	3,016	2,928
		Yes	3,299	3,209
Pooled SEM			26	25
Simple effect means				
Corn	10	No	3,415^{bc}	3,321^{bc}
	7	No	3,432^{bc}	3,345^{bc}
	4	No	3,483^{abc}	3,324^{bc}
	10	Yes	3,769^{a}	3,651^{a}
	7	Yes	3,635^{ab}	3,554^{ab}
	4	Yes	3,392^{bc}	3,326^{bc}
Barley	10	No	2,813^{ef}	2,729^{ef}
	7	No	2,328^{g}	2,283^{g}
	4	No	2,534^{fg}	2,486^{fg}
	10	Yes	3,198^{cd}	3,079^{cd}
	7	Yes	2,862^{ef}	2,778^{ef}
	4	Yes	2,917^{de}	2,840^{de}
Pooled SEM			63	61
P-values for main the effects and interactions				
Feedstuff			<0.001	<0.001
Adaptation length			<0.001	<0.001
Enzyme			< 0.001	<0.001
Feedstuff × Adaptation length			0.002	0.001
Feedstuff × Enzyme			<0.001	0.003
Adaptation length × Enzyme			0.023	0.100
Adaptation length × Enzyme × Feedstuff			0.059	0.142

n = 36 for main effects of feedstuff and Enzyme; n = 24 for main effects of adaptation length; n = 6 for simple effects means; SEM- standard error of the means. a-g Means in a column, and within a group, but with no common superscripts are significantly different.

Table 4.5: Influence of method, adaptation length and Enzyme on AME and AMEn of corn and barley

Item	Method	Enzyme	Adaptation length, d	Feedstuff	AME (kcal/kg)	AMEn (kcal/kg)
The main effects means for method						
	TCM				3,158	3,068
	Index				2,880	2,839
Pooled SEM					45	47
The main effects means for Enzyme						
		No			2,918	2,849
		Yes			3,134	2,954
Pooled SEM					45	47
The main effects means for adaptation length						
			10		3,030	2,954
			7		2,986	2,926
			4		3,055	2,994
Pooled SEM					55	58
The main effect means for feedstuff						
				Corn	3,377	3,309
				Barley	2,654	2,591
Pooled SEM					45	47
Simple effects means						
	TCM					
		Corn	No	10	$3,415^{abcd}$	$3,321^{ab}$
				7	$3,432^{abcd}$	$3,345^{ab}$
				4	$3,483^{abc}$	$3,324^{ab}$
			Yes	10	$3,769^{a}$	$3,651^{a}$
				7	$3,635^{ab}$	$3,554^{a}$
				4	$3,392^{bcd}$	$3,326^{ab}$
		Barley	No	10	$2,813^{ghij}$	$2,729^{cdef}$
				7	$2,328^{k}$	$2,283^{g}$
				4	$2,534^{ijk}$	$2,486^{defg}$
			Yes	10	$3,198^{cdef}$	$3,079^{bc}$
				7	$2,862^{fghi}$	$2,778^{cde}$
				4	$2,917^{efgh}$	$2,840^{cd}$
	Index					
		Corn	No	10	$2,783^{hijk}$	$2,726^{cde}$
				7	$3,139^{defg}$	$3,085^{bc}$
				4	$3,458^{abcd}$	$3,435^{ab}$

		Yes	10	3,308^{bcde}	3,301^{ab}
			7	3,459^{abcd}	3,434^{ab}
			4	3,352^{bcd}	3,334^{ab}
	Barley	No	10	2,498^{ijk}	2,461^{defg}
			7	2,451^{jk}	2,388^{efg}
			4	2,414^{k}	2,366^{fg}
		Yes	10	2,548^{ijk}	2,474^{efg}
			7	2,572^{hijk}	2,534^{defg}
			4	2,661^{hijk}	2,634^{defg}
Pooled SEM				32	33
P-value for main effects and interactions					
Method				< 0.001	<0.001
Adaptation length				0.171	0.323
Enzyme				< 0.001	<0.001
Feedstuff				<0.001	<0.001
Method × Enzyme				0.068	0.166
Method × Adaptation length				< 0.001	<0.001
Enzyme × Adaptation length				0.003	0.022
Enzyme × Feedstuff				0.111	0.419
Method × Feedstuff				0.642	0.791
Adaptation length × Feedstuff				<0.001	< 0.001
Method × Enzyme × adaptation length				0.821	0.919
Method × Enzyme × Feedstuff				0.001	< 0.005
Method × Adaptation length × Feedstuff				0.055	0.039
Enzyme × Adaptation length × Feedstuff				<0.001	< 0.001
Method × Enzyme × Adaptation length × Feedstuff				0.201	0.069

n = 72 for main effects of method and enzyme; n = 48 for main effects for adaptation length; n = 12 for simple effect means

a-k Means in a column, and within a group, but with no common superscripts are significantly different.

Table 4.6: Influence of experimental diets on caeca short chain fatty acid content (mM)

Diets	Adaptation length, d	Enzyme	Acetate	Propionate	Butyrate	Total SCFA
The main effect means for diet type						
Reference diet			58.7	1.37	9.13	69.2
Reference diet + 300 g/kg corn			68.3	1.99	11.9	82.2
Reference diet + 300 g/kg barley			70.2	2.93	11.0	84.5
Pooled SEM			2.33	0.37	0.66	3.00
The main effect means for adaptation length						
	10		61.3	2.40	9.81	73.7
	7		67.6	1.91	10.9	80.5
	4		67.4	1.90	11.1	80.5
Pooled SEM			2.33	0.37	0.66	3.00
The main effect means for Enzyme						
		No	62.4	2.11	9.86	74.5
		Yes	68.6	2.02	11.4	82.2
Pooled SEM			1.91	0.30	0.54	2.45
Simple effects means						
Reference diet	10	No	47.7	1.78	5.76	55.2
	7	No	58.9	0.97	9.26	69.1
	4	No	54.0	1.53	8.98	64.5
	10	Yes	59.1	2.33	10.3	71.7
	7	Yes	70.6	0.73	10.8	82.1
	4	Yes	61.6	0.88	9.71	72.3
Reference diet + 300 g/kg corn	10	No	56.4	1.27	9.26	67.0
	7	No	69.4	2.66	11.6	83.7
	4	No	73.6	2.50	13.9	90.0
	10	Yes	73.9	2.18	12.5	88.8
	7	Yes	65.8	2.77	11.7	83.1
	4	Yes	61.8	0.62	12.1	82.2
Reference diet + 300 g/kg barley	10	No	69.4	3.70	10.9	84.6
	7	No	64.6	2.02	10.1	76.7
	4	No	70.4	2.92	9.47	82.7
	10	Yes	65.4	3.51	11.0	80.7
	7	Yes	74.7	2.27	12.3	90.0
	4	Yes	75.9	2.73	12.5	91.1
Pooled SEM			5.72	0.9	1.62	7.35
P-value for the main effects and interactions						
Diet			0.003	0.017	0.019	0.001
Adaptation length			0.193	0.501	0.442	0.303

Enzyme	0.039	0.827	0.064	0.041
Diet × Adaptation length	0.714	0.446	0.836	0.799
Enzyme × Adaptation length	0.731	0.417	0.592	0.603
Diet × Enzyme	0.581	0.937	0.652	0.616
Diet × Adaptation length × Enzyme	0.363	0.855	0.508	0.339

n = 36 for main effects of diets and adaptation length, n = 54 for main effects of Enzyme, n = 6 for simple effect means; reference diet was wheat-soybean meal based.

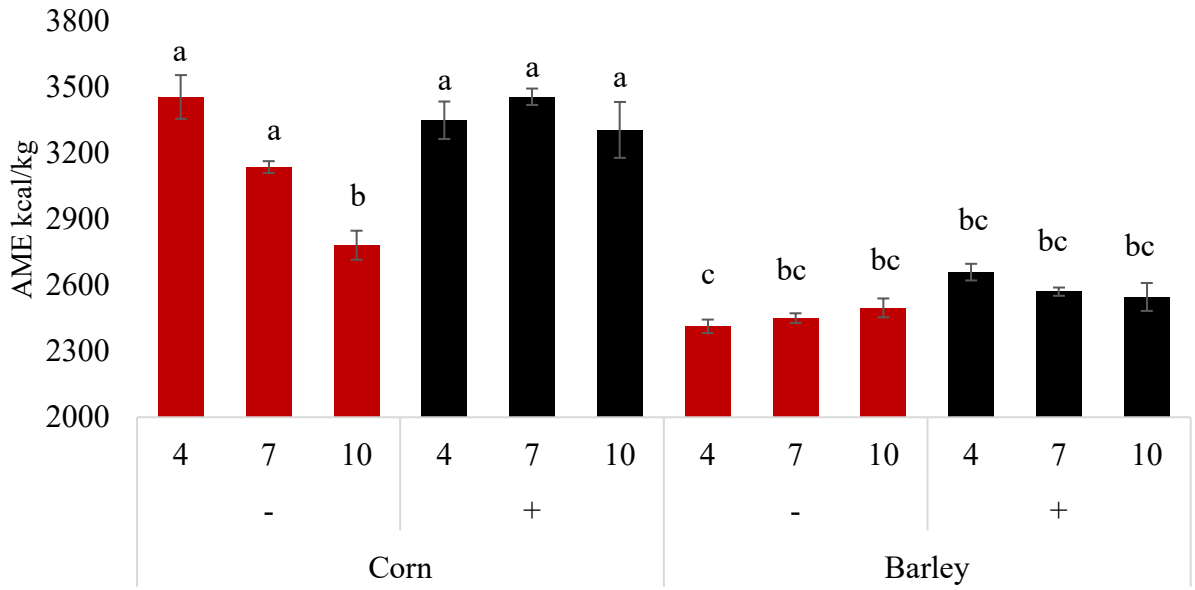

Figure 4.1: Influence of adaptation length and enzyme supplementation on AME of corn and barley determined by difference method using index method . Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 6.

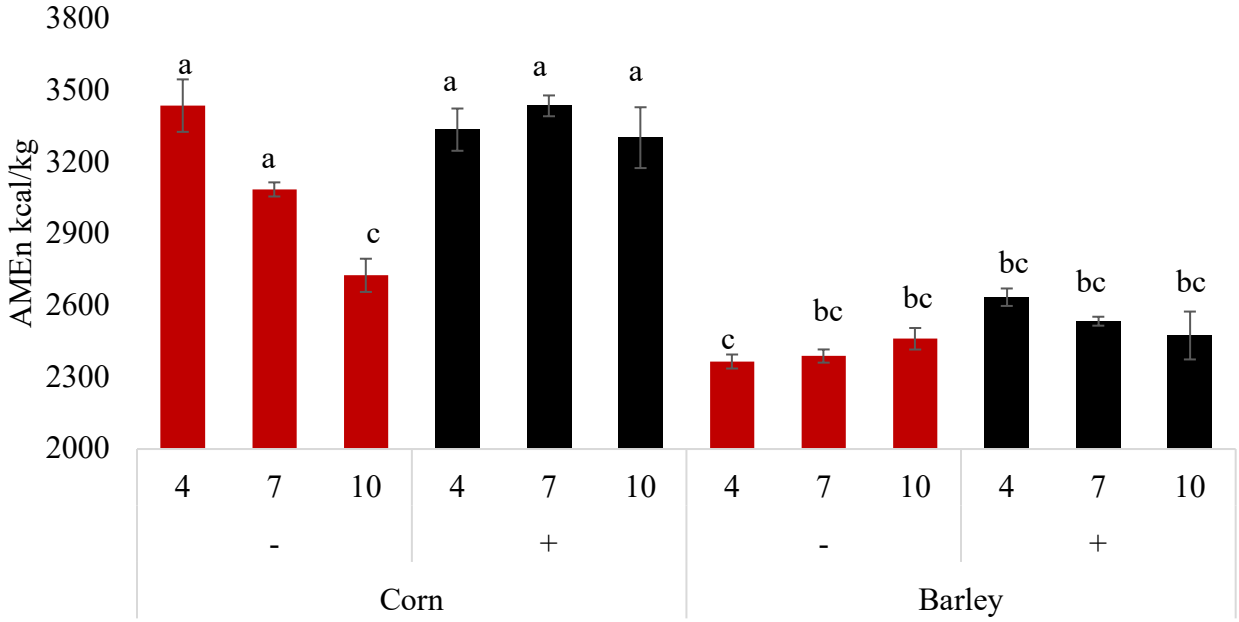

Figure 4.2: Influence of adaptation length and enzyme supplementation on AMEn of corn and barley determined by difference method using index method. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10 days. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 6.

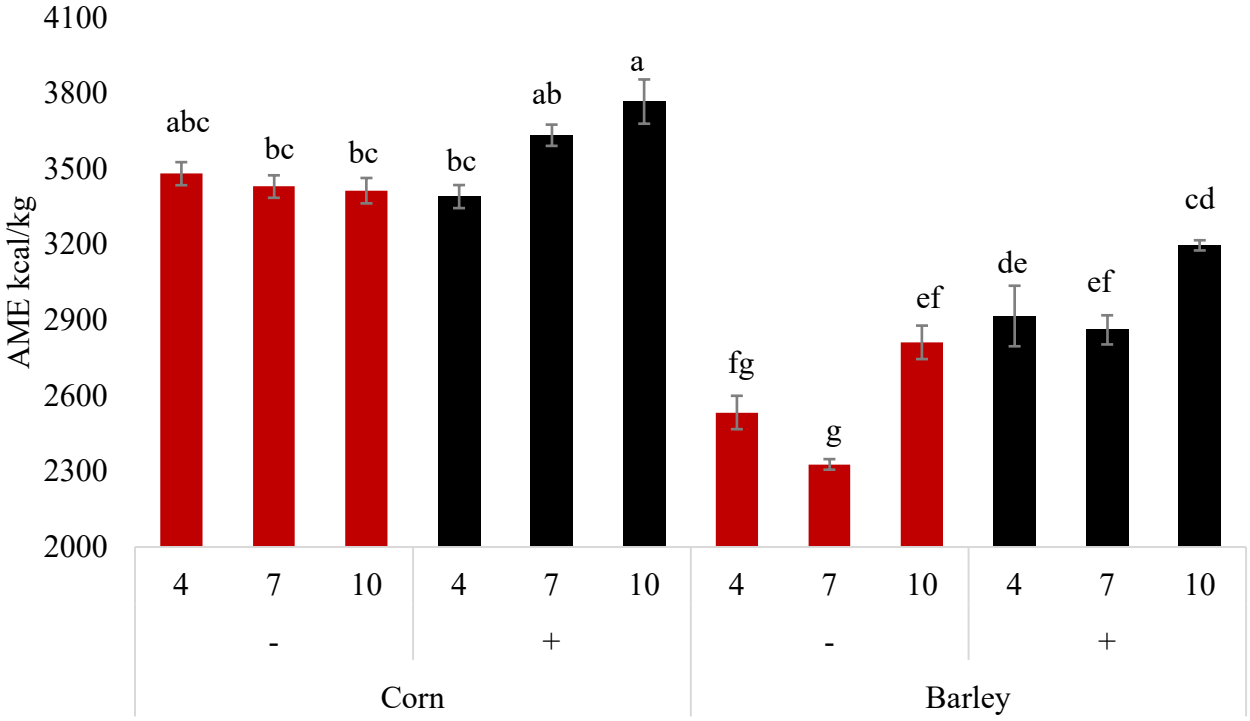

Figure 4.3: Influence of adaptation length and enzyme supplementation on AME of corn and barley determined by difference method using total collection method. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 6.

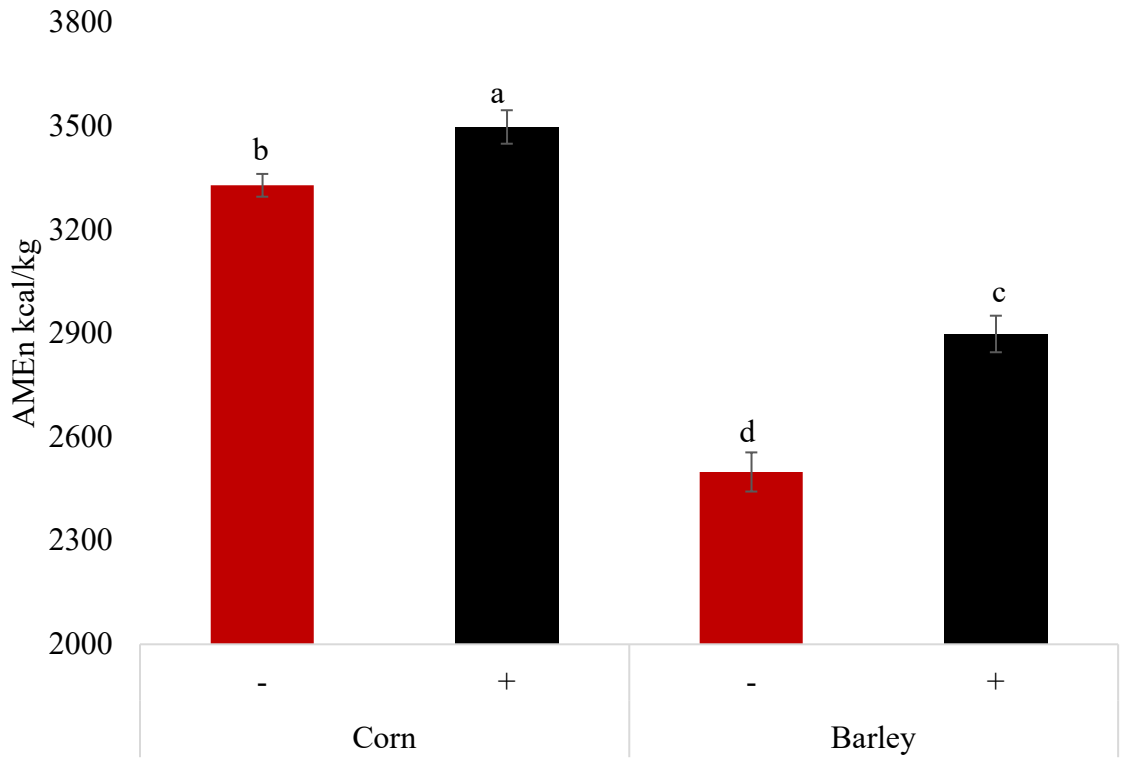

Figure 4.4: Influence of enzyme supplementation on AMEn of corn and barley determined by difference method using total collection method. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 18.

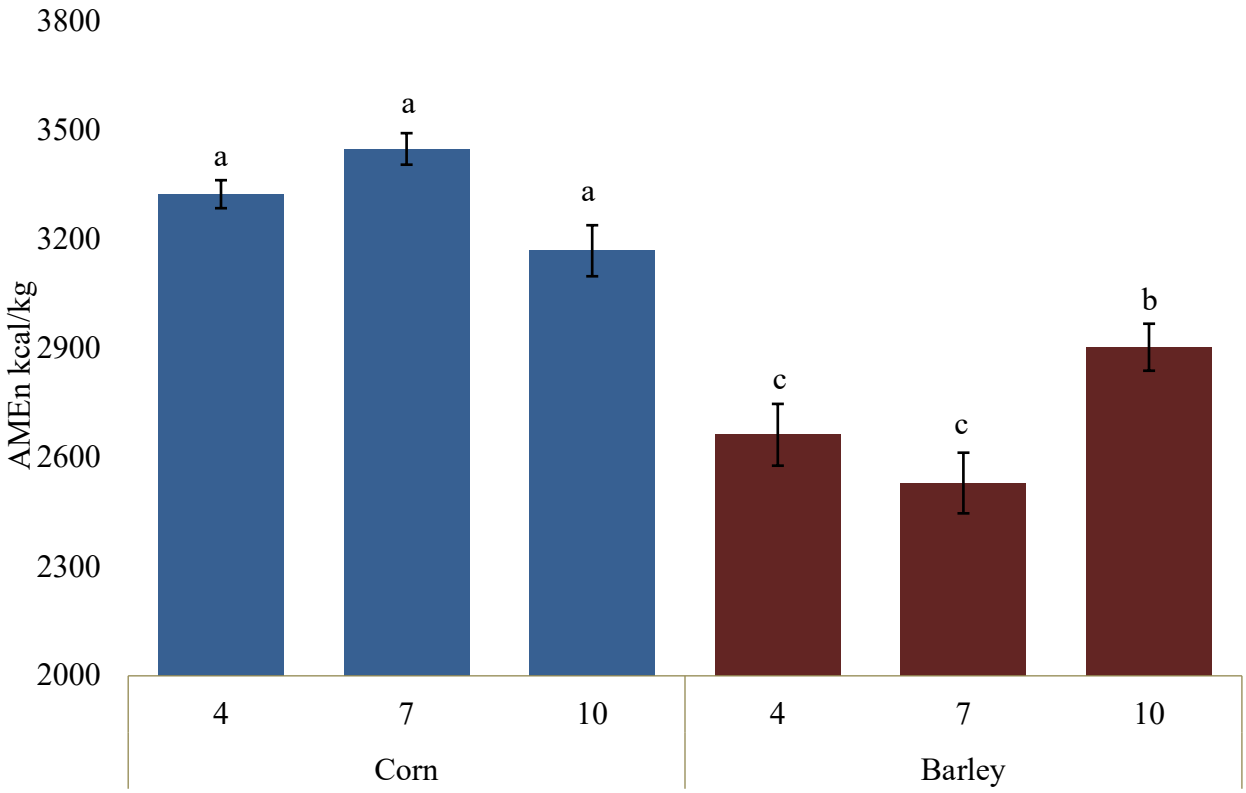

Figure 4.5: Influence of adaptation length on AMEn of corn and barley when determined difference method using total collection method. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 12.

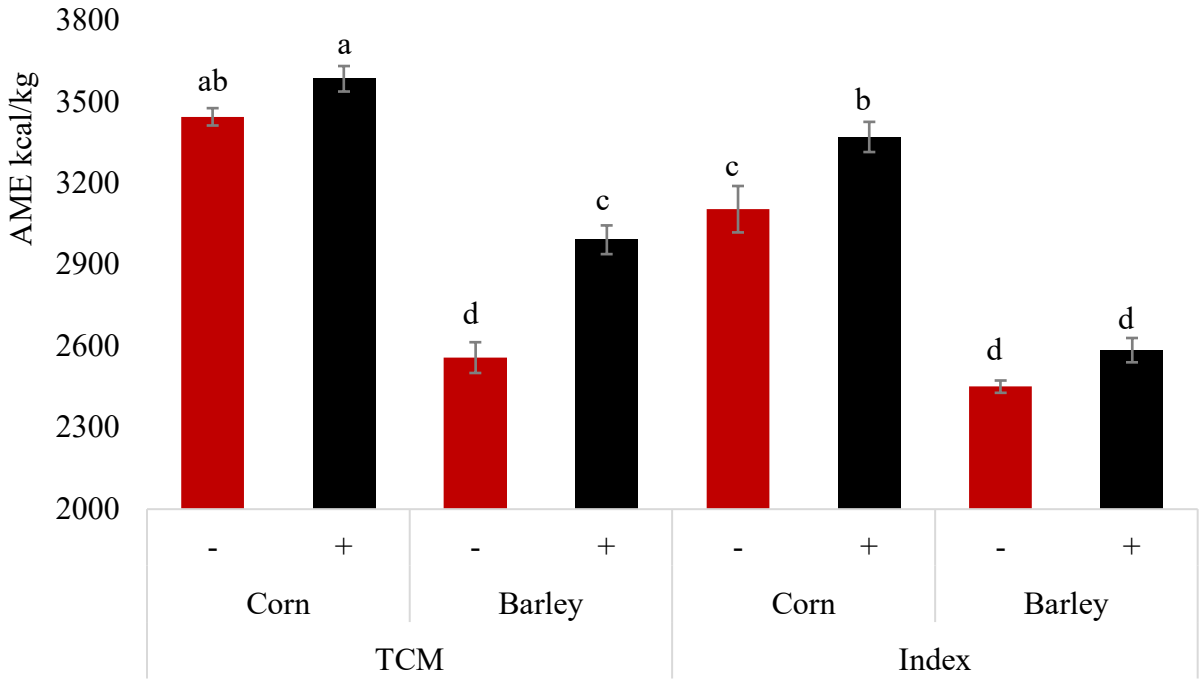

Figure 4.6: Influence of assay method and enzyme supplementation on AME of corn and barley. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 18.

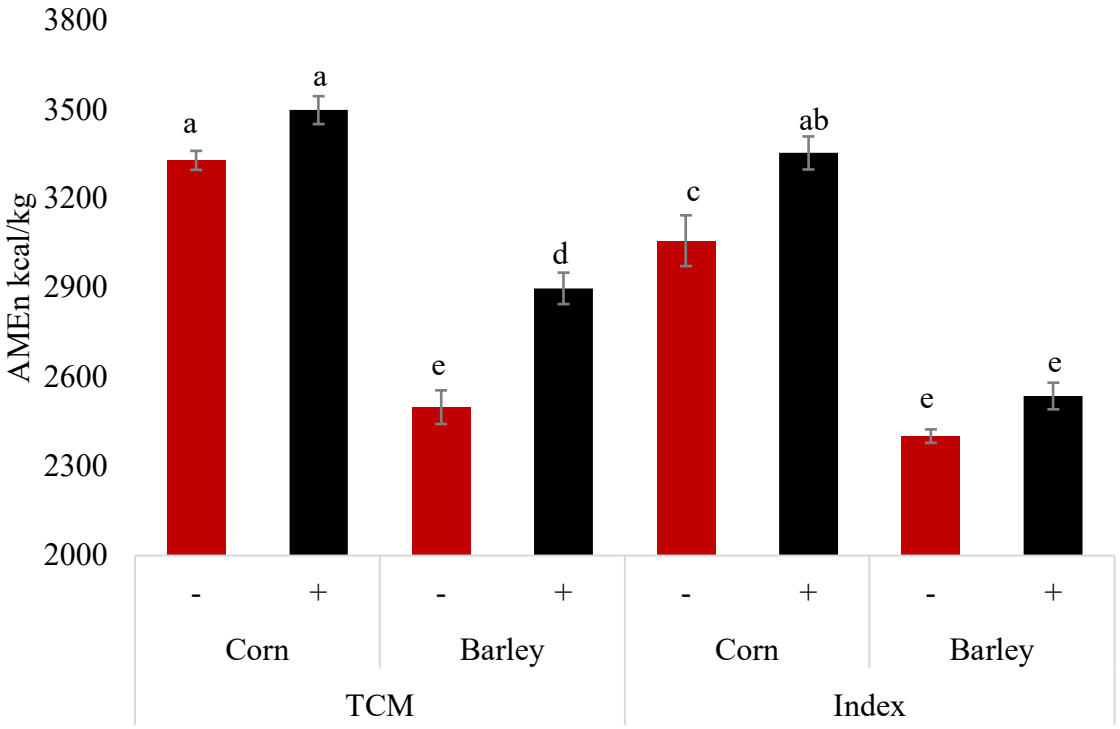

Figure 4.7: Influence of assay method and enzyme supplementation on AMEn of corn and barley. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 18.

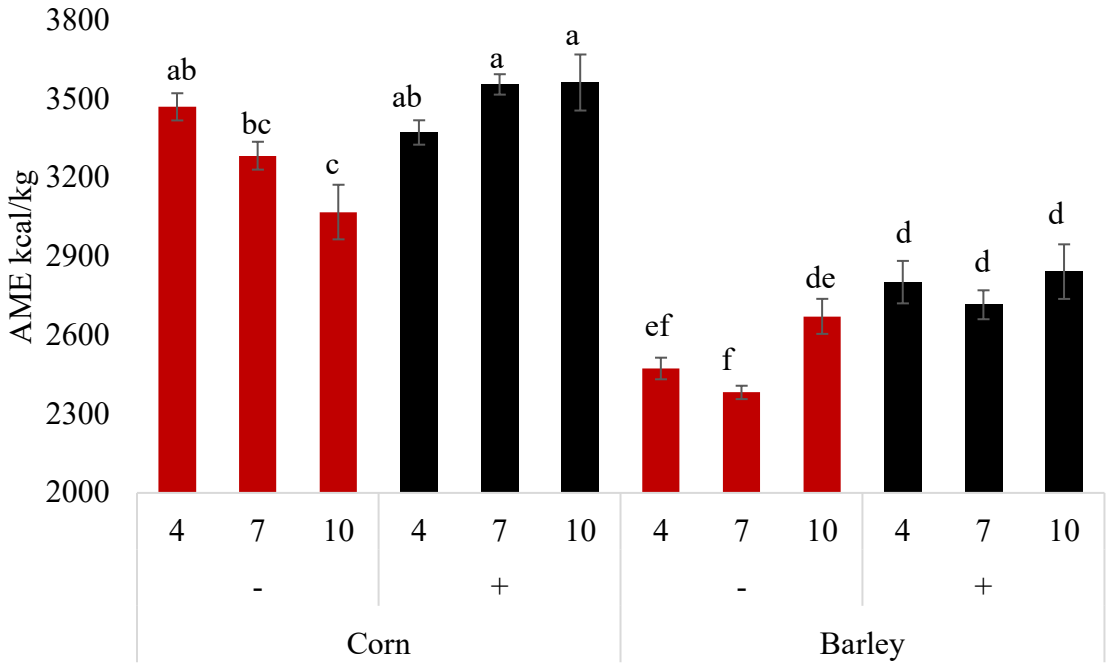

Figure 4.8: Influence of adaptation length and enzyme supplementation on AME of corn and barley. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 6.

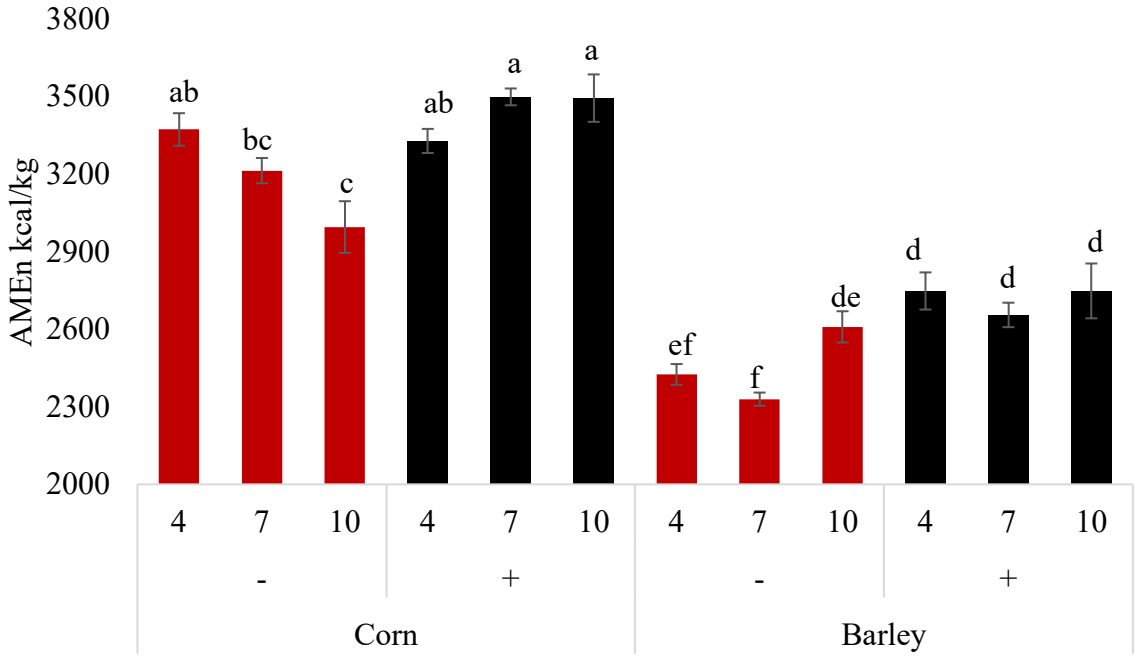

Figure 4.9: Influence of enzyme supplementation and adaptation length on AMEn of corn and barley. Birds were randomly allocated to 18 treatments in a 3 × 2 × 3 factorial with factors as 3 diets; wheat-SBM based reference diet (RD), RD with corn included at 300 g/kg, and RD with barley included at 300 g/kg inclusion level; 2 enzyme levels, without and with enzyme; 3 adaptation lengths, 4, 7 and 10. Excreta collected at day 21 was used to determine AME of diets by total collection and index method, and AME of corn and barley determined by difference method. Bars (mean ± SEM) with no common superscripts differ significantly ($P < 0.05$). '-' without enzyme' '+' with enzyme; 4, 7 and 10 are days of adaptation length. n = 6.