# Effects of Assay Methodology and Standardization for Endogenous Losses of Energy on Determination and Additivity of Metabolizable and Digestible Dietary Energy for Broilers

by

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## ABSTRACT

Efficient broiler production requires providing birds with diets with appropriate energy density. A lack of additivity of dietary energy could lead to under- or over-supplying energy to broilers. Additivity may be affected by assay methodology, standardization for endogenous losses of energy (**EEL**), or the energy calculation. A series of experiments was conducted to evaluate diet suitability for estimating EEL, determine effect of assay methodology and energy calculation on individual feed ingredients, and to assess effect of these techniques on additivity of ingredient energy in blended diets. In Chapter 3, Experiments 1 and 2 were conducted to determine a diet appropriate for estimation of EEL in broilers. Broilers were provided with semi-purified diets (8 replicate cages per dietary treatment) from 18 to 23 D of age. At 23 D of age, ileal digesta was collected and evaluated for the presence of glucose. Gross energy and $TiO_2$ concentrations were measured to determine EEL. Differences in EEL were compared utilizing ANOVA and regression. In Experiment 1 (Chapter 3), broilers were provided with diets primarily composed of dextrose, dextrose and cellulose, starch and dextrose, or starch, dextrose, and casein. In Experiment 2 (Chapter 3), broilers were provided with dextrose-based diets containing 0, 5, 10, or 15% casein. Based on these experiments, a dextrose-based diet containing 10% casein is appropriate for estimating EEL as it minimizes ileal glucose recovery ($P < 0.0001$) while allowing broilers to maintain BW ($P < 0.0001$). In Chapters 4 and 5, a series of 3 experiments was conducted to evaluate effect of methodology and calculation on additivity. In each experiment, broilers were provided with experimental diets (16 replicate cages per dietary treatment) at 18 D of age, a 48-h balance study was

conducted from 21 to 23 D of age, and ileal digesta was collected at 24 and 25 D of age. Gross energy, $TiO_2$ concentration, and CP were determined in dried feed, digesta, and excreta samples to determine AME, $AME_n$, standardized ME (**SME**), apparent ileal digestible energy (**AIDE**), and standardized ileal digestible energy (**SIDE**). Differences in energy were analyzed utilizing ANOVA and contrasts, while additivity was assessed utilizing a 1-sample 1-side T-test where $H_0=0$. Significance was considered $P \leq 0.05$, while additivity was determined when $P > 0.05$. Three experiments evaluated energy in cereal grains or soybean meal (**SBM**). Experiment 1 (Chapters 4 and 5) determined energy of corn and wheat or SBM utilizing the direct method. Experiment 2 (Chapters 4 and 5) determined energy of corn or SBM utilizing the direct method as well as the substitution method at two substitution rates (15 and 30% for corn; 10 and 20% for SBM). Experiment 3 (Chapters 4 and 5) determined the energy of corn or SBM utilizing the direct method and the substitution method (20 and 30% substitution for corn and SBM, respectively). The direct method underestimated the determined energy of both corn and SBM compared with the substitution method ($P \leq 0.0032$). In Chapter 6, 2 experiments evaluating effects of assay methodology and energy calculation on additivity of ingredients were conducted. Additivity was determined ($P \geq 0.08$) using AME, SME, AIDE, and SIDE when determined based on the substitution method, but not ($P \leq 0.0019$) when determined based on the direct method. These experiments indicated that methodology affects determined energy of corn and SBM, which then impacts the additivity of energy. However, standardization for EEL did not provide energy values more additive than apparent values.

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## LIST OF ABBREVIATIONS

**AA**.....Amino acid

**AIDE**..... Apparent ileal digestible energy

**AME** ..... Apparent metabolizable energy

**AME$_n$**.....Nitrogen-corrected apparent metabolizable energy

**ANOVA** .....Analysis of variance

**BW**.....Body weight

**CP** ..... Crude protein

**DE**..... Digestible energy

**DEB** ..... Dietary electrolyte balance

**dLys**.....Digestible lysine

**DM**.....Dry matter

**dMet** .....Digestible methionine

**dThr**.....Digestible threonine

**EAAL** .....Endogenous losses of amino acids

**EEL**.....Endogenous losses of energy

**EL** ..... Endogenous losses

**GE**..... Gross energy

**ME** .....Metabolizable energy

**SBM** .....Soybean meal

**SEM**.....Standard error of the mean

**SIDE** ..... Standardized ileal digestible energy

**SME**..... Standardized metabolizable energy

# I. INTRODUCTION

Dietary energy affects broiler production as it is required for essential maintenance functions as well as production (Leeson and Summers, 2001). Thus, providing broilers with appropriate energy balance is essential for efficient growth. However, feed costs represent between 50 to 70% of live production costs (Skinner et al., 1992), with dietary energy comprising up to 70% of feed costs (Donohue and Cunningham, 2009). Due to rising costs of energy and commodities, compounded with the competition between utilization of commodities for feed, fuel production, or human food, energy costs are expected to continue to increase. Additionally, the broiler market is producing increasingly heavier birds that respond to higher energy density more than smaller market weight broilers do, further increasing the importance of appropriate dietary energy balance. Therefore, efficient production requires feeding broilers diets formulated to contain the lowest energy density possible, while maintaining desired performance.

Accurate formulation for any dietary component requires knowing the response of the bird as well as the nutrient content of the available feed ingredients and the availability of those nutrients. While total nutrients may be evaluated in vitro, nutrient availability is typically evaluated in vivo in broiler chickens. Available energy is typically determined post-cecal in broilers as AME, equal to gross energy minus fecal and urinary energy (Wu et al., 2020). While this is the most common measurement of energy utilized in databases of feedstuffs, it has some problems, including high variability, lack of additivity, sensitivity to feed intake, and lack of sensitivity to enzyme effects (Wu et al., 2020). The high variability of the assay may lead to inaccurate energy values and could cause the

formulation of diets with a higher or lower energy balance than optimal. A lack of additivity would indicate interactions between ingredients where the energy contribution of an ingredient is affected by other ingredients or dietary factors (Dale and Fuller, 1980). Apparent ME is uncorrected for endogenous losses of energy (**EEL**) such as sloughed epithelial cells and mucins. Standardization, or correcting for EEL, may help to reduce variation and improve additivity of energy assays (Jonsson and McNab, 1983; Kong and Adeola, 2013). Due in part to the variability and lack of additivity of AME values, effects of carbohydrases can be difficult to detect. With the increase in use of carbohydrases, there is increased need to be able to evaluate the efficacy of these enzymes.

While much research is available discussing energy determination assays (Mateos et al., 2019; Wu et al., 2020), limited data directly compare different energy assay methodologies. The effect of experimental diet as well as calculation method on determined energy should be evaluated for different feed ingredients. Furthermore, while there are assays designed to measure EEL, these assays typically require fasting broilers and may not be practical with modern welfare concerns, resulting in a need for different procedures. Despite the additivity of energy from feed ingredients being a fundamental assumption of formulation, the effects of assay methodology on additivity of energy have not been well studied. With the rising cost of energy-contributing ingredients and the increased importance in dietary energy with the shift towards production of larger broilers, it is important to improve the measurement of dietary energy for more accurate formulation. The current research was designed to evaluate energy assay methodologies that may alleviate some of the inaccuracies with the current AME assay. This included

assessment of standardization for EEL, digestible energy, and dietary methodology, and also evaluated the effect of those techniques on additivity of ingredients.

# II. LITERATURE REVIEW

## ENERGY IN BROILER PRODUCTION

Dietary energy is essential for physiological functions in broilers including basal metabolism, immune function, thermoregulation, growth, and muscle accretion (Leeson and Summers, 2001). It has been demonstrated that increased dietary energy can increase BW gain in broilers (Dozier et al., 2011) and can decrease feed conversion ratio (Johnson et al., 2020). However, the cost of supplying dietary energy accounts for approximately 50 to 70% of feed ingredient costs (Skinner et al., 1992), while feed costs account for up to 69% of live production costs (Donohue and Cunningham, 2009; Noblet et al., 2021). Furthermore, energy sources are a limited resource and are utilized for human food, animal feed, and fuel (Noblet et al., 2021). Therefore, broilers should be precisely provided with energy necessary to maximize performance objectives and economic outcomes. This can be accomplished by adequately providing the nutrients needed by the bird. Precision formulation requires knowing the nutrient or energy need of the bird, which can be obtained through conducting response studies. It also requires measuring the total nutrient or gross energy (**GE**) of the ingredient in vitro, and determining the amount of the energy or nutrient that the bird can utilize. While this can be estimated well in vitro in ruminants (Getachew et al., 2002), in vitro assays are not sufficiently defined to estimate digestible energy or ME for broilers (Zaefarian et al., 2021). Thus, in vivo assays must be used to determine nutrient utilization or energy balance for broilers.

### *Energy in Broiler Diets*

Energy is not a nutrient itself, but rather a property of other nutrients (van der Klein et al., 2020). Energy is measured as heat and can be obtained from catabolism of carbohydrates, protein or amino acids (**AA**), and lipids. While oil and carbonaceous ingredients are primarily fed to provide energy (Leeson and Summers, 2001), energy is also obtained from proteinaceous ingredients, supplemental AA, and other ingredients.

Nutrients from the ingredients are digested in the mouth, proventriculus, gizzard and small intestine until they are in a form that can be absorbed (Cunningham and Klein, 2007), primarily monosaccharides (Shim et al., 2003), free AA or di- or tri-peptides (Krehbiel and Matthews, 2003), or free fatty acids and 2-monoglycerides (Sklan, 1979) for carbohydrates, proteins, and lipids, respectively. These nutrients are absorbed through specialized active transport systems using the $Na^{+}K^{+-}$ATPase pump, secondary mechanisms utilizing ionic gradient from sodium to facilitate absorption, sodium co-transport, or passive diffusion (Cunningham and Klein, 2007). Nutrient absorption can also be altered by environmental factors such as temperature (Larbier et al., 1993) or through disease challenges (Persia et al., 2006). Because of the variety of nutrients utilized for energy, the interaction between nutrients that affect the digestion and absorption of other nutrients, and the influence of environmental factors and disease, many factors can affect energy absorption. This in turn affects determined energy values.

## ENERGY ASSAY METHODOLOGY

### *Energy Partitioning*

Bioassays are utilized to measure the energy of feed ingredients and diets for poultry. Various methods have been utilized to determine energy utilization of broilers, but essentially all methods compare the caloric intake with the caloric output, leading to the assays collectively being called balance assays.

Energy assays are based on the concept of energy partitioning, as originally described by Armsby and Fries (1915). Briefly, the GE of a feedstuff is determined using calorimetry. Digestible energy is GE less fecal energy, while ME is DE less urinary and gaseous energy. Metabolizable energy is partitioned into the heat increment, or heat used for digestion, and then net energy. Net energy is the energy available for maintenance and production. Metabolizable energy is most commonly used for broilers (Wu et al., 2020); thus, an advantage of that system is its wide recognition and large literature base. However, DE has the advantage over ME of being prececal, which minimizes variance caused by microbial activity, and providing values that are less affected by contamination than excreta (Wu et al., 2020). Net energy is not commonly used in diet formulation for broilers due to the relatively small proportion of energy that the heat increment represents, as well as the resources required to measure net energy.

### *Balance Assay Procedure*

An assay for determining ME was described by Hill and Anderson (1958) and Hill et al. (1960). This method related the energy in feed consumed to the energy in excreta and is still commonly used to determine AME. This method was expanded on by Sibbald (1975) using predetermined feed allotments and evaluating endogenous losses of energy

(**EEL**). Farrell et al. (1978) further modified the AME assay using trained cockerels. Mateos et al. (2019) described the typical balance assay procedure as follows. Test diets are provided to broilers for several days for acclimation. During the collection period, feed disappearance and excreta output are quantified, and excreta samples are collected. Gross energy of the feed and excreta samples are evaluated on a DM basis using bomb calorimetry, and the total energy intake and excretion are calculated using the measured feed disappearance and excreta weight. While the general premise of balance studies is simple, there are many factors that must be determined when conducting a balance study. An appropriate balance assay design should be selected to provide the test ingredient. Several experimental designs are available for AME or $AME_n$ balance studies, including the direct method, utilization of ingredients with known ME, difference or substitution method, or regression method (Wu et al., 2020; Khalil et al., 2021). In the direct method, the test ingredient is the only ingredient fed (Sibbald, 1976). Hill and Anderson (1958) suggested using a basal diet with an inclusion of an ingredient with a known ME, such as glucose, and substituting in the test ingredient for the known ingredient. A similar method (Carpenter and Clegg, 1956) suggests feeding diets composed of only ingredients with known ME such as glucose blended with the test ingredient, which eliminates the need for a basal diet as in the method suggested by Hill and Anderson (1958). Another method utilizing basals is called the difference or substitution method. It evaluates energy in a practical corn and soybean-meal (**SBM**) basal diet compared with a diet where a percentage of the basal diet was compared with the test ingredient (Sibbald and Kramer, 1978). Two final methods are utilization of simple (Dozier et al., 2008; Adeola and Ileleji, 2009) or multiple (Young et al., 1977; Noblet et al., 1993) linear regression to determine energy.

These methods, as well as other concerns with energy determination assays, are thoroughly discussed in a review by Wu et al. (2020).

Beyond experimental design, diet preparation, including nutrient profile, inclusion rate of test ingredient and interactions between feed ingredients, as well as feed form, should be considered when determining energy utilization. Maximum test ingredient inclusion rate when using the substitution method is determined based on palatability and nutrient balance (Lopez and Leeson, 2008), and the inclusion rate may influence determined energy. Adeola and Ileleji (2009) fed broilers diets varying in inclusion of dried distillers' grains with solubles in either corn- and SBM-based diets or semi-purified diets and indicated that utilization of semi-purified diets may overestimate AME and $AME_n$ compared with utilization of corn- and SBM-based diets. These authors suggested this effect may be due to associative effects of the semi-purified diet and cautioned that diet type may affect determined energy. Balance studies are typically conducted with mash diets (Lee and Kong, 2019), but Khalil et al. (2021) noted that feed form affects determined $AME_n$, with mash diets potentially underestimating $AME_n$ of cereals compared with feeding pelleted diets.

The feeding method is important as feed intake affects determined AME due to the effect of EEL (Guillaume and Summers, 1970; Sibbald, 1975), as well as due to the potential for feed wastage or measurement error when feeding ad libitum (Wu et al., 2020). Furthermore, diet composition directly affects the glucose absorption of the bird and should therefore be chosen carefully. Wu et al. (2020) described different dietary methods available for determining energy. Direct feeding provides only the test ingredient. Standard ingredient substitution substitutes the test ingredient into an ingredient such as dextrose

with a known energy value. Basal substitution measures energy of a basal diet and a basal diet substituted with the test ingredient simultaneously. Multiple linear regression substitutes an ingredient into a basal diet at increasing inclusions. Additional methods include the direct plus method, or the test ingredient plus vitamins and minerals; and the multiple linear regression method (Noblet et al., 1993).

*True Metabolizable Energy Rooster Assay and Standardization*

Energy values can be expressed as apparent, true, or standardized. Apparent ME is the most common, while much work by Sibbald (1976, 1978) described true energy. True or standardized ME or DE can be calculated by determining EEL. Multiple methods exist for this determination, including fasting the birds (Sibbald 1976), gavaging the birds with a glucose solution (McNab and Blair, 1988), or feeding a semi-purified diet (Khalil et al., 2020). All methods assume that no exogenous feed reaches the terminal ileum so that the contents of the terminal ileum can be used to estimate EEL. In the precision-fed rooster assay, broilers are fasted for 24 hours, gavaged with 30 g test diet or ingredient, and excreta is collected quantitatively for 48 hours (Parsons et al., 1982). Feed and excreta or digesta are analyzed for moisture, energy, and possibly nitrogen to calculate TME.

*Nitrogen correction*

One correction unique to energy is the correction for nitrogen excretion, commonly studied as $AME_n$. The purpose of this metric is to correct the birds to a N equilibrium for comparison (Hill and Anderson, 1958), based on the assumption that oxidation of protein will lead to the production of uric acid, and that this oxidation occurs at a rate that varies with growth (Lopez and Leeson, 2007). A review by Lopez and Leeson (2007) suggested that the value of using $AME_n$ as compared with AME is dependent on bird age and the feed

ingredient being evaluated. As expected, those authors found that adult roosters had higher $AME_n$ values than growing broilers, while the reverse was found with AME. Additionally, these authors reported that $AME_n$ did reduce variation of energy in feed ingredients. Crude protein content of the test ingredient did affect the degree of penalty for N correction. As with correction for EEL, nitrogen-corrected energy is also determined as a calculation utilizing the N in excreta, so N-corrected and uncorrected can both be determined with any ME sample.

### *Confounding Variables in Energy Assays*

Innate variability exists with energy utilization based on the digestive capacity of individual birds, which is increased in ingredients that are less digestible (Cowieson et al., 2020). This innate variability is compounded by differing methodology in the literature and between laboratories (Ravindran et al., 2017), including diet formulation, ingredient inclusion, feed form, marker, adaptation period, bird age, collection method, collection location, and drying method. Further variability is introduced with the lack of consistent methodology in estimating endogenous losses (**EL**) (Adedokun et al., 2011). Additionally, the diet composition may have a substantial effect on determined AME due to interactions between ingredients, either additive or anti-nutritive. Variation is introduced in apparent ME or DE due to variations in feed intake (Guillaume and Summers, 1970), and in ME due to feed, water, or feather contamination of excreta (Khalil et al., 2020; Wu et al., 2020).

## ADDITIVE AND ASSOCIATAVE EFFECTS OF ENERGY

Broiler diets are formulated assuming additivity, or that the energy of the complete diet is equal to the sum of the energy of the components (Sibbald et al., 1960). However,

it is established that broiler diets are not additive in that there are interactions between ingredients where one ingredient affects the absorption of another (Dale and Fuller, 1980). Additivity is contrasted to either an associative effect, where the determined energy of the diet is greater than the calculated energy, or an antinutritive effect, where the sum of the determined energy is lower than the calculated energy. This can be assessed by determining the energy of multiple feed ingredients and a diet composed of those ingredients and calculating the predicted energy of the blended diet using the energy of the individual feed ingredients. Babatunde et al. (2020) described the statistical analysis for determining additivity as follows: $H_0$: Determined – Predicted = 0; $H_1$: Determined – Predicted ≠ 0; and $P > 0{:}05$ = Additive. Dale and Fuller (1980) evaluated additivity of feed ingredients and demonstrated that a diet composed of corn, SBM, and poultry biproduct meal had higher determined energy than predicted, while diets that contained corn, SBM, and either corn gluten meal or fish meal had lower determined energy than predicted.

The assumption of additivity is important for energy determination assays in formulating commercial diets. If energy is measured utilizing the substitution method, the resulting values are only valid when there is additivity between the test ingredient and the basal diet (Hong et al., 2001; Aardsma and Parsons, 2017). If ingredients are not additive for formulation in practical settings, the energy value is only accurate in the conditions under which it was measured (Aardsma et al., 2017), limiting the usability of data.

Reasons for a lack of additivity may include physical or chemical effects. An example of a physical affect is fat inclusion (Mateos et al., 1980; Golian and Maurice, 1992), where increasing the dietary fat may lead to an extra-caloric effect. Conversely, diets containing high concentrations of non-starch polysaccharides may exhibit an antinutritive effect where

the fiber limits the absorption of other nutrients (Jorgensen et al., 1996). Furthermore, chemical properties of diets, such as the dietary ion concentration, may affect additivity as many nutrients utilize Na and K for active transport (Sklan and Noy, 2000; Ravindran et al., 2008). Therefore, a diet deficient in Na exhibits reduced determined energy compared with a diet with adequate Na. Similarly, data may indicate that the carbohydrate profile of the diet affects absorption (Khoddami et al., 2018; Chrystal et al., 2020).

An additional factor that may cause a lack of additivity is EEL. Endogenous losses of energy cause AME to be affected by feed intake while TME, which is corrected for EEL, is not sensitive to feed intake (Jonsson and McNab, 1983). Thus, standardization may lead to values less sensitive to feed intake and more additive. Kong and Adeola (2013) demonstrated that standardized digestibility of some AA are more additive than their respective apparent AA digestibility; therefore, a similar effect may be observed with EEL. These mechanisms lend support to measuring energy in assays that utilize complete diets such as the substitution or regression methods. In addition to improving accuracy of formulation, improved additivity may lead to assays that are more sensitive to enzyme effects.

## STANDARDIZATION OF ENDOGENOUS LOSSES OF ENERGY

Energy balance assays assume that all excreted components, whether in the ileal digesta or the excreta, are from the feed. This is not the case, as the digesta and excreta both contain energy of endogenous origin. Endogenous losses of energy represent non-dietary losses comprised of mucin, sloughed epithelium, digestive enzymes, or other endogenous sources (Nyachoti et al., 1997), and may be as high as 347 kcal/kg DM intake

for broilers (Khalil et al., 2020). Total EL can be divided into basal or specific losses. Basal losses are not related to diet composition and are independent of feed intake (Rodehutscord et al., 2004; Stein et al., 2007. Specific losses are caused by ingredient characteristics such as fiber or antinutritional factors and increase with feed intake (Schulze et al., 1995; Jansman et al., 2002; Lemme et al., 2004). True ileal digestibility corrects for both specific and basal EL, while standardized digestibility only corrects for basal losses (Stein et al., 2007). While these terms are not the same, standardized digestibility is sometimes called true digestibility. The purpose of both standardized and true digestibility is to correct for the effects of feed intake on determined energy. Thus, these values should be more additive in formulation (Stein et al., 2005).

To quantify EL, semi-purified diets that are either devoid of the nutrient of interest or are highly digestible must be fed (Adedokun et al., 2011; Khalil et al., 2020). These diets may contain corn starch, dextrose, casein, minerals, and other ingredients, depending on the objectives of the study. As it is impractical to feed an energy-free diet, EEL are quantified utilizing a highly DE diet composed primarily of dextrose or by utilizing fasted roosters.

Much of the early research into developing an assay for determining EEL was conducted by Sibbald and colleagues in the 1970s and 1980s. The basis for EEL was developed due a study demonstrating that AME with increased feed intake (Sibbald, 1975). The author hypothesized that metabolic fecal energy and endogenous urinary energy had a proportionally greater effect at low feed intake and suggested developing a new assay to adjust for this effect. The following year Sibbald described an assay for TME (1976), which corrected AME for EEL by fasting roosters for 24 hours and then force-feeding a

predetermined allotment of dextrose. As dextrose is considered completely digestible, the excreta from that period should approximate the EL and can be used to correct the AME of roosters.

A series of studies utilizing Single-Combed White Leghorn roosters evaluated factors that may affect endogenous flow rates. Sibbald and Price (1978) evaluated roosters of varying BW and determined that BW only accounted for 23% of the variation in endogenous flow. Similarly, Sibbald (1978) observed no relationship between endogenous flow rate and age of roosters ranging from 24 days of age to adult. Another study utilized diets with varying concentrations of sand and cellulose and demonstrated that neither fiber nor abrasive feed ingredients led to consistent trends in EEL (Sibbald, 1980). The large variation between endogenous flow rates of birds indicates that standardization for EL could reduce variability when determining DE or ME.

***Components of Endogenous Losses of Energy***

Endogenous losses are composed primarily of epithelial sloughing and digestive secretions, primarily mucin (Adedokun et al., 2011). Epithelial sloughing is constantly occurring, with enterocytes typically turning over every 3 to 5 days (Ghiselli et al., 2021). It can be either apoptosis, which is non-inflammatory and occurs under normal physiological conditions, or necrosis, which occurs under pathological conditions (Ramachandran et al., 2000). Necrotic epithelial sloughing should be minimized when measuring EL, as EL should be estimated under normal physiological conditions (Cowieson and Ravindran, 2007).

Intestinal mucus functions primarily to protect the epithelium from pathogens (Honda and Takeda, 2009) and from gastric acid (Rocco et al., 2006), and to aid in

controlling the passage of nutrients (Moran, 1982; Macierzanka et al., 2019). The AA profile of EL indicate that mucin is a key component of endogenous flow (Moran, 2016), as EL and mucin have similar AA composition (Lien et al., 1997; Ravindran et al., 2004; Golian et al., 2008; Soleimani et al., 2010). Therefore, factors influencing mucin production, including intestinal health or disease (Cook and Bird, 1973; Collier et al., 2008), probiotic supplementation (Aliakbarpour et al., 2012; Tsirtsikos et al., 2012) and dietary threonine inclusion (Horn et al., 2009) may influence the extent of EEL.

***Factors Affecting Endogenous Losses of Energy***

While limited data delineate factors affecting EEL, many papers describing factors that may affect endogenous AA losses (**EAAL**). Endogenous AA losses measure the AA composition of the endogenous flow, while EEL measures the energy of the endogenous flow. Therefore, it is reasonable to expect that factors that increase EAAL will also increase EEL. Many factors may influence EL including age, strain, health status of the bird, diet composition, and DM intake. Younger chicks have increased EL than older chicks (Ravindran and Hendriks, 2004a; Adedokun et al., 2007), such that Barua et al. (2021) demonstrated that EL decrease quadratically with age. Conversely, utilization of chicks, roosters, and laying hens led to different AA composition of losses but did not affect total endogenous N losses (Ravindran and Hendriks, 2004b). Endogenous losses have been reported to increase linearly with *Eimeria* challenges (Teng et al., 2021), as intestinal diseases increase mucin production and turnover (Collier et al, 2008; Adedokun et al., 2012), thus increasing endogenous flow. Adedokun et al. (2011) reviewed factors affecting endogenous flow and suggested that increased mucin turnover can be caused by increased dietary fiber or increased antinutritive factors including phytate in addition to disease

challenges. Similarly, EAAL may increase with higher dietary protein, due to the increased enzyme production (Adedokun et al., 2007). Additionally, diet composition, including N content, Ca content, dietary electrolyte balance, and starch:dextrose ratios can affect EL (Adedokun et al., 2018; Adedokun et al., 2019; Chrystal et al., 2020; Zhou et al., 2022). The aforementioned studies indicate that diet, age, and health status affect EL. An implication of these results is that methodology does affect estimation of EL, and thus should be chosen carefully.

## KNOWLEDGE GAPS

While a variety of papers discuss assay methodology for determination of energy, data evaluating effect of assay methodology on determined energy are limited. Wu et al. (2020) and Mateos et al. (2019) delineated many of the flaws of the current energy literature, including a lack of consistency in both terminology and methodology. The authors indicate that further research evaluating energy determination assays is warranted.

Diet formulation for broilers is trending towards utilization of standardized values. Standardized values are commonly used for AA (Bryden and Li, 2010) and work is being conducted to evaluate methods for standardizing mineral utilization (Walk et al., 2021). Therefore, in order to use consistent values throughout, standardized energy values should be used. Thus, research evaluating methodology for evaluating EEL is warranted. While there are data describing methodology for standardization of energy employing the TME assay (Sibbald, 1976), this methodology has some shortcomings. The assay utilizes roosters that have been fasted 24 hours and may lead to errors in estimation due to altered physiological state of the bird. Furthermore, welfare guidelines for research are becoming

stricter, limiting the use of fasting (Noblet et al., 2021). This warrants evaluation of alternative methods of estimating EL that do not involve feed restriction.

Much research has assessed factors affecting EAAL (Adedokun et al., 2011). Many of these factors affecting EAAL, such as protein or AA content, dietary electrolyte balance, feed intake, and starch to dextrose ratios, may also affect EEL, but information is sparse evaluating factors affecting EEL. Diets for the determination of EEL must contain completely digestible energy, a constraint that does not apply to diets for estimation of EAAL. Therefore, research should be conducted to elucidate an optimal diet for determination of EEL.

After determination of an appropriate diet for EEL estimation, research should evaluate effects of standardization on additivity and error. As TME increases additivity of ingredients by removing the effect of feed intake (Stein et al., 2007), it would follow that standardized ME or DE would be more additive than apparent ME or DE. As EL are highly variable, standardization should reduce variability between birds and studies compared with AME or apparent DE, but this concept has not been evaluated.

Sibbald et al. (1960) reported that additivity is a foundational assumption of energy assays. However, with the exception of the effect of fat (Aardsma et al., 2017; Aardsma and Parsons, 2017), recent literature has sparse data delineating the true additivity of energy in feed ingredients. With the increased utilization of including co-products in poultry diets, it is possible that the assumption of additivity may become questioned (Flores and Castanon, 1991).

Finally, variation exists between energy assay methodology in the literature, resulting in a wide range of values (Mateos et al., 2019). This emphasizes the need for

controlling factors that may affect energy values and uniformity of methodology among research groups. Some issues with current methodologies include utilization of nutritionally imbalanced diets, variable energy of ingredients considered to have standard energy values, and calculation errors (Wu et al., 2020).

Providing birds with appropriate dietary energy is essential for efficient broiler production. Available literature regarding optimization of energy assays for broilers is incomplete. Further research delineating EEL and assay methodology on determined energy for broiler chickens is warranted. These data may allow for determination of energy values that are more additive in formulation or are more sensitive to effects of exogenous enzymes. To address these knowledge gaps, a series of experiments (Table 2.1) was designed to evaluate methodology for estimation of EEL and to evaluate different methodology and calculation methods to improve the additivity and sensitivity of energy determination assays.

## REFERENCES

Aardsma, M. P., and C. M. Parsons. 2017. A slope-ratio precision-fed rooster assay for determination of relative metabolizable energy values for fats and oils. Poult. Sci. 96:108–117.

Aardsma, M. P., R. D. Mitchell, and C. M. Parsons. 2017. Relative metabolizable energy values for fats and oils in young broilers and adult roosters. Poult. Sci. 96:2320–2329.

Adedokun, S. A., A. J. Pescatore, M. J. Ford, T. Ao, and J. P. Jacob. 2018. Investigating the effect of dietary calcium levels on ileal endogenous amino acid losses and standardized ileal amino acid digestibility in broilers and laying hens. Poult. Sci. 97:131–139.

Adedokun, S. A., A. J. Pescatore, M. J. Ford, J. P. Jacob, and A. Helmbrecht. 2017. Examining the effect of dietary electrolyte balance, energy source, and length of feeding of nitrogen-free diets on ileal endogenous amino acid losses in broilers. Poult. Sci. 96:3351–3360.

Adedokun, S. A., K. M. Ajuwon, L. F. Romero, and O. Adeola. 2012. Ileal endogenous amino acid loss: Response of broiler chickens to fiber and mild coccidial vaccine challenge. Poult. Sci. 91:899–907.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Comparison of ileal endogenous amino acid flows in broiler chicks and turkey poults. Poult. Sci. 86:1682–1689.

Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. Poult. Sci. 88:579–585.

Aliakbarpour, H. R., M. Chamani, G. Rahimi, A. A. Sadeghi, and D. Qujeq. 2012. The Bacillus subtilis and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. Asian Austral. J. Anim. 25:1285-1293.

Armsby, H. P., and J. A. Fries. 1915. Net energy values of feeding stuffs for cattle. J. Agric. Res. 3:435–491.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Responses of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. 99:3997-4008.

Barua, M., M. R. Abdollahi, F. Zaefarian, T. J. Wester, C. K. Girish, P. V. Chrystal, and V. Ravindran. 2021. Basal ileal endogenous amino acid flow in broiler chickens as influenced by age. Poult. Sci. 100:101269.

Bryden, W. L., and X. Li. 2010. Amino acid digestibility and poultry feed formulation: expression, limitations and application. Rev. Bras. Zootec. 39:279-287.

Carpenter, K. J., and K. M. Clegg. 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Sci. Food Agr. 7: 45-51.

Chrystal, P. V., A. F. Moss, A. Khoddami, V. D. Naranjo, P. H. Selle, and S. Y. Liu. 2020. Effects of reduced crude protein levels, dietary electrolyte balance and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein and amino acid metabolism. Poult. Sci. 99:1421-1431.

Collier, C. T., C. L. Hofacre, A. M. Payne, D. B. Anderson, P. Kaiser, R. I. Mackie, and H. R. Gaskins. 2008. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting Clostridium perfringens growth. Vet. Immunol. Immunopathol. 122:104–115.

Cook, R., and F. Bird. 1973. Duodenal villus area and epithelial cellular migration in conventional and germ free chicks. Poult. Sci. 52:2276-2280.

Cowieson, A. J., and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98:745–752.

Cowieson, A. J., M. M. Bhuiyan, J. O. B. Sorbara, G. Pappenberger, M. B. Pedersen, and M. Choct. 2020. Contribution of individual broilers to variation in amino acid digestibility in soybean meal and the efficacy of an exogenous monocomponent protease. Poult. Sci. 99:1075-1083.

Cunningham, J. G., and B. G. Klein. 2007. Textbook of Veterinary physiology. Fourth edition. Saunders Elsevier, Philadelphia, PA.

Dale, N. M., and H. L. Fuller. 1980. Additivity of true metabolizable energy values as measured with roosters, broiler chicks, and poults. Poult. Sci. 59:1941-1942.

Donohue, M., and D. L. Cunningham. 2009. Effects of grain and oilseed prices on the costs of US poultry production. J. Appl. Poult. Res. 18:325–337.

Dozier, W. A., III, B. J. Kerr, A. Corzo, M. T. Kidd, T. E. Weber, and K. Bregendahl. 2008. Apparent metabolizable energy of glycerin for broiler chickens. Poult. Sci. 87:317–322.

Dozier, W. A., III, C. K. Gehring, A. Corzo, and H. A. Olanrewaju. 2011. Apparent metabolizable energy needs of male and female broilers from 36–47 days of age. Poult. Sci. 90:804–814.

Farrell, D. J. 1978. Rapid determination of metabolisable energy of foods using cockerels. Br. Poult. Sci. 19:303-308.

Flores, M. P., and J. I. R. Castanon. 1991. Effect of level of feed input on true metabolizable energy values and their additivity. Poult. Sci. 70:1381-1385.

Getachew, G., G. M. Crovetto, M. Fondevila, U. Krishnamoorthy, B. Singh, M. Spanghero, H. Steingass, P. H. Robinson, and M. M. Kailas. 2002. Laboratory variation of 24 h in vitro gas production and estimated metabolizable energy values of ruminant feeds. Anim. Feed Sci. Technol. 102:169-180.

Ghiselli, F., B. Rossi, M. Felici, M. Parigi, G. Tosi, L. Fiorentini, P. Massi, A. Piva, and E. Grilli. 2021. Isolation, culture, and characterization of chicken intestinal epithelial cells. BMC Mol. Cell Biol. 22:1-14.

Golian, A., and D. V. Maurice. 1992. Dietary poultry fat and gastrointestinal transit time of feed and fat utilization in broiler chickens. Poult. Sci. 71:1357–1363.

Golian, A., W. Guenter, D. Hoehler, H. Jahanian, and C. M. Nyachoti. 2008. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. Poult. Sci. 87:706–712

Guillaume, J., and J. D. Summers. 1970. Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolizable energy. Can. J. Anim. Sci. 50:363-369.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603

Hill, F. W., D. L. Anderson, R. Renner, and L. B. Carew, Jr. 1960. Studies on the metabolizable energy of grain and grain products for chickens. Poult. Sci. 39:573–579.

Honda, K., and K. Takeda. 2009. Regulatory mechanisms of immune responses to intestinal bacteria. Mucosal Immunol. 2:187–196.

Hong, D., D. Ragland, and O. Adeola. 2001. Additivity and associative effects of metabolizable energy and amino acid digestibility in barley and canola meal for White Pekin ducks. Poult. Sci. 80:1600–1606

Horn, N. L., S. S. Donkin, T. J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. Poult. Sci. 88:1906-1914.

Jansman, A. J. M., W. Smink, P. Van Leeuwen, M. and Rademacher. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim. Feed Sci. Technol. 98:49–60.

Johnson, C. A., T. Duong, R. E. Latham, R. B. Shirley, and J. T. Lee. 2020. Effects of amino acid and energy density on growth performance and processing yield of mixed-sex Cobb 700 3 MV broiler chickens. J. Appl. Poult. Res. 29:269–283.

Jonsson, G., and J. M. McNab. 1983. A comparison of methods for estimating the metabolizable energy of a sample of grass meal. Br. Poult. Sci. 49:349-359.

Jorgensen, H., X. Q. Zhao, K. E. Knudsen, and B. O. Eggum. 1996. The influence of dietary fiber source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379–395.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, and V. Ravindran. 2021. Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. Anim. Feed Sci. Technol. 271:114754.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, F., and V. Ravindran. 2020. Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poult. Sci. 99:6809-6817.

Khoddami, A., P. V. Chrystal, P. H. Selle, and S. Y. Liu. 2018. Dietary starch to lipid ratios influence growth performance, nutrient utilisation and carcass traits in broiler chickens offered diets with different energy densities. PLoS One 13:e205272.

Kong, C., and O. Adeola. 2013. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Pekin ducks. Poult. Sci. 92:2381–2388.

Krehbiel, C. R., and J. C. Matthews. 2003. Absorption of amino acids and peptides. In D'Mello, J. R. F. Amino acids in animal nutrition— 2nd. Ed. pp. 41-71. CABI Publishing. Cambridge, MA.

Larbier, Z. M., A. M. Chagneau, and P. A. Geraert. 1993. Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. Poult. Sci. 72:289–295.

Lee, J., and C. Kong. 2019. Comparison of energy values estimated by direct and indirect methods for broiler chickens. Int. J. Poult. Sci. 18:244–248.

Leeson, S., and J. D. Summers. 2001. Scott's Nutrition of the Chicken. 4th ed. University Books, Guelph, Ontario, Canada.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J. 60:423–437.

Lien, K. A., W. C. Sauer, and M. Fenton. 1997 Mucin output in ileal digesta of pigs fed a protein-free diet. Z. Ernährungswiss. 36:182-190.

Lopez, G., and S. Leeson. 2007. Relevance of nitrogen correction for assessment of metabolizable energy with broilers to forty-nine days of age. Poult. Sci. 86:1696–1704.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Macierzanka, A., A. R. Mackie, and L. Krupa. 2019. Permeability of the small intestinal mucus for physiologically relevant studies: Impact of mucus location and ex vivo treatment. Sci. Rep—UK. 9:1-12.

Mateos, G. G., and J. L. Sell. 1980. Influence of carbohydrate and supplemental fat source on the apparent metabolizable energy of the diet. Poult. Sci. 59:2129–2135.

Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2019. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. J. Appl. Poult. Res. 28:506–525.

McNab, J. M., and J. C. Blair. 1988. Modified assay for true and apparent metabolisable energy based on tube-feeding. Br. Poult. Sci. 29:697–707.

Moran, E. T., Jr. 2016. Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation and villus integrity. J. An. Feed Sci. Technol. 221:284-303.

Moran, E. T., Jr., B. Hunter, P. Ferket, L. G. Young, and L. G. McGirr. 1982. High tolerance of broilers to vomitoxin from corn infected with Fusarium graminearum. Poult. Sci. 61:1828–1831.

Noblet, J., H. Fortune, C. Dupire, and S. Dubois. 1993. Digestible, metabolizable and net energy values of 13 feedstuffs for growing pigs: effect of energy system. Anim. Feed Sci. Technol. 42:131– 149.

Noblet, J., S. B. Wu, and M. Choct. 2021. Methodologies for energy evaluation of pig and poultry feeds: A review. Animal Nutrition. 8:185-203.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149–163.

Parsons, C. M., L. M. Potter, and B. A. Bliss. 1982. True metabolizable energy corrected to nitrogen equilibrium. Poult. Sci. 61:2241–2246.

Persia, M. E., E. L. Young, P. L. Utterback, and C. M. Parsons. 2006. Effects of dietary ingredients and Eimeria acervulina infection on chick performance, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 85:48–55.

Ramachandran, A., M. Madesh, and K. A. Balasubramanian. 2000. Apoptosis in the intestinal epithelium: its relevance in normal and pathophysiological conditions. J. Gastroen. Hepatol. 15:109-120.

Ravindran, V., A. J. Cowieson, and P. H. Selle. 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poult. Sci. 87:677–688.

Ravindran, V., and W. H. Hendriks. 2004a. Recovery and composition of endogenous protein collected at the terminal ileum as influenced by the age of broiler chickens. Aust. J. Agric. Res. 55:705–709.

Ravindran, V., and W. H. Hendriks. 2004b. Endogenous amino acid flows at the terminal ileum of broilers, layers and adult roosters. Anim. Sci. 79:265–271.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2004. Endogenous amino acid flow in the ileum: Quantification using three techniques. Br. J. Nutr. 92:217–223.

Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations. Anim. Feed Sci. Technol. 225:62–72

Rocco, A., P. Borriello, D. Compare, P. De Colibus, L. Pica, A. Iacono, and G. Nardone. 2006. Large Brunner's gland adenoma: case report and literature review. World J. Gastroentero. 12:1966.

Rodehutscord, M., M. Kapocius, R. Timmler, and A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45:85–92.

Schulze, H., H. S. Saini, J. Huisman, M. Hessing, W. Van Den Berg, and M. W. Verstegen. 1995. Increased nitrogen secretion by inclusion of soya lectin in the diets of pigs. J. Sci. Food Agri. 69:501-510.

Shim, Y. J., H. K. Doo, S. Y. Ahn, Y. S. Kim, J. K. Seong, I. S. Park, and B. H. Min. 2003. Inhibitory effect of aqueous extract from the gall of Rhus chinensis on alpha-glucosidase activity and postprandial blood glucose. J. Ethnopharmaco. 85:283-287.

Sibbald, I. R. 1975. The effect of level of feed intake on metabolizable energy values measured with adult roosters. Poult. Sci. 54:1990-1998.

Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci. 55:303–308.

Sibbald, I. R., 1978. The effect of the age of the assay bird on the true metabolizable energy values of feeding stuffs. Poult. Sci. 57:1008-1012.

Sibbald, I. R. 1980. Metabolizable energy in poultry nutrition. BioScience. 30:736-741.

Sibbald, I. R., and J. K. G. Kramer. 1978. The effect of the basal diet on the true metabolizable energy value of fat. Poult. Sci. 57:685–691.

Sibbald, I. R., and K. Price. 1975. Variation in the metabolizable energy values of diets and dietary components fed to adult roosters. Poult. Sci. 54:448-456.

Sibbald, I. R., and K. Price. 1978. The metabolic and endogenous energy losses of adult roosters. Poult. Sci. 57:556-557.

Sibbald, I. R., J. D. Summers, and S. J. Slinger. 1960. Factors affecting the metabolizable energy content of poultry feeds. Poult. Sci. 39:544-556.

Skinner, J. T., A. L. Waldroup, and P. W. Waldroup. 1992. Effects of dietary nutrient density on performance and carcass quality of broilers 42 to 49 days of age. J. Appl. Poult. Res. 1:367–372.

Sklan, D. 1979. Digestion and absorption of lipids in chicks fed triglycerides or free fatty acids: Synthesis of monoglycerides in the intestine. Poult. Sci. 58:885–889.

Sklan, D., and Y. Noy. 2000. Hydrolysis and absorption in the small intestines of post hatch chicks. Poult. Sci. 79:1306–1310.

Soleimani, A. F., A. Kasim, A. R. Alimon, A. Meimandipour, and I. Zulkifli. 2010. Ileal endogenous amino acid flow of broiler chickens under high ambient temperature. Anim. Physiol. Anim. Nutr. 94:641–647.

Stein, H. H., C. Pedersen, A. R. Wirt, and R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387–2395.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282–285.

Teng, P. Y., J. Choi, Y. Tompkins, H. Lillehoj, and W. Kim. 2021. Impacts of increasing challenge with Eimeria maxima on the growth performance and gene expression of biomarkers associated with intestinal integrity and nutrient transporters. Vet. Res. 52:1–12.

Titus, H. W. 1956. Energy values of feedstuffs for poultry. Pages 10–14 in Proc. Semiannual Meet. Nutr. Counc. Am. Feed Manufacturers Assoc., St. Louis, MO.

Tsirtsikos, P., K. Fegeros, C. Balaskas, A. Kominakis, and K. C. Mountzouris. 2012. Dietary probiotic inclusion level modulates intestinal mucin composition and mucuosal morphology in broilers. Poult. Sci. 91:1860–1868.

van der Klein, S. A. S., J. A. More-Bayona, D. R. Barreda, L. F. Romero, and M. J. Zuidhof. 2020. Comparison of mathematical and comparative slaughter methodologies for determination of heat production and energy retention in broilers. Poult. Sci. 99:3237–3250.

Walk, C. L., L. F. Romero, and A. J. Cowieson. 2021. Towards a digestible calcium system for broiler chicken nutrition: a review and recommendations for the future. Anim. Feed Sci. Technol. 276:114930.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. Poult. Sci. 99:385–406.

Young, L. G., and G. C. Ashton, and G. C. Smith. 1977. Estimating the energy value of some feeds for pig using regression equations. J. Anim. Sci. 44:765–771.

Zaefarian, F., A. J. Cowieson, Pontoppidan, M. R. Abdollahi, and V. Ravindran. 2021. Trends in feed evaluation for poultry with emphasis on in vitro techniques. Animal Nutrition, 7:268-281.

Zhou, H., W. Wu, T. Mahmood, Y. Chen, Y. Xu, Y. Wang, Y., and J. Yuan. 2022. Comparison of endogenous amino acid losses in broilers when offered nitrogen-free diets with differing ratios of dextrose to corn starch. Sci. Rep—UK. 12:1-11.

**Table 2.1.** Outline of objectives for experiments designed to evaluate methodology for estimation of endogenous losses of energy and to evaluate different methodology and calculation methods to improve the additivity and sensitivity of energy determination assays.

| Objective | Experiments | Description |
|---|---|---|
| 1. | Endogenous losses – 3.1 | Evaluate semi-purified diets and ingredients for estimation of endogenous losses of energy. |
| 2. | Endogenous losses – 3.2 | Evaluate effect of dietary amino acid density on estimation of endogenous losses of energy. |
| 3. | Cereal grain energy – 4.1<br>Soybean meal energy – 5.1<br>Additivity– 6.1 | Evaluate additivity of energy in cereal grains and soybean meal utilizing different methods of calculating energy. Energy balance was determined utilizing the direct method. |
| 4. | Cereal grain energy – 4.2<br>Soybean meal energy – 5.2 | Compare direct method of energy determination with the substitution method at two inclusion rates for corn and soybean meal. |
| 5. | Cereal grain energy – 4.3<br>Soybean meal energy – 5.3<br>Additivity– 6.3 | Evaluate additivity of corn and soybean meal using direct and substitution methods. |

# III. EVALUATION OF SEMI-PURIFIED DIETS FOR GLUCOSE RECOVERY TO ESTIMATE BASAL ENDOGENOUS LOSSES OF ENERGY IN BROILER CHICKENS

## ABSTRACT

Standardization of energy for basal endogenous losses of energy (**EEL**) may reduce variability of energy assays or increase assay sensitivity. Two experiments were designed to evaluate suitability of diets for estimating EEL by evaluating presence of glucose in the terminal ileum. In both experiments, day-old Yield Plus × Ross 708 chicks were placed into 32 battery cages (9 chicks/cage; 8 replicates/diet) and were provided common starter diets. At 18 D of age, birds were provided with semi-purified experimental diets. In experiment 1, diets primarily consisted of 1) dextrose, 2) dextrose and cellulose, 3) dextrose and starch, or 4) dextrose, starch, and casein. In experiment 2, diets were dextrose-based with 5% cellulose and 0, 5, 10, and 15% casein. Excreta samples were collected at 21 and 22 D of age. Birds were necropsied for collection of digesta at 23 D of age. Glucose recovery, $TiO_2$ concentration, and gross energy were determined in excreta and terminal ileal digesta samples and glucose content was analyzed in digesta samples. Growth performance characteristics and EEL were analyzed with ANOVA, preplanned orthogonal contrasts were utilized in experiment 1, and regression was utilized in experiment 2. In experiment 1, birds provided with the dextrose diet had insufficient digesta for analysis,

while glucose was recovered in ileal digesta of chicks provided diets containing starch ($P \leq 0.0013$). Chicks fed the diet containing casein had increased BW gain ($P < 0.0001$) compared with chicks receiving other diets. No glucose was recovered in terminal ileal digesta in chicks provided with any diet in experiment 2 ($P = 0.09$). Body weight gain was maximized in chicks provided with 10 or 15% casein ($P < 0.0001$). Casein inclusion rate did not affect EEL ($P = 0.38$). These experiments demonstrate that broilers have measurable basal EEL that may affect ingredient additivity in diet formulation, and that dextrose-based semi-purified diets with 5% cellulose and 10% casein are appropriate for estimating EEL in broilers.

## INTRODUCTION

Energy accounts for a large proportion of dietary costs for broilers (Noblet and van Milgen, 2004); therefore, it is important to properly evaluate energy contribution of ingredients. While apparent metabolizable energy (**AME**) is the most common method of energy determination for broilers (Wu et al., 2020), standardized methodology that corrects for endogenous losses is widely utilized for amino acids (**AA**) and is becoming more emphasized for minerals (Walk et al., 2021). Thus, standardization of energy to correct for endogenous losses of energy (**EEL**) should be considered for consistency with other dietary components. Endogenous losses of energy include sloughed epithelial cells and mucins, which may lead to underestimation of energy and increased variation when utilizing apparent methodologies (Wolynetz and Sibbald, 1984; Nyachoti et al., 1997). The increase in variation of determined energy may be caused by increased variation due to differences in EEL (Sibbald and Price, 1978) or differences in feed intake (Guillaume and

Summers, 1970). Sibbald (1976) proposed methodology for estimation of TME where the feed ingredient was force-fed to one group of roosters, while a second set of roosters was fasted to estimate EEL. However, methodologies that require fasting animals may become less practical due to animal welfare considerations (Noblet et al., 2021). Additionally, employing a semi-purified diet where all dietary energy is absorbed may allow for estimation of EEL under more physiologically normal conditions. Recent research utilizing a semi-purified diet for estimation of EEL demonstrated that EEL can be measured using this methodology, but the study utilized only one dextrose-based diet and was not designed to measure effect of diet on EEL. (Khalil et al., 2020). Research evaluating endogenous AA losses demonstrated that diet type affected endogenous flow rate (Golian et al., 2008; Kong and Adeola, 2013); therefore, it is reasonable to hypothesize that diet type may affect EEL.

Recent literature has not evaluated the effect of different diets on glucose recovery or estimation of EEL. The objectives of this research were to determine optimal diets for estimation of EEL in broiler chickens.

## MATERIALS AND METHODS

All experimental procedures involving live animals were approved by the Auburn University Animal Care and Use Committee (PRN 2019-3619 and 2020-3761).

### *Bird Husbandry*

Both experiments employed identical bird husbandry. In each experiment, 288 Yield Plus × Ross 708 male chicks (Aviagen North America, Huntsville, AL) were procured from a commercial hatchery at day of hatch. All chicks were vaccinated for

Newcastle disease, Marek's disease, and infectious bronchitis. Additionally, all chicks received a 1× dose of Coccivac B52® via spray cabinet at the hatchery. Chicks were reared in 32 battery cages (9 chicks per cage; 68 × 68 × 38 cm; Petersime, Gettysburg, OH). Each cage contained a trough feeder and waterer. Batteries were housed in a solid-sided room equipped with evaporative cooling pads and forced-air furnaces for temperature control. Temperature was set at 33°C at placement and decreased to a final setpoint of 25°C at 18 D of age. Photoperiod was 23L:1D and 20L:4D from 1 to 7 and 8 to 23 D of age, respectively. Chicks were provided with common starter diets from 1 to 17 D of age (Table 3.1). Birds and feed were weighed at the beginning and end of the experimental periods (18 and 23 D of age, respectively) for determination of BW gain and feed disappearance.

***Dietary Treatments- Experiment 1***

In experiment 1, chicks were randomly provided with 4 experimental diets from 18 to 23 D of age (Table 3.2). All diets were formulated to contain 0.96% Ca, 0.48% non-phytate P, and 0.20% Na, with a dietary electrolyte balance of 180 mEq. Additionally, all diets contained 0.50% $TiO_2$ as an indigestible marker. The dextrose, starch, and casein diets contained 3,060 kcal/kg AME, with the cellulose diet having a 21% decrease in AME due to the 20% inclusion of cellulose. The dextrose diet contained 93.12% dextrose as the sole energy contributing ingredient, with the balance of the diet comprised of vitamins and minerals. The cellulose diet was formulated similarly to the dextrose diet with the addition of 20% cellulose (Solka-Floc, International Fiber Corporation, North Tonawanda, NY) to evaluate the effect of reduced caloric intake. The starch diet contained a 1:1 ratio of starch: dextrose. The protein diet contained the same dextrose percentage as the starch diet but included 10% casein at the expense of starch for a starch:dextrose ratio of 0.76.

***Dietary Treatments- Experiment 2***

In experiment 2, chicks were randomly provided with 4 experimental diets from 18 to 23 D of age (Table 3.3). All diets were dextrose based with 5% cellulose and contained 0, 5, 10, or 15% casein added at the expense of dextrose. All diets contained 0.88% Ca, 0.44% non-phytate P, and 0.21% Na, with a dietary electrolyte balance that ranged from 162 to 163 mEq. Apparent ME ranged from 3,001 kcal/kg in the 0% casein diet to 3,099 kcal/kg in the 15% casein diet. Crude protein ranged from 0% in the 0% casein diet to 13.08% in the 15% casein diet. All diets contained 0.50% $TiO_2$ as an indigestible marker.

***Measurements, Sample Collection, and Chemical Analysis***

Birds were weighed at 1, 18, and 23 D of age to determine BW and BW gain. Feed disappearance was measured from 18 to 23 D of age. At 18 D of age, broilers were provided with experimental diets. From D 21 to 23, a 48-h excreta collection was conducted. Feed disappearance and total excreta voided was measured for 48-h. Multiple sub-samples were collected using weigh boats placed in the collection pans to limit contamination by feed or other foreign material and to allow for accurate determination of excreta DM. Samples were placed in plastic bags and stored at -20°C until analysis. Fifty g subsamples of excreta were measured quantitively into sample cups and were lyophilized using a VirTis Genesis 25ES freeze dryer (SP Industries, Inc., Warminster, PA). After drying, digesta and excreta were ground utilizing an electric coffee grinder (Capresso Infinity 560 burr grinder, Montvale, NJ). Gross energy was determined in dried diets in quadruplicate and in dried excreta in duplicate (800 mg per sample) utilizing an isoperibol oxygen bomb calorimeter (Model no. 6300, Parr Instruments, Moline, IA).

At 23 D of age, 8 birds per cage were randomly selected and euthanized utilizing $CO_2$ asphyxiation in accordance with the guidelines of the American Veterinarian Medical Association (American Veterinarian Medical Association, 2013) for collection of ileal digesta. Following asphyxiation, chicks were cervically dislocated to prevent reversibility of euthanasia. Digesta samples were collected from the duodenum, jejunum, upper 2/3 of the ileum, and terminal ileum by gently flushing the tract sections with distilled, deionized water (Adedokun et al., 2011). The terminal ileum was defined as 2 cm proximal to the ileocecal junction to 1/3 of the way from the ileocecal junction to Meckel's diverticulum (Kluth et al., 2005). Digesta samples were pooled by cage and stored at -20°C until analysis. Digesta was lyophilized in a VirTis Genesis 25ES freeze dryer (SP Industries, Inc., Warminster, PA). Gross energy of digesta samples was determined in duplicate while GE of diets was determined in quadruplicate (0.800 g samples) utilizing an isoperibol oxygen bomb calorimeter (Model no. 6300, Parr Instruments, Moline, IA). Titanium dioxide concentration was determined in feed in quadruplicate and in digesta in duplicate according to the method described by Short et al. (1996). Samples were ashed in a muffle furnace (Thermo Scientific Thremolyne Muffle Ashing Oven F30400, Waltham, MA) at 580° C for 12 h. Ashed samples were rinsed into beakers with 10 mL of 7.4 M HCl and were heated on hotplates at 200° C until sample was dissolved and solutions were clear. The solution was then rinsed with 10 mL water into another beaker containing 20 mL 30% hydrogen peroxide and 60 mL water. After allowing color to develop for 48 hours, samples were measured for absorbance in a spectrophotometer (SpectraMax Plus 384, Molecular Devices LLC, San Jose, CA) at 410 nm. Titanium concentration was calculated by comparing absorbance with standards utilizing simple linear regression.

Glucose presence was determined qualitatively in digesta and excreta samples prior to lyophilization. Glucose presence was determined utilizing Quantofix glucose test strips (CTL Scientific Supply Corp., Deer Park, NY) as a method of verifying glucose absorption without consuming sample. The primary of measurement was verification of whether or not exogenous glucose was present.

***Calculations***

Endogenous losses were calculated according to the following equation using excreta:

$$EEL\ \left({}^{kcal}/_{kg\ DMI}\right) = \frac{GE_{dried\ excreta}\ \times (Excreta\ (kg) \times Excreta\ DM\%)}{FD, kg\ DMI}$$

where EEL in kcal/kg DM intake is calculated as caloric output divided by feed disappearance on a DM basis. Caloric output was calculated as the GE of the dried excreta multiplied by the total excreta weight during the 48-h collection period multiplied by the DM of the excreta.

Endogenous losses were calculated according to the following equation using digesta:

$$EEL\ \left({}^{kcal}/_{kg\ DMI}\right) = GE_{digesta}\ \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}}\right)$$

where EEL in kcal/kg DM intake is equal to GE of lyophilized digesta, multiplied by the ratio of $TiO_2$ in the diet divided by the $TiO_2$ in the digesta.

With the exception of the dextrose diet in Experiment 1, all diets contained cellulose. As cellulose is completely indigestible for broilers, EEL should be adjusted for

the cellulose content. To correct the equations in the diets containing exogenous cellulose the following equation was utilized:

$$EEL_{corrected}\left({}^{kcal}/_{kg\ DMI}\right) = EEL - \left(\frac{Cellulose\ inclusion, \%}{100} \times GE_{cellulose}\right)$$

where the corrected EEL in kcal/kg DM intake is equal to the uncorrected EEL minus the energy contribution from the dietary cellulose.

***Statistical Analysis***

Both experiments were arranged as randomized complete block designs where pen location was the blocking factor and individual cages were the experimental unit. In each experiment, measurements were composed of 8 replicate cages per treatment, and analyses were performed in duplicate. In both experiments, glucose recovery in the terminal ilium was analyzed using one-sample one-tail T-test where $H_0$=0. Differences between proportions of cages from which digesta samples were positive for glucose recovery were determined utilizing Fisher's Exact Test. Mortality percentages were arcsine transformed prior to statistical analysis. All statistical significance was considered at $P \leq 0.05$.

In Experiment 1, differences in growth performance and EEL between the 4 dietary treatments were evaluated utilizing ANOVA (SAS 9.4, 2016) using the following mixed effects model:

$$Y_{ij} = \mu + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu$ represents the overall mean; $\rho_i$ is identically and independently normally distributed random block effects with a mean 0 and variance $\sigma^2$; $\tau_j$ is the random effect of treatment $j$ with mean 0 and variance $\sigma^2$; and $\varepsilon_{ij}$ represents the random error that is independently and normally distributed with mean 0 and variance $\sigma^2$. Additionally,

preplanned orthogonal contrasts were planned between the dextrose and cellulose treatments to determine the effect of adding dietary cellulose, between the dextrose and starch treatments to determine the effect of starch:dextrose ratio, and between the starch and protein diets to determine the effect of casein.

Differences in growth performance and EEL in Experiment 2 were analyzed utilizing simple linear regression based on casein inclusion percentage using PROC MIXED in SAS (SAS 9.4, 2016) using the following model:

$$Y_i = \beta_0 + \beta_1 x_1 + \varepsilon_i$$

where $\beta_0$ and $\beta_1$ represent the intercept and the slope, respectively, which are fixed but unknown parameters, $x_1$ represents the casein inclusion, and $\varepsilon_i$ is the random error that is independently and normally distributed with mean 0 and variance $\sigma^{2.}$ The casein inclusions were evenly spaced (0, 5, 10, or 15%) to allow for normal distribution of data. Additionally, ANOVA was conducted using PROC MIXED (SAS 9.4, 2016) according to the model in Experiment 1.

## RESULTS AND DISCUSSION

### *Diet Analysis, Experiment 1*

Chemical analyses of diets utilized in Experiment 1 (Table 3.2) were in agreement with calculated values, with the exception of the analyzed cellulose concentrations in the starch and protein diets. Analyzed cellulose concentrations were 16.4 and 17.0% in the starch and protein diets, respectively, compared with the expected values of 9.9 and 10.5%. The increased cellulose concentration could cause an overestimation of EEL due to a higher content of indigestible feedstuffs in the excreta if formulated values were utilized to correct

EEL. Therefore, analyzed values were utilized EEL for cellulose content. Crude protein values and $TiO_2$ recovery were in agreement with expected values.

***Growth Performance, Experiment 1***

Body weight of broilers at 18 D of age (Table 3.3) was similar between treatments (635 g, $P = 0.78$). At 23 D of age, broilers provided with the protein diet had higher BW than broilers receiving other dietary treatments ($P < 0.0001$). Additionally, broilers that received the protein diet were the only birds that had a positive BW gain of 8 g ($P < 0.0001$), while broilers receiving other diets lost between 45 to 53 g during the experimental period. Feed disappearance ranged from 284 to 308 g and was similar ($P = 0.24$) between treatments. Broilers that received the cellulose diet had reduced ($P < 0.0001$) caloric intake of 702 kcal during the experimental period compared with consumption of 868 to 942 kcal by broilers provided with other dietary treatments. No mortality was observed during the experimental period. It is well documented that dietary AA concentration is associated with increased BW gain (Smith and Pesti, 1998; Dozier et al., 2008). The protein diet was the only diet containing AA, which may have allowed broilers to maintain their BW.

***Glucose Recovery, Experiment 1***

Glucose was recovered in the excreta of broilers from all treatments (Table 3.4). This was likely due to the diet being in a powder form that readily dissolved in the liquid excreta. Utilization of a collection method where a collection container is sutured directly to the vent of the broilers such as the method described for measuring AME in ducks (Adeola et al., 1997) may help mitigate glucose contamination. However, mucin glycoproteins that comprise a large portion of endogenous losses are not readily reabsorbed

by broilers (Fuller, 1994). Additionally, Kadim et al. (2002) evaluated the difference between endogenous flows of AA when measured in ileal digesta and excreta. The authors found no differences between endogenous AA flow in the two locations with the exception of aspartic acid and glutamic acid, which were higher ($P < 0.05$) in the excreta than the ileal digesta. Furthermore, Ragland et al. (1999) evaluated the difference in TME between cecectomized and conventional roosters and found that changes in TME were dependent on feed ingredient utilized. This indicates that cecal functions may have limited effects on semi-purified diets as utilized in the current experiments. While limited data describing differences between EEL estimates in ileal digesta and excreta are available, there may be similarities between factors affecting endogenous AA flow and EEL. This indicates that measurement of EEL in the ileum may be appropriate for standardization of ME.

Glucose was detected ($P = 1.00$) in the duodenum and jejunum of broilers in all cages, regardless of dietary treatment. Detection of glucose in duodenal and jejunal samples was expected, due to the duodenum and jejunum being the location of the approximately 65 and 20% of starch absorption, respectively (Riesenfeld et al., 1980). Glucose was detected in 100% ($P = 1.00$) of the upper and terminal ileum samples of broilers provided the starch or protein diets, but only in 62.5 and 12.5% of upper ($P < 0.0001$) and terminal ($P < 0.0001$) ileal samples, respectively, from broilers provided with the dextrose diets. No glucose ($P = 1.00$) was detected in upper or lower ileal samples from broilers provided with the cellulose diet. The starch and protein diets contained 42 and 31% cornstarch, respectively. These diets were utilized as studies have demonstrated effects of starch:dextrose ratio on endogenous losses of AA (Kong and Adeola, 2013; Kong et al., 2014) and, as far as the authors know, a lack of data defining effects of starch:dextrose

ratios on EEL. However, the current experiment demonstrated that utilization of cornstarch is not appropriate when estimating EEL due to residual exogenous glucose in ileal digesta. This may be due to incomplete starch absorption in the ileum (Yuan et al., 2017).

***Estimation of Endogenous Losses of Energy, Experiment 1***

Endogenous losses of energy could not be estimated from excreta samples due to glucose contamination (Table 3.4). Additionally, the starch and protein diets did not appear to be suitable for estimation of EEL due to the recovery of glucose in the terminal ileal samples of all broilers provided with the starch diets, and in 75% of samples from broilers provided with the protein diets, as an assumption of estimation of EEL utilizing semi-purified diets is that all ingested nutrients are either completely digestible or completely indigestible (Lemme et al., 2004). Despite the diets containing starch not being fully digestible, EEL was calculated for these samples by measuring the glucose content of the sample and subtracting that energy from the estimated EEL. While this does provide EEL values for comparison, such methodology introduces error and is less accurate than providing broilers with a diet that is fully digestible. Endogenous losses of energy were calculated to be 249, 252, and 242 kcal/kg DM intake for broilers provided with the cellulose, starch, and protein diets, respectively. No differences were observed between these values utilizing ANOVA ($P = 0.97$) or contrasts ($P \geq 0.81$).

While many studies have evaluated effect of diets on endogenous losses of AA Adedokun et al., 2011; Adedokun et al., 2012; Adedokun et al., 2018), limited data describe methodology for determination of EEL beyond the method of fasting roosters (Sibbald and Price, 1978), which yielded EEL of 10.44 kcal/bird/24 h, or gavaging roosters with dextrose (McNab and Blair, 1988), which provided an EEL estimate of 14.8 kcal/bird/48

h. More recently, Khalil et al. (2022) evaluated effect cellulose concentration on EEL and demonstrated that EEL increased ($P$ = 0.001) from 88 kcal/kg DM intake when the test diet had no cellulose to 430 kcal/kg DM intake in diets containing 7.5% cellulose. However, the authors acknowledged that the values were not corrected for the cellulose content in the digesta which may confound the true effect of cellulose on EEL. Broilers provided with diets containing 5% cellulose had EEL of 289 kcal/kg, while in a different experiment from the same laboratory (Khalil et al., 2020) reported EEL of 347 kcal/kg when utilizing similar diets. This indicates that EEL may be variable within birds, as endogenous losses of AA are also inconsistent (Cowieson et al., 2020). The EEL estimate for the cellulose diet reported in the current experiment (249 kcal/kg DM intake) is within the range of the reported literature.

***Diet Analysis, Experiment 2***

Diets in experiment 2 had analyzed composition close to calculated values (Table 3.5). Crude protein increased from 0.06% to 13.12% with increased dietary casein inclusion. Titanium dioxide recovery was slightly lower than calculated values, ranging from 0.46 to 0.47% compared with a formulated inclusion of 0.50%. Cellulose content ranged from 4.40 to 5.30%.

***Growth Performance, Experiment 2***

Initial broiler BW at the start of Experiment 2 was similar between treatments (722 g, $P$ = 0.36; Table 3.6). Body weight and BW gain during the experiment increased linearly ($P$ < 0.0001) with increasing inclusion of casein, ranging from BW gain of -55 g in broilers receiving diets without casein to 49 g in broilers that received diets with 15% casein. Feed disappearance was similar between treatments ($P$ = 0.19), ranging from 230 to 242 g during

the experimental period. This led to a linear increase ($P < 0.0001$) in CP intake due to the gradient CP content of the diets, ranging from 0.1 g in broilers provided with diets with no casein to 31.6 g in broilers provided with diets containing the highest content of casein. As stated previously, the changes in BW gain are likely associated with the varied protein content of the diets, as the relationship between AA intake and growth is well defined (Smith and Pesti, 1998; Dozier et al., 2008). While some studies have reported that broilers adjust their feed intake to an AA requirement (Summers et al., 1992), this was not observed in the current experiment and may only occur within a practical range of AA densities. It is not likely the differing energy content of the diet affected consumption as Cho (2012) demonstrated that modern broilers may not adjust feed intake to energy density. Additionally, the powdered form and the hygroscopicity of the diet may further limit the ability of the broiler to adjust feed intake to dietary nutrient density.

***Glucose Recovery, Experiment 2***

Glucose was recovered in all jejunal digesta samples (Table 3.7), regardless of dietary treatment. Glucose recovery was similar ($P = 0.77$) between treatments in the upper ileum, with 50% of samples from broilers provided diets with no casein and 25% of samples from broilers provided with any diet containing casein demonstrating in positive glucose recovery. In the terminal ileum, glucose recovery was also similar between treatments ($P = 0.54$). No glucose was recovered from terminal ileal samples from broilers provided with 5% casein, while glucose was recovered in 25% of samples from all other treatments. The consistent detection of glucose in the upper ileum indicates that EEL should be measured in the terminal ileum to limit energy from exogenous sources. This agrees with Kluth et al. (2005) who demonstrated that net disappearance of CP, AA, and

GE was higher in the proximal ileum compared with the terminal ileum, with some intermediate values when considering the middle section of the ileum. Weurding et al. (2001) measured starch digestion of various feedstuffs through the posterior jejunum, anterior ileum, and posterior ileum, and reported numeric increases in digestibility throughout the length of the tract but did not detect differences ($P > 0.05$) for starch digestibility for any ingredient between the anterior and posterior ileum. The study utilized a sample size of 6 cages, however, and had insufficient replication to detect actual differences. Numeric differences ranged from increases in digestibility coefficient of 0.2 in tapioca pellets to 9.4 in beans (Weruding et al., 2001), indicating there may be starch digestion occurring in the proximal ileum.

***Estimation of Endogenous Losses of Energy, Experiment 2***

Endogenous losses of energy ranged from 175 kcal/kg DM intake in broilers receiving diets with no casein to 194 kcal/kg DM intake in broilers that received diets containing 5% casein (Table 3.7). No differences were detected between treatments when utilizing ANOVA ($P = 0.50$), linear regression ($P = 0.32$; $R^2 = 0.04$), or quadratic regression ($P = 0.57$; $R^2 = 0.05$). This differs from the response of endogenous AA flow in broilers based on dietary casein inclusion. Adedokun et al. (2007) and Ravindran et al. (2008) demonstrated that increased dietary AA content through the inclusion of casein increased endogenous nitrogenous flow. Ravindran et al. (2008) suggested that by supplying the chick with AA, the chick is able to produce more endogenous proteins. The EEL output may vary from endogenous losses of AA, as endogenous losses of AA only measure nitrogenous compounds, while EEL are affected by other components such as bile (Mutucuramana and Ravindran, 2021), which may contain energy in the form of fatty acids

(Tancharoenrat et al., 2022), or sloughed glycocalyx, or membrane associated mucin (Moran, 2016), which is composed of carbohydrates as well as proteins (Carlstedt et al., 1993). The lack of effect of casein inclusion on EEL flow in the current experiment may indicate that EEL and endogenous losses of AA are not necessarily the same, and that experimental diets may affect EEL flow differently than the flow of AA. The EEL determined in Experiment 2 was numerically lower than the EEL determined in Experiment 1 (165 to 195 vs. 249 kcal/kg). This could be caused by the 20% inclusion rate of casein in Experiment 1 compared with 5% in Experiment 2. Khalil et al. (2022) suggested that increased dietary cellulose could cause mechanical damage to the intestinal wall or could cause an increase in mucin production. As demonstrated by the relationship between BW gain and casein inclusion, dietary casein affected the physiological status of the bird, but this did not translate to a change in the EEL.

One limitation of this method is that it only estimates basal endogenous losses, or the losses that are independent of feed intake, and not the specific losses that are caused by dietary components (Ravindran, 2021). This indicates that total EEL may be underestimated in ingredients high in fiber or antinutrients. To determine specific EEL, a study utilizing linear regression of the test ingredient would need to be utilized to determine the total EEL (Rodehutscord et al., 2004). Basal EEL could be determined utilizing a method such as the one described herein, and the specific EEL could be determined by subtracting the basal EEL from the total EEL (Ravindran, 2021). While the terminology standardized and true are often interchanged when referring to digestible or metabolizable energy, standardized values are only corrected for basal EEL while true values are corrected for basal and specific EEL (Stein et al., 2007).

Endogenous losses of energy were calculated to be 249 kcal/kg DMI in Experiment 1, and from 175 to 195 kcal/kg in Experiment 2. Endogenous losses of this degree, if unaccounted for, could lead to increased variability in determined energy values between birds due to variability in endogenous flow and could cause a lack of additivity of feed ingredient values. A dextrose-based diet containing cellulose and casein, but devoid of cornstarch appears to be an appropriate method for estimating EEL. The BW gain of broilers receiving 10% casein was similar to that of broilers receiving 15% casein; thus, inclusion of casein at 10% may be sufficient to maintain a normal physiological state of the chick.

## REFERENCES


Adedokun, S. A., A. J. Pescatore, M. J. Ford, T. Ao, and J. P. Jacob. 2018. Investigating the effect of dietary calcium levels on ileal endogenous amino acid losses and standardized ileal amino acid digestibility in broilers and laying hens. Poult. Sci. 97:131–139.

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. Poult. Sci. 86:2598-2607.

Adedokun, S. A., K. M. Ajuwon, L. F. Romero, and O. Adeola. 2012. Ileal endogenous amino acid loss: Response of broiler chickens to fiber and mild coccidial vaccine challenge. Poult. Sci. 91:899–907.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Adeola, O., D. Ragland, and D. King. 1997. Feeding and excreta collection techniques in metabolizable energy assays for ducks. Poult. Sci. 76:728-732.

American Veterinary Medical Association. 2013. AVMA guidelines for the euthanasia of animals: 2013 edition. Schaumburg, IL, American Veterinary Medical Association.

Carlstedt, I., A. Herrmann, H. Karlson, J. Sheehan, L. A. Fransson, and G. C. Hansson. 1993. Characterization of different glycosylated domains from the insoluble mucin complex of rat small intestine. J. Biol. Chem. 268:18771–18781.

Cho, M. 2012. The impact of diet energy and amino acid content on the feed intake and performance of broiler chickens. Master Thesis, University of Saskatchewan. SK. Canada.

Cowieson, A. J., M. M. Bhuiyan, J. O. B. Sorbara, G. Pappenberger, M. B. Pedersen, and M. Choct. 2020. Contribution of individual broilers to variation in amino acid digestibility in soybean meal and the efficacy of an exogenous monocomponent protease. Poult. Sci. 99:1075-1083.

Dozier, W. A. III, A. Corzo, M. T. Kidd, and M. W. Schilling. 2008. Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. Poult. Sci. 87:1385–1391.

Fuller, M. F. 1994. Amino acid requirements for maintenance body protein accretion and reproduction in pigs. Pages 155– 184 in Amino Acids in Farm Animal Nutrition. CAB International, Wallingford, UK.

Golian, A., W. Guenter, D. Hoehler, H. Jahanian, and C. M. Nyachoti. 2008. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. Poult. Sci. 87:706–712.

Guillaume, J., and J. D. Summers. Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolizable energy. Can. J. Anim. Sci. 50:363-369.

Kadim, I. T., P. J. Moughan, and V. Ravindran. 2002. Ileal amino acid digestibility assay for the growing meat chicken--comparison of ileal and excreta amino acid digestibility in the chicken. Br. Poult. Sci. 43: 588-597.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, F., and V. Ravindran. 2020. Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poult. Sci. 99:6809-6817.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, P. V. Chrystal, and V. Ravindran. 2022. Influence of age and dietary cellulose levels on ileal endogenous energy losses in broiler chickens. Poult. Sci. 101:101948.

Kluth, H., K. Mehlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271–279.

Kong, C., and O. Adeola. 2013. Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in broiler chickens. Poult. Sci. 92:1276-1282.

Kong, C., D. Ragland, and O. Adeola. 2014. Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in pigs. Asian-Austral. J. Anim. Sci. 27:1124–1130.

Lemme, A., V. Ravindran, and W. L Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J. 60:423-438.

McNab, J. M., and J. C. Blair. 1988. Modified assay for true and apparent metabolisable energy based on tube-feeding. Br. Poult. Sci. 29:697–707.

Moran, E. T., Jr. 2016. Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation and villus integrity. Anim. Feed. Sci. Technol. 221:284-303.

Mutucumarana, R. K. and V. Ravindran. 2021. Measurement of endogenous phosphorus losses in broiler chickens. Poult. Sci. 58:58-63.

Noblet, J., and J. Van Milgen. 2004. Energy value of pig feeds: Effect of pig body weight and energy evaluation system. Anim. Sci. 82:E229-E238.

Noblet, J., S. B. Wu, and M. Choct. 2021. Methodologies for energy evaluation of pig and poultry feeds: A review. Animal Nutrition. 8:185-203.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Can. J. Anim. Sci. 77:149–163.

Ragland, D., C. R. Thomas, R., G. Elkin, D. J. Shafer, and O. Adeola. 1999. The influence of cecectomy on metabolizable energy and amino acid digestibility of select feedstuffs for White Pekin ducks. Poult. Sci. 78:707-713.

Ravindran, V. 2021. Progress in ileal endogenous amino acid flow research in poultry. J. Anim. Sci. Biotechnol. 12:5.

Ravindran, V., P. C. H. Morel, S. M. Rutherfurd, and D. V. Thomas. 2008. Endogenous flow of amino acids in the avian ileum is increased by increasing dietary peptide concentrations. Br. J. Nutr. 101:822-828.

Riesenfeld, G., D. Sklan, A. Bar, A., U. Eisner, and S. Hurwitz. 1980. Glucose absorption and starch digestion in the intestine of the chicken. J. Nutr. 110:117-121.

Rodehutscord, M., M. Kapocius, R. Timmler, and A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45:85-92.

SAS Institute. 2016. SAS® User's Guide. Statistics. Version 9.4 ed. SAS Inst. Inc., Cary, NC.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci. 55:303–308.

Sibbald, I. R., and K. Price. 1980. The metabolic and endogenous energy losses of adult roosters. Poult. Sci. 57:556-557.

Smith, E. R., and G. M. Pesti. 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. Poult. Sci. 77:276-281.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282–285.

Summers, J. D., D. Spratt, and J. L. Atkinson. 1992. Broiler weight gain and carcass composition when fed diets varying in amino acid balance, dietary energy, and protein level. Poult. Sci. 71:263-273.

Tancharoenrat, P., F. Zaefarian, and V. Ravindran. 2022. Composition of chicken gallbladder bile. Br. Poult. Sci. 63:548-551.

Walk, C. L., L. F. Romero, and A. J. Cowieson. 2021. Towards a digestible calcium system for broiler chicken nutrition: a review and recommendations for the future. Anim. Feed Sci. Technol. 276:114930.

Weurding, R. E., A. Veldman, W. A. Veen, P. J. van der Aar, and M. W. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2335.

Wolynetz, M. S., and I. R. Sibbald. 1984. Relationships between apparent and true metabolizable energy and the effects of a nitrogen correction. Poult. Sci. 63:1386-1399.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. Poult. Sci. 99:385–406.

Yuan, J., X. Wang, D. Yin, M. Wang, X. Yin, Z. Lei, and Y. Guo. 2017. Effect of different amylases on the utilization of cornstarch in broiler chickens. Poult. Sci 96:1139-1148.

**Table 3.1.** Ingredient and calculated nutrient composition of common starter diet fed to male Yield Plus × Ross 708 broilers from 1 to 17 D of age (Experiments 1 and 2).

| Ingredient | Inclusion, % | Calculated Nutrient | Diet, % (unless otherwise noted |
|---|---|---|---|
| Corn | 50.81 | $AME_n$ (kcal/kg)[4] | 3,053 |
| Soybean Meal | 40.89 | Crude Protein | 23.26 |
| Vegetable Oil | 4.38 | Digestible Lysine | 1.23 |
| Defluorinated Phosphate | 1.89 | Digestible Methionine | 0.64 |
| Calcium Carbonate | 0.78 | Digestible Threonine | 0.84 |
| Sodium Chloride | 0.46 | Digestible TSAA | 0.93 |
| DL-Methionine | 0.34 | Calcium | 1.01 |
| Mineral Premix[1] | 0.10 | Phosphorus-AV | 0.48 |
| Vitamin Premix[2] | 0.10 | Sodium | 0.22 |
| L-Threonine | 0.09 | | |
| L-Lysine | 0.08 | | |
| Choline[3] | 0.08 | | |

[1]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[4]$AME_n$- nitrogen-corrected apparent metabolizable energy.

**Table 3.2.** Ingredient composition and calculated nutrient composition of semi-purified diets fed to male Yield Plus × Ross 708 broilers from 18 to 23 D of age (Experiment 1).

| | Dextrose | Cellulose | Starch | Protein |
|---|---|---|---|---|
| Ingredient (%) | | | | |
| Dextrose | 93.120 | 73.120 | 41.600 | 41.600 |
| Casein[1] | 0.000 | 0.000 | 0.000 | 10.000 |
| Starch | 0.000 | 0.000 | 41.600 | 31.450 |
| Solka-Floc[2] | 0.000 | 20.000 | 9.920 | 10.500 |
| Sodium Bicarbonate | 0.300 | 0.300 | 0.300 | 0.300 |
| Defluorinated Phosphate | 0.610 | 0.610 | 0.610 | 0.610 |
| Dicalcium Phosphate | 1.770 | 1.770 | 1.770 | 1.295 |
| Sodium Chloride | 0.220 | 0.220 | 0.220 | 0.220 |
| Potassium Sulfate | 1.450 | 1.450 | 1.450 | 1.450 |
| Limestone | 1.250 | 1.250 | 1.250 | 1.290 |
| Magnesium Oxide | 0.200 | 0.200 | 0.200 | 0.205 |
| Choline chloride-70[3] | 0.380 | 0.380 | 0.380 | 0.380 |
| Vit Premix[4] | 0.100 | 0.100 | 0.100 | 0.100 |
| Min Premix[5] | 0.100 | 0.100 | 0.100 | 0.100 |
| Titanium Dioxide[6] | 0.500 | 0.500 | 0.500 | 0.500 |
| | | | | |
| Calculated Analysis (% unless otherwise noted) | | | | |
| AME (kcal/kg) | 3,060 | 2,403 | 3,060 | 3,060 |
| CP (%) | 0 | 0 | 0 | 8.720 |
| Ca (%) | 0.962 | 0.962 | 0.962 | 0.962 |
| P (%) | 0.482 | 0.482 | 0.482 | 0.482 |
| K (%) | 0.598 | 0.598 | 0.598 | 0.598 |
| Na (%) | 0.200 | 0.200 | 0.200 | 0.200 |
| Cl (%) | 0.212 | 0.212 | 0.212 | 0.212 |
| S (%) | 0.281 | 0.281 | 0.281 | 0.275 |
| Mg (ppm) | 1,315 | 1,315 | 1,315 | 1,315 |
| Mn (ppm) | 127 | 127 | 127 | 126 |
| Choline (ppm) | 1,701 | 1,701 | 1,701 | 1,701 |
| DEB[7] | 180 | 180 | 180 | 180 |
| Starch:Dextrose | 0.00 | 0.00 | 1.00 | 0.76 |
| | | | | |
| Analyzed Composition, DM | | | | |
| CP[8], % | 0.03 | 0.07 | 0.04 | 9.07 |
| GE[9], kcal/kg | 3,161 | 3,324 | 3,557 | 3,698 |
| $TiO_2$[10], % | 0.48 | 0.51 | 0.55 | 0.50 |
| Cellulose[11], % | 0.762 | 18.172 | 16.431 | 16.975 |

[1]Acid casein (Fonterra, Auckland, New Zealand).
[2]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[4]Vitamin premix included per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[5]Mineral premix included per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[6]Titanium dioxide was included at 0.5% in all diets as an indigestible marker.
[7]Dietary electrolyte balance was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[8]Crude protein was determined using an Elementar Rapid N Cube.
[9]Gross energy was calculated in a Parr 6300 Calorimeter.
[10]$TiO_2$ concentrations were determined using according to Short et al., 1996.
[11] Cellulose was analyzed using AOAC 991.43.

**Table 3.3.** Growth performance of male Yield Plus × Ross 708 broilers fed semi-purified diets from 18 to 22 D of age (Experiment 1).[1]

| Treatment[2] | BW, g | BW Gain, g | Feed Disappearance, g | ME Intake, kcal |
|---|---|---|---|---|
| Dextrose | 586[b] | -53[b] | 299 | 915[a] |
| Low Energy | 575[b] | -50[b] | 292 | 702[b] |
| Starch | 581[b] | -45[b] | 308 | 942[a] |
| Protein | 659[a] | 8[a] | 284 | 868[a] |
| SEM[3] | 9 | 3 | 8 | 24 |
| | | | *Probabilities* | |
| ANOVA | <0.0001 | <0.0001 | 0.24 | <0.0001 |
| Dextrose vs. Cellulose | 0.34 | 0.47 | 0.57 | <0.0001 |
| Dextrose vs. Starch | 0.69 | 0.07 | 0.46 | 0.45 |
| Low energy vs. Starch | 0.61 | 0.23 | 0.20 | <0.0001 |
| Starch vs. Protein | <0.0001 | <0.0001 | 0.05 | 0.0450 |

[1]Broilers were fed a common corn and soybean meal-based diet from 1 to 17 D of age. Broilers had similar ($P$ = 0.78) BW of 635 g at 18 D of age. No mortality occurred from 18 to 22 D of age.
[2]The dextrose diet contained 91% dextrose; the cellulose diet contained 73% dextrose and 20% cellulose; the starch diet contained 42% dextrose, 42% starch, and 10% cellulose; and the protein diet contained 42% dextrose, 31% starch, 10% casein, and 11% cellulose. Diets were supplemented with vitamins and minerals to equal 100%, and all diets contained 0.5% $TiO_2$ as an indigestible marker.
[3]Pooled standard error.

**Table 3.4.** Endogenous energy losses (**EEL**) and glucose recovery percentages from male Yield Plus × Ross 708 broilers fed semi-purified diets from 18 to 23 D of age calculated using ileal digesta based on DM feed disappearance (Experiment 1).

| | EEL[1] | Proportion of samples with glucose recovery[2] | | |
|---|---|---|---|---|
| Treatments[4] | kcal/kg DM intake | Jejunum | Upper Ileum[3] | Terminal Ileum[3, 4] |
| Dextrose[5] | - | 1.00 | 0.63 | 0.13 |
| Cellulose | 249 | 1.00 | 0.00 | 0.00 |
| Starch | 252 | 1.00 | 1.00 | 1.00 |
| Protein | 242 | 1.00 | 1.00 | 0.75 |
| SEM[6] | 32 | - | - | - |
| | | *Probabilities* | | |
| ANOVA | 0.97 | - | - | - |
| Cellulose vs. Starch | 0.97 | - | - | - |
| Starch vs. Protein | 0.81 | - | - | - |
| Fisher's Exact test | - | 1.00 | <0.0001 | <0.0001 |

[1]Endogenous loss values were corrected for cellulose and glucose content. Endogenous losses were compared utilizing ANOVA and contrasts.
[2]Glucose recovery was measured utilizing Quantofix glucose test strips (CTL Scientific Supply Corp., Deer Park, NY) as a method of verifying glucose absorption. Proportions are based on 8 replicate cages per diet. Proportions of samples containing glucose were compared utilizing Fisher's Exact Test.
[3]The ileum was considered the segment of the small intestine from Meckel's diverticulum to 2 cm proximal of the ileo-cecal junction. The upper ileum was considered to be the 2/3 of the ilium proximal Meckel's diverticulum while the lower ileum wis the 1/3 of the ileum proximal the ileo-cecal junction.
[4] Glucose presence in terminal ileum was analyzed as a one-sample one-tail T-test where $H_0$=0. The probability of glucose detection in the terminal ilium in broilers receiving the dextrose, cellulose, starch, and protein diets was $P = 0.18$. $P = 1.00$, $P < 0.0001$, and $P = 0.0013$, respectively.
[5]The dextrose diet contained 91% dextrose; the low energy diet contained 91% dextrose and 20% cellulose; the starch diet contained 42% dextrose, 42% starch, and 10% cellulose; and the protein diet contained 42% dextrose, 31% starch, 10% casein, and 11% cellulose. Diets were supplemented with vitamins and minerals to equal 100%, and all diets contained 0.5% $TiO_2$ as an indigestible marker.
[6]The amount of digesta collected from birds fed the dextrose diet was insufficient for analysis and was omitted from statistical analysis.
[7]Pooled standard error.

**Table 3.5.** Diet composition of test diets provided to male Yield Plus × Ross 708 broilers from 18 to 23 D of age (Experiment 2).

| | Casein Inclusion, % | | | |
|---|---|---|---|---|
| Ingredient, % | 0 | 5 | 10 | 15 |
| Dextrose | 88.451 | 83.668 | 78.886 | 74.103 |
| Casein[1] | 0.000 | 5.000 | 10.000 | 15.000 |
| Solka-Floc[2] | 5.000 | 5.000 | 5.000 | 5.000 |
| Sodium Bicarbonate | 0.200 | 0.200 | 0.200 | 0.200 |
| Defluorinated Phosphate | 0.610 | 0.610 | 0.610 | 0.610 |
| Dicalcium Phosphate | 1.570 | 1.335 | 1.095 | 0.860 |
| Sodium Chloride | 0.320 | 0.320 | 0.320 | 0.320 |
| Potassium Sulfate | 1.390 | 1.390 | 1.390 | 1.390 |
| Potassium Chloride | 0.060 | 0.060 | 0.060 | 0.060 |
| Limestone | 1.117 | 1.135 | 1.157 | 1.175 |
| Magnesium Oxide | 0.202 | 0.202 | 0.202 | 0.202 |
| Choline Chloride-70[3] | 0.380 | 0.380 | 0.380 | 0.380 |
| Vitamin Premix[4] | 0.100 | 0.100 | 0.100 | 0.100 |
| Mineral Premix[5] | 0.100 | 0.100 | 0.100 | 0.100 |
| Titanium Dioxide[6] | 0.500 | 0.500 | 0.500 | 0.500 |
| | | | | |
| Calculated nutrient composition (% unless otherwise stated) | | | | |
| Crude Protein | 0 | 4.36 | 8.72 | 13.08 |
| AME (kcal/kg) | 3,001 | 3,034 | 3,067 | 3,099 |
| Ca | 0.879 | 0.879 | 0.879 | 0.879 |
| P | 0.440 | 0.440 | 0.440 | 0.440 |
| K | 0.603 | 0.603 | 0.604 | 0.604 |
| Na | 0.212 | 0.212 | 0.213 | 0.213 |
| Cl | 0.300 | 0.300 | 0.300 | 0.300 |
| DEB[7] (mEq) | 162 | 162 | 163 | 163 |
| Mg (ppm) | 1,313 | 1,299 | 1,285 | 1,270 |
| Mn (ppm) | 126 | 126 | 125 | 124 |
| S | 0.268 | 0.265 | 0.262 | 0.259 |
| Choline (ppm) | 1,701 | 1,701 | 1,701 | 1,701 |
| | | | | |
| Analyzed nutrients (DM) | | | | |
| CP[8], % | 0.062 | 4.086 | 7.523 | 13.122 |
| GE[9], kcal/kg | 3,169 | 3,273 | 3,360 | 3,440 |
| $TiO_2$[10], % | 0.458 | 0.472 | 0.467 | 0.464 |
| Cellulose[11], % | 5.277 | 4.353 | 5.223 | 5.060 |

[1]Acid casein (Fonterra, Auckland, New Zealand).
[2]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[4]Vitamin premix included per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[5]Mineral premix included per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[6]Titanium dioxide was included at 0.5% in all diets as an indigestible marker. Analyzed recovery ranged from 0.495 to 0.524%.
[7]Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[8]Crude protein was determined using an Elementar Rapid N Cube.
[9]Gross energy was calculated in a Parr 6300 Calorimeter.
[10]$TiO_2$ concentrations were determined using according to Short et al., 1996.
[11] Cellulose was analyzed using AOAC 991.43.

**Table 3.6.** Growth performance and feed disappearance measurements of male Yield Plus × Ross 708 broilers fed semi-purified diets from 18 to 23 D of age (Experiment 2).[1]

| Casein inclusion, % | BW, g | BW gain[2], g | Feed Disappearance, g | Protein intake, g |
|---|---|---|---|---|
| 0 | 669[c] | -55[c] | 230 | 0.1[d] |
| 5 | 714[b] | -8[b] | 241 | 9.8[c] |
| 10 | 752[a] | 16[ab] | 242 | 18.2[b] |
| 15 | 751[a] | 49[a] | 239 | 31.6[a] |
| SEM[3] | 12 | 11 | 5 | 0.1 |
| | | | *Probabilities* | |
| ANOVA | <0.0001 | <0.0001 | 0.15 | <0.0001 |
| Linear | <0.0001 | <0.0001 | 0.19 | <0.0001 |
| Quadratic | <0.0001 | <0.0001 | 0.16 | <0.0001 |
| $R^2$ Linear | 0.43 | 0.72 | 0.06 | 0.98 |
| $R^2$ Quadratic | 0.66 | 0.73 | 0.13 | 0.99 |

[1]Values within columns with different superscripts are significantly different ($P < 0.05$).
[2]Initial BW was similar between treatments ($P = 0.36$) at 722 g. No mortality occurred from 18 to 22 D of age.
[3]Standard error of the mean.

**Table 3.7.** Endogenous energy losses (**EEL**) and glucose recovery percentages from male Yield Plus × Ross 708 broilers fed semi-purified diets from 18 to 23 D of age calculated using ileal digesta based on DM feed disappearance (Experiment 2).

| | EEL[1] | Proportion of samples with glucose recovery[2] | | |
|---|---|---|---|---|
| Casein inclusion, % | kcal/kg DM intake | Jejunum | Upper Ileum[3] | Terminal Ileum[3,4] |
| 0 | 175 | 1.00 | 0.50 | 0.25 |
| 5 | 194 | 1.00 | 0.25 | 0.00 |
| 10 | 178 | 1.00 | 0.25 | 0.25 |
| 15 | 195 | 1.00 | 0.25 | 0.25 |
| SEM[6] | 12 | - | - | - |
| | | *Probabilities* | | |
| ANOVA | 0.50 | - | - | - |
| Linear | 0.32 | - | - | - |
| Quadratic | 0.57 | - | - | - |
| $R^2$ Linear | 0.04 | - | - | - |
| $R^2$ Quadratic | 0.05 | - | - | - |
| Fisher's Exact Test | - | 1.00 | 0.77 | 0.54 |

[1]Endogenous loss values were corrected for cellulose content. Samples in which glucose was detected were omitted from calculations. Endogenous losses were compared utilizing ANOVA and contrasts.

[2]Glucose recovery was measured utilizing Quantofix glucose test strips (CTL Scientific Supply Corp., Deer Park, NY) as a method of verifying glucose absorption. Proportions are based on 8 replicate cages per diet. Proportions of samples containing glucose were compared utilizing Fisher's Exact Test.

[3]The ileum was considered the segment of the small intestine from Meckel's diverticulum to 2 cm proximal of the ileo-cecal junction. The upper ileum was considered to be the 2/3 of the ilium proximal Meckel's diverticulum while the lower ileum wis the 1/3 of the ileum proximal the ileo-cecal junction.

[4]Glucose presence in terminal ileum was analyzed as a one-sample one-tail T-test where $H_0$=0. The probability of glucose detection in the terminal ilium in provided with the 0, 5, 10, or 15% diets was $P$ = 0.09. $P$ = 1.00, $P$ = 0.09, and $P$ = 0.09, respectively.

[5]The amount of digesta collected from birds fed the dextrose diet was insufficient for analysis and was omitted from statistical analysis.

[6]Pooled standard error.

# IV. EVALUATION OF ENERGY ASSAY METHODOLOGY FOR BROILER DIETS: 1. CEREAL GRAINS

## ABSTRACT

Three experiments were conducted to evaluate assay methodology for energy determination of cereal grains in broiler diets. In Experiment 1, broilers were supplied with corn- or wheat-based diets utilizing the direct method, where all dietary energy was supplied by the test ingredient. In Experiment 2, broilers were provided with corn-based diets utilizing the direct method, or the substitution method, in which corn was substituted into basal diets at 15 or 30%. In Experiment 3, determined energy of corn was evaluated utilizing the direct method and substitution at 30%. In each experiment, broilers were provided with common starter diets to 17 D of age and experimental diets were placed at 18 D of age. Metabolizable energy was determined based on a 48-h balance assay from 21 to 23 D of age, and birds were necropsied for collection of terminal ileal digesta at 24 and 25 D of age. Endogenous losses of energy were determined in all experiments by providing 16 cages of birds with a semi-purified diet. In each experiment, 9 chicks were placed in each battery cage with 16 replicated cages per diet. Body weight, BW gain, and feed disappearance were measured throughout the experiments. Energy was calculated on DM basis utilizing the different methodologies as AME, $AME_n$, standardized ME, apparent ileal digestible energy, or standardized ileal digestible energy. Data were analyzed utilizing

one-way ANOVA in all experiments and contrasts were utilized in Experiment 2. In Experiment 1, AME for corn and wheat was 3,460 and 3,036 kcal/kg, respectively. In Experiment 2, AME was lower when calculated using the direct method than substitution at 15 or 30%, with no differences between the two substitution methods (3,290, 3,635, and 3,636 kcal/kg, respectively; $P = 0.0004$). In Experiment 3, AME of corn was similarly lower when utilizing the direct method compared with the substitution method (3,431 or 3,675 kcal/kg, respectively; $P = 0.0025$). These data indicate that assay methodology affects determined energy values of cereal grains.

## INTRODUCTION

Dietary energy density affects broiler performance as it is required for maintenance functions as well as protein accretion (Latshaw and Moritz, 2009). A majority of the energy in broiler diets originates from cereal grains such as corn or wheat. Corn is more favorable than wheat due to its carbohydrate profile that is highly available for broiler chickens (Slominski, 2011), but wheat is commonly used in some regions due to availability (Amerah, 2015). Due to rising costs of cereal grains and other dietary energy sources (Donohue and Cunningham, 2009), it is important to formulate diets to optimize the efficiency and minimize the cost of broiler production. Accurate formulation requires appropriate assay methodology for energy determination. Different methodologies are available for measuring energy, including the direct method and the substitution method, where the direct method utilizes the test ingredient as the sole source of energy, while the substitution method measures energy in a basal diet and in a basal diet with the test ingredient substituted to the basal diet (Wu et al., 2020). In addition to diet methodology,

different calculation and collection methods are available, including AME, $AME_n$, standardized ME (**SME**), apparent ileal digestible energy (**AIDE**), and standardized ileal digestible energy (**SIDE**). Apparent ME values are most common in feed ingredient databases. However, AME is variable and may not be additive in diet formulation (Wu et al., 2020). Utilization of digestible energy (**DE**) measured pre-cecal or standardized energy, which is corrected for endogenous losses of energy (**EEL**) may mitigate some variation in AME.

While many papers describe different energy determination methodologies, limited studies directly compare the results of different methodology. The objectives of these experiments were to determine whether assay methodology affects either determined energy or variance of determined energy of cereal grains fed to broiler chickens.

## MATERIALS AND METHODS

All procedures involving the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2020-3796, 2021-3895, and 2022-4050).

### *Dietary Treatments*

Broilers were provided a common starter diet from 1 to 17 D of age in Experiments 1, 2, and 3 (Table 4.1). The same semi-purified diet was utilized in all experiments for determination of endogenous losses of energy (EEL). The semi-purified diet was dextrose-based and contained 10% casein (Tables 4.2, 4.3, and 4.4). In Experiment 1, energy contribution of corn and wheat was evaluated utilizing the direct method, where all dietary energy was provided by the test ingredient (Table 4.2). The corn diet contained 93.66%

corn and the wheat diet contained 94.40% wheat. The remainder of the diet consisted of vitamins and minerals to formulate the diets to the specifications of the primary breeder (Aviagen, 2022) and titanium dioxide as an indigestible marker. In Experiment 2, diets were designed to address why the determined energy was lower than expected in Experiment 1. Diets consisted of a direct corn diet identical to the one utilized in Experiment 1, as well as 4 diets (2 basal diets and 2 test diets) utilized to evaluate the substitution method at two inclusion rates (Table 4.3). Two basal diets were utilized so the substituted diets at 15 or 30% would be formulated to a similar nutrient density of 1.15, 0.87, and 0.77% digestible Lys, Met + Cys, and Thr, respectively. The basal consisted of all energy-contributing ingredients, while the vitamins, minerals, and titanium dioxide were added separately as to not be diluted by substitution. Experiment 3 was designed to further develop the results of Experiment 2 by evaluating additivity of different methods (presented in companion manuscript). Experiment 3 consisted of the same corn direct method described previously, one basal diet, and one substituted diet where 30% additional corn was substituted into the diet at the expense of the basal. The basal and substituted diets were similar to those utilized in Experiment 2, but without added oil. Wheat diets were not utilized in Experiments 2 or 3 due to space limitations.

***Broiler Husbandry***

Three energy balance experiments (Experiments 1, 2, and 3) were conducted in broilers from 18 to 25 D of age. Broiler husbandry was similar in all experiments. Soybean meal and ingredient additivity were evaluated concurrently with the cereal grains and data are presented in the two companion manuscripts. Male Yield Plus × Ross 708 chicks (Aviagen Inc., Huntsville, AL) were obtained at day of hatch from a commercial hatchery.

All chicks were vaccinated against Marek's, Newcastle, infectious bronchitis, and coccidiosis. Nine chicks were placed into each battery cage (68 × 68 × 38 cm; Petersime, Gettysburg, OH), which contained a trough feeder and waterer. Batteries were housed in solid sided houses equipped with forced air furnaces, evaporative cooling pads, vent boards, and electronic controllers for temperature control. Temperature was set to 33°C at placement and decrease to 25°C 18 D of age. Photoperiod was 23L:1D from 1 to 7 D of age and 20L:4D after 7 D of age.

***Measurements, Sample Collection, and Chemical Analysis***

Birds were weighed at 1, 18, and 23 D of age to calculate BW and BW gain. Feed disappearance was measured from 18 to 23 D of age and mortality was recorded daily. From 21 to 23 D of age, a 48-h balance assay was conducted to determine ME. Feed disappearance and excreta output were measured at 24 h intervals. Excreta samples were collected from multiple locations from the pan below each cage and were pooled into collection bags for analysis (approximately 1.5 kg/cage). Samples were homogenized and a 50 g subsample was dried. In Experiments 1 and 2, samples were lyophilized (VirTis Genesis 25ES, SP Industries Inc., Warminster, PA). In Experiment 3, samples were dried in a forced-air oven (Thermo Scientific Heratherm UT 20 P oven, Waltham, MA) at 55°C for 48 h (Jacobs et al., 2011). Dried sample was ground through a coffee grinder (Capresso Infinity 560 burr grinder, Montvale, NJ) and 0.80 g of dried diets and excreta were analyzed for gross energy (**GE**) utilizing an isoperibol oxygen bomb calorimeter (Model 6300 for Experiments 1 and 2, Model 6400 for Experiment 3, Parr Instruments, Moline, IA). Nitrogen content of dried excreta and diets was measured utilizing duplicate 0.25 g samples. In Experiments 1 and 2, N content was analyzed with a combustion analyzer

(Rapid N Cube, Elementar, Hanau, Germany; AOAC 968.06, AOAC International, 2006). In Experiment 3, N content was analyzed by a commercial laboratory (University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO; method 990.03 AOAC International, 2006).

At 24 and 25 D of age, ileal digesta was collected from 8 chicks per cage to determine digestible energy. In Experiments 1 and 2, half of the replicate cages were necropsied on D 24 and D 25. In Experiment 3, all cages were necropsied at 24 D of age. Chicks were euthanized utilizing CO2 asphyxiation. Digesta was collected from the terminal one-third of the ileum to 2 cm proximal to the ileo-cecal junction (Kluth et al., 2005) by gently flushing the intestinal section with distilled, deionized water (Adedokun et al., 2011). Samples were pooled by pen and were stored on ice until transfer to freezer. Samples were dried, ground, and assessed for GE in the same manner described for excreta. Additionally, titanium dioxide concentration was determined in dried digesta in duplicate (200 mg) and in dried diet (600 mg) in quadruplicate (Short et al., 1996). Samples were ashed for 12 h at 580°C in a muffle furnace (Thermo Scientific Thermolyne Muffle Ashing Oven F30400, Waltham, MA). Samples were then rinsed into glass beakers with 10 mL 7.4 M sulfuric acid and heated on hotplates at 200 °C until dissolved. After cooling, 10 mL water was used to rinse solutions into 100 mL beakers containing 60 mL water and 20 mL 30% hydrogen peroxide was added, and water was brought up to 100 mL. Solutions were measured for absorbance at 410 nm after 48 h (SpectraMax Plus 384, Molecular Devices LLC., San Jose, CA). Titanium concentration was calculated by comparing absorbance with standards using simple linear regression.

Standardization for EEL was evaluated in all experiments utilizing 16 cages with 9 chicks per cage utilizing the same husbandry as broilers receiving the other treatments. Standardization for ME and DE was calculated based on the energy in the terminal ileum due to contamination of excreta by dietary glucose. All digesta samples utilized for standardization were tested for the presence of glucose using qualitative test strips (Quantofix glucose test strips, CTL Scientific Supply Corp., Deer Park, NY) to verify absorption of dietary energy.

***Calculations***

Endogenous losses of energy (kcal/kg DM intake) were calculated as follows:

$$EEL\ \left({}^{kcal}/_{kg\ DMI}\right) = GE_{digesta} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}}\right) - (Cellulose\ \%\ \times GE_{cellulose})$$

where $GE_{digesta}$ represents the GE of the dried digesta and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the dried semi-purified diet and digesta, respectively. The diet utilized for standardization of EEL contained cellulose, which is completely indigestible. Thus, the calculation is corrected for the content of indigestible cellulose by subtracting the GE of the cellulose multiplied by the dietary inclusion of cellulose.

Apparent ME, $AME_n$, and SME of diets were calculated using the following three equations:

$$AME = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD(kg)}$$

$$AME_n = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)] - \{8.22(kcal/g) \times [N_{in}(g) - N_{ex}(g)]\}}{FD(kg)}$$

$$SME = EEL\ (kcal/kg) + \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD(kg)}$$

where $GE_{in}$ represents the total GE intake by the birds during the collection period, $GE_{ex}$ represents the GE of voided excreta during the collection period, FD (kg) represents the total feed disappearance during the collection period on a DM basis, and $N_{in}$ and $N_{ex}$ represent the total N intake and excretion during the experimental period. Nitrogen corrected AME was corrected with a factor of 8.22 kcal/g (Hill and Anderson, 1958).

Apparent ileal DE and SIDE of diets were calculated with the following two equations:

$$AIDE\ \left({}^{kcal}/_{kg}\right) = GE_{diet} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)$$

$$SIDE\left({}^{kcal}/_{kg}\right) = GE_{diet} \times \left(\frac{EEL}{GE_{diet}} + \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)\right)$$

where $GE_{diet}$ and $GE_{digesta}$ represent the GE of the diet and digesta, respectively, on a DM basis and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the diet and digesta, respectively.

Apparent ME, $AME_{n,}$ SME, AIDE, and SIDE in corn or wheat on a DM basis was calculated with the following two equations.

$$Direct\ ingredient\ energy\ \left({}^{kcal}/_{kg}\right) = \frac{Diet\ energy}{Ingredient\ \%} \times 100$$

$$Substituted\ ingredient\ energy\ \left({}^{kcal}/_{kg}\right) = \frac{Energy_{td} - (Energy_{bde} \times Inclusion_{td})}{Inclusion_{ti}} \times 100$$

Energy of corn or wheat using the direct method was calculated by dividing the energy of the test diet by the inclusion rate of the corn or wheat and multiplying by 100. Energy utilizing the substitution method was calculated by subtracting the determined energy of

the energy contributing ingredients of the basal diet ($Energy_{bde}$, calculated as energy of the basal diet divided by the inclusion percent of energy contributing ingredients) multiplied by the inclusion rate of the test diet ($Inclusion_{td}$) from the determined energy of the substituted diet ($Energy_{td}$) to determine the energy value provided by the test ingredient. The energy provided by the test ingredient was then divided by the inclusion rate of the test ingredient ($Inclusion_{ti}$) and multiplied by 100 to determine the energy of the test ingredient in kcal/kg DM.

***Statistical Analyses***

Dietary treatments were randomly allocated in each experiment as a randomized complete block design where pen location was the blocking factor. Cage (9 chicks/cage) was considered the experimental unit with each dietary treatment was represented with 16 replicate cages. Initial BW at 18 D of age, final BW, BW gain, feed disappearance, and mortality were evaluated in each experiment using a one-way ANOVA with PROC MIXED in SAS (2016) according to the following model:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where $\mu$ is the overall mean, $\tau_i$ are the factor level effects of the $i^{th}$ dietary treatment where the $\Sigma\tau_i = 0$, $\beta_j$ are the random block effects, which are identically and independently normally distributed, and $\varepsilon_{ij}$ represents the random error of the $i^{th}$ treatment and the $j^{th}$ block and are identically and independently normally distributed. Similarly, differences in energy determination method as well as energy calculation method for both diets and test ingredients were analyzed with one-way ANOVA using the same model as for growth performance characteristics. In Experiment 2, preplanned orthogonal contrasts were utilized to analyze the difference in growth performance and determined energy between

Corn direct vs. Corn 15, Corn direct vs. Corn 30, and Corn 15 vs. Corn 30. Mortality was arcsine transformed prior to analysis. Statistical significance was considered at $P \leq 0.05$.

## RESULTS AND DISCUSSION

### *Diet Analysis*

In Experiment 1, diet analysis (Table 4.2) was close to calculated values. Crude protein of the semi-purified diet was 8.76% compared with a calculated CP of 8.72%. Crude protein of the corn and wheat diets was slightly higher than calculated values (8.29 vs. 7.12% CP for corn; 10.85 vs. 8.77% CP for wheat). Titanium dioxide concentrations 0.46 to 0.50%, compared with a formulated value of 0.50%. In Experiment 2, analyzed composition of diets (Table 4.3) was close to calculated values. Crude protein of basal and substituted diets was higher than formulated but was consistent between diets. Titanium dioxide recovery ranged from 0.49% in the direct diet to 0.58% in the 15-substituted diet. In Experiment 3, analyzed CP (Table 4.4) values were close to calculated values and $TiO_2$ recovery ranged from 0.44 to 0.48%. Analyzed CP of cereal diets was higher than calculated CP in Experiments 1 and 2. This was likely because the CP of the corn and wheat used in the test diets was higher than the calculated CP values used for formulation.

### *Growth Performance*

In Experiment 1 (Table 4.5), BW of chicks at the beginning of the experimental period was 702 g and was similar between treatments ($P \geq 0.07$). Body weight gain from 18 to 23 D of age was similar between broilers receiving corn and wheat diets (36 vs. 31 g, respectively; $P = 0.39$). Broilers provided with the wheat diets had increased feed disappearance compared with broilers provided with corn diets from 18 to 23 D of age (367

vs. 396 g, respectively; $P = 0.0033$). In Experiment 2, broilers in all treatments had similar initial BW (750 g, $P = 0.43$). Broilers provided the direct diet had the lowest BWG of 66 g, while BWG was maximized in broilers provided with either of the basal diets or the 15 substituted diet ($P < 0.0001$). Feed disappearance was higher in broilers provided with any of the basal or substituted diets compared with the direct diet ($P < 0.0001$). Broilers receiving the 15 substituted or 30 substituted diet had increased ($P < 0.0001$) BW, BW gain, and feed disappearance compared with broilers receiving the direct diet. Mortality was not influenced by dietary treatments ($P \leq 0.29$). In Experiment 3, broilers had similar BW at the start of the experimental period (609 g, $P = 0.46$). Body weight gain of broilers provided with the basal or substituted diet was higher than broilers receiving the direct diet (311, 299, and 130 g, respectively; $P < 0.0001$). Feed disappearance was highest in broilers provided with the substituted diet and lowest in the direct diet, with the basal diet being intermediary ($P < 0.0001$).

Body weight gain responses were likely due to the nutrient composition of the diets. The amino acid content provided by the direct corn and wheat diets is lower than recommendations of the primary breeder (Aviagen, 2022). The overall reduction in dietary CP and amino acid density may have led to the minimal BW gain observed in the broilers receiving the direct diet in each experiment due to the importance of amino acids for tissue accretion (Kidd et al., 2004; Dozier et al., 2008).

***Effect of Energy Calculation***

In Experiment 1, only the direct method was utilized to determine the energy of corn and wheat (Table 4.6). Apparent ME, $AME_n$, SME, AIDE, and SIDE of corn was increased compared with wheat ($P = 0.001$). For corn, determined energy was highest ($P$

= 0.0003) when calculated as SIDE and lowest when calculated as AME or $AME_n$, with AIDE being similar to SIDE, AME, and $AME_n$. Endogenous losses of energy were calculated to be 150 kcal/kg DM intake. Determined energy of wheat followed the same pattern ($P < 0.0001$) as corn. It is well documented that corn typically has increased dietary energy compared with wheat (NRC, 1994; Khalil et al., 2020). In Experiments 2 and 3, differences were more pronounced between calculation method when evaluating diet rather than methodology, due to increased variance.

In Experiments 1 and 2, AIDE was numerically higher than AME and SIDE was higher than SME. This aligns with energy partitioning, as ME is equal to DE minus urinary energy (Latshaw and Moritz, 2009). In Experiment 3, corn had similar energy ($P > 0.05$) when calculated utilizing AME and AIDE, but AME was numerically higher than AIDE when utilizing the substitution method. This could be caused by energy derived from cecal activity (Yang et al., 2020), which is not accounted for in the energy partitioning model. Additionally, $AME_n$ was lower ($P \leq 0.0136$) than other calculations in Experiments 1 and 3, while SME was higher ($P < 0.0001$) than AME and SIDE was higher than AIDE ($P < 0.0001$). This is expected due to the calculations used to compute these values (Wolynitz and Sibbald, 1984).

Determined energy values for corn obtained utilizing the substitution method were generally lower than published values for AME $AME_n$, and SME, despite having higher determined energy than when utilizing the direct method. The substitution method provided AME values that ranged from 3,272 to 3,308 kcal/kg when converted to as-fed basis, compared with published values of 3,470 kcal/kg (NRC, 1994) or 3,364 kcal/kg (Rostagno et al., 2017). One explanation for this could be the increased utilization of corn

for ethanol production (USDA Feed Grains Yearbook, 2017). Corn with increased amylose:amylopectin ratios may produce higher ethanol yields (Wu et al., 2006). However, broilers fed corn with higher amylose:amylopectin ratios may have reduced determined energy (Zhou et al., 2010) or reduced digestibility coefficients (Ma et al., 2020). Gelatinization during the pelleting process is more likely to retrograde amylose to resistant starch than amylopectin (Moran, 2019), which could reduce the ME or DE of corn. Furthermore, chemical characteristics including the lipid, protein, or antinutrient profile, as well as growing and processing conditions, can affect the energy of corn (Cowieson, 2005; Gehring et al., 2013). Another explanation for the reduced determined energy in the current experiment is that the chicks utilized were vaccinated against coccidiosis, which could have impaired nutrient digestion. Gautier and Rochell (2020) observed reductions ($P < 0.05$) in DM and N digestibility in vaccinated chicks compared with unvaccinated chicks when provided with a test diet containing corn substituted at 30% of the basal diet. Furthermore, the authors reported decreased ($P < 0.05$) determined energy of corn, soybean meal, and dried distiller's grains with solubles when determined in vaccinated chicks compared with unvaccinated chicks. A variety of factors may have led to the differences in determined energy between experiments herein.

***Effect of Assay Methodology***

In Experiment 2, determined AME and SME of corn was higher ($P \leq 0.0005$) utilizing the substitution method at either inclusion rate than the direct method (Table 4.7). The determined AME (3,625 vs. 3,636 kcal/kg) and SME (3,796 vs. 3,797 kcal/kg) of corn using the substitution method was similar ($P = 0.89$) at 15 or 30% substitution. No significant differences were detected between the different determination methods for

determined $AME_n$, AIDE, or SIDE ($P \geq 0.45$). Endogenous losses of energy were calculated to be 134 kcal/kg DM intake. The SEM of the corn energy measured using the direct method was 16 when calculated utilizing AME, SME, or $AME_n$, and 18 when calculated with AIDE and SIDE. The error was inflated with both substitution methods with the error of determined energy ranging from 64 to 122 kcal/kg when corn was substituted at 15% and from 61 to 94 kcal/kg when corn was substituted at 30%. This is a result of the assumption in the substitution calculation that there was no variance in the energy the birds obtained from the basal portion of the diet. Thus, all dietary variance is attributed caloric contribution of the test ingredient, despite the test ingredient only composing 15 or 30% of the diet.

Experiment 3 compared the direct method with the substitution method utilizing a similar design as Experiment 2, but with 30% as the only substitution rate (Table 4.8). All energy calculation methods were higher ($P \leq 0.0403$) when energy was determined with the substitution method than the direct method. Endogenous losses of energy were calculated to be 161 kcal/kg DM intake. The SEM was inflated when utilizing the substation method compared with the direct method in a similar manner as in Experiment 2. The error for AME, $AME_n$, and SME was 19 kcal/kg when utilizing the direct method and 62 kcal/kg when utilizing substitution. The error for AIDE and SIDE followed a similar pattern and was 26 kcal/kg when utilizing the direct method and 69 kcal/kg when utilizing substitution.

The test diets utilized in each experiment were balanced for vitamin and mineral composition, so no vitamin or mineral deficiencies were expected (Table 4.2, 4.3, and 4.4). Therefore, the main differences between the diets were the chemical composition, such as

amino acid content or starch:protein ratio (Truong et al., 2015). The reduction in DE and ME values when determined with the direct method compared with the substitution method could be partially due to an undesirable starch:protein ratio or due to rapidly digestible starch. The absorption of starch and protein in the duodenum and jejunum is interrelated, but the relationships are currently not well defined (Truong et al, 2015). However, Weurding et al. (2001) demonstrated that corn had more rapid starch digestion than many other feed ingredients, while Selle et al. (2014) demonstrated that providing broilers with gradually digestible starch compared with rapidly digestible starch led to increased determined energy. These authors hypothesized that the gradually digestible starch complemented amino acid absorption and protein anabolism better than rapidly digestible starch, allowing the birds to better utilize the dietary nutrients. The direct method diets in the current experiments may have had a starch profile that was more rapidly digestible than diets containing soybean meal due to the high inclusion of corn, which could have led to the reduced determined energy.

Additionally, dietary fat content has been demonstrated to interact with other nutrients to increase utilization of dietary components (Sibbald and Kramer, 1978; Mateos and Sell, 1980). In the current experiments, the basal and substituted diets were the only diets containing supplemental fat. The difference in determined AME between the substituted-30 and direct diet was 346 and 244 kcal/kg in Experiment 2 and 3, respectively. This could indicate that supplemental fat provided an extra-caloric benefit to the determined energy of corn utilizing the substitution method in Experiment 2.

The changes in the feed composition may have led to physiological changes in broilers provided the direct diet such as reduced surface area or reduced enzyme activity

(Swatson et al., 2002), which could have further limited nutrient digestion and absorption. In addition to the overall decrease in BW gain when broilers are provided with a diet low in CP density such as the direct diets, some studies have demonstrated that reductions in BW may coincide with duodenal weight of broilers (Wijtten et al., 2010). The reduced duodenal weight could reduce nutrient absorption by reducing the available surface area of the duodenum. Furthermore, providing broilers with diets that have unbalanced amino acid ratios has been reported to reduce ($P \leq 0.002$) villus length, crypt depth, and villus surface area, and to reduce ($P \leq 0.008$) the mucosal protein content as well as maltase and sucrase activity (Swatson et al., 2002). This may further decrease the absorption capacity of the intestines in broilers that receive diets with inadequate or imbalanced amino acid specifications. Beyond the effects of CP in general, specific amino acids may be instrumental in digestive dynamics. Threonine is reported to be required in adequate quantities to maintain the mucin in the intestine (Fernandez et al., 1994). Insufficient Thr to maintain the intestinal mucin may lead to conditions that impair energy utilization (Dozier et al., 2001). This could have further reduced energy utilization in broilers provided with the direct diet, as the digestible Lys:Thr ratio in the direct diet was below the published requirement for optimal growth performance (Dozier et al., 2015; 2016). However, the Thr requirement for optimal growth and intestinal maintenance may differ. The results of these experiments indicate that diet composition affects nutrient digestion and absorption, possibly through affecting intestinal physiology of the broilers.

These experiments demonstrated that experimental methodology affects determined energy of corn and may also affect determined energy of other cereals such as wheat. Furthermore, they indicate that there are impactful interactions between feed

ingredients and nutrients. The substitution method may more accurately estimate energy available to the broiler than the direct method and may provide values that are more additive as well. Substitution of a test ingredient at the highest practical inclusion rate into a nutritionally balanced diet, such as the basal diets reported in these experiments, may accurately determine ingredient energy while minimizing variance.

## REFERENCES


Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Amerah, A. M. 2015. Interactions between wheat characteristics and feed enzyme supplementation in broiler diets. Anim. Feed Sci. Technol. 199:1–9.

AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD.

Aviagen. 2022. Ross 708 Broiler: Nutrient Specifications. Aviagen, Huntsville, AL.

Coweison, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed. Sci. Technol. 119:293-305.

Donohue, M., and D. L. Cunningham. 2009. Effects of grain and oilseed prices on the costs of US poultry production. J. Appl. Poult. Res. 18:325–337.

Dozier, W. A., III, A. Corzo, M. T. Kidd, and M. W. Schilling. 2008. Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. Poult. Sci. 87:1385–1391.

Dozier, W. A., III, E. T. Moran, Jr., and M. T. Kidd. 2001. Male and female broiler responses to low and adequate dietary threonine on nitrogen and energy balance. Poult. Sci. 80:926-930.

Dozier, W. A., III, K. J. Meloche, P. B. Tillman, and Z. Jiang. 2015. Growth performance of male broilers fed diets varying in digestible threonine to lysine ratio from 1 to 14 days of age. J. Appl. Poult. Res. 24:475-462.

Dozier, W. A., III, P. B. Tillman, and Z. Jiang. 2016. Growth performance and carcass characteristics of male broilers fed diets varying in digestible threonine to lysine ratio from 21 to 49 days of age. J. Appl. Poult. Res. 25:571-580.

Fernandez, S. R., S. Aoyagi, Y. Han, C. M. Parsons, and H. Baker. 1994. Limiting order of amino acid in corn and soybean meal cereal for growth of the chick. Poult. Sci. 73:1887–1896.

Gautier, A. E., and S. J. Rochell. 2020. Influence of coccidiosis vaccination on nutrient utilization of corn, soybean meal, and distillers dried grains with solubles in broilers. Poult. Sci. 99:3540-3549.

Gehring, C. K., A. J. Cowieson, M. R. Bedford, and W. A. Dozier, III. 2013. Identifying variation in the nutritional value of corn based on kernel characteristics. World Poult. Sci. J. 69:299-311.

Jacobs, B. M., J. F. Patience, W. A. Dozier, K. J. Stalder, and B. J. Kerr. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. J. Anim. Sci. 89:2624–2630.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, F., and V. Ravindran. 2020. Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poult. Sci. 99:6809-6817.

Kidd, M. T., C. D. McDaniel, S. L. Branton, E. R. Miller, B. B. Boren, and B. I. Fancher. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. J. Appl. Poult. Res. 13:593–604.

Kluth, H., K. Mehlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271–279.

Latshaw, J. D., and J. S. Moritz. 2009. The partitioning of metabolizable energy by broiler chickens. Poult. Sci. 88:98–105.

Ma, J., T. Yang, M. Yang, Z. Yan, L. Zhao, L. Yao, J. Chen, Q. Chen, B. Tan, T. Li, and J. Yin. 2020. Effects of dietary amylose/amylopectin ratio and amylase on growth performance, energy and starch digestibility, and digestive enzymes in broilers. J. Anim. Physiol. An. N. 104:928-35.

Mateos, G. G., and J. L. Sell. 1980. Influence of carbohydrate and supplemental fat source on the apparent metabolizable energy of the diet. Poult. Sci. 59:2129–2135.

Moran, E. T., Jr. 2019. Starch: Granule, Amylose-Amylopectin, Feed Preparation, and Recovery by the Fowl's Gastrointestinal Tract. J. Appl. Poult. Res. 28:566-586.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Rostagno, H. S. 2017. Brazilian Tables for Poultry and Swine, Composition of Feedstuffs and Nutrient Requirements. 4th ed Federal University of Vicosa, Minas Gerais, Brazil.

SAS Institute. 2016. SAS® User’s Guide. Statistics. Version 9.4 ed. SAS Inst. Inc., Cary, NC.

Selle, P. H., S. Y. Liu, J. Cai, R. A. Caldwell, and A. J. Cowieson. 2014. Graded inclusions of sodium metabisuphite in sorghum-based diets: 1. Reduction of disulphide cross-linkages in vitro and enhancement of energy utilisation and feed conversion efficiency in broiler chickens. Anim. Feed. Sci. Technol. 190:59-67.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Sibbald, I. R., and J. K. G. Kramer. 1978. The effect of the basal diet on the true metabolizable energy value of fat. Poult. Sci. 57:685–691.

Slominski, B. A. 2011. Recent advances in research on enzymes for poultry. Poult. Sci. 90:2013–2023.

Swatson, H. K., R. Gous, P. A. Iji, and R. Zarrinkalam. 2002. Effect of dietary protein level, amino acid balance, and feeding level on growth, gastrointestinal tract, and mucosal structure of the small intestine in broiler chickens. Anim. Res. 51:501–515.

Titus, H. W. 1956. Energy values of feedstuffs for poultry. Pages 10–14 in Proc. Semi-Annu. Mtg., Nutr. Council, Am. Feed Manufact. Assoc. Am. Feed Manufact. Assoc, St. Louis, MO.

Truong, H. H., S. Y. Liu, and P. H. Selle. 2015. Starch utilisation in chicken-meat production: the foremost influential factors. Anim. Prod. Sci. 56:797–814.

USDA. Feed Grains Yearbook. USDA, Washington, DC. https://www.ers.usda.gov/data-products/feed-grains-database/feed-grains-yearbook-tables/ Sept. 2022.

Weurding, R. E., A. Veldman, W. A. G. Veen, P. J. van der Aar, and M. W. A. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2334.

Wijtten, P. J. A., E. Hangoor, J. Sparla, and M. W. A. Verstegen. 2010. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. Poult. Sci. 89:1424–1439.

Wolynetz, M. S., and I. R. Sibbald. 1984. Relationships between apparent and true metabolizable energy and the effects of a nitrogen correction. Poult. Sci. 63:1386-1399.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. Poult. Sci. 99:385–406.

Wu., X., R. Zhao, D. Wang, S. R. Bean, P. A. Seib, M. R. Tuinstra, M. Campbell, and A. O'Brien. 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. Cereal Chem. 83:569-575.

Yang, Z., V. R. Pirgozliev, S. P. Rose, S. Woods, H. M. Yang, Z. Y. Wang, and M. R. Bedford. 2020. Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens. Poult. Sci. 99:320-330.

Zhou, Z., H. F. Wan, Y. Li, W. Chen, Z. L. Qi, P. Peng, and J. Peng. 2010. The influence of the amylopectin/amylose ratio in samples of corn on the true metabolizable energy value for ducks. Anim. Feed Sci. Technol 157:99-103.

**Table 4.1.** Ingredient and calculated nutrient composition of common starter diet fed to male Yield Plus × Ross 708 broilers from 1 to 17 D of age (Experiments 1, 2, and 3).

| Ingredient | Inclusion, % | Calculated Nutrient | Diet, % |
|---|---|---|---|
| Corn | 50.81 | Crude Protein | 23.26 |
| Soybean Meal | 40.89 | Digestible Lysine | 1.23 |
| Vegetable Oil | 4.38 | Digestible Methionine | 0.64 |
| Defluorinated Phosphate | 1.89 | Digestible Threonine | 0.84 |
| Calcium Carbonate | 0.78 | Digestible TSAA | 0.93 |
| Sodium Chloride | 0.46 | Calcium | 1.01 |
| DL-Methionine | 0.34 | Phosphorus-AV | 0.48 |
| Mineral Premix[1] | 0.10 | Sodium | 0.22 |
| Vitamin Premix[2] | 0.10 | | |
| L-Threonine | 0.09 | $AME_n$ (kcal/kg)[4] | 3,053 |
| L-Lysine | 0.08 | | |
| Choline[3] | 0.08 | | |

[1]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[4]$AME_n$- nitrogen-corrected apparent metabolizable energy.

**Table 4.2.** Ingredient and calculated nutrient composition of diets fed to male Yield Plus × Ross 708 from 18 to 25 D of age (Experiment 1).

| Ingredient, % | Semi-purified | Corn | Wheat |
|---|---|---|---|
| Dextrose | 78.88 | - | - |
| Corn | - | 93.66 | - |
| Wheat | - | - | 94.40 |
| SBM | - | - | - |
| Casein[2] | 10.00 | - | - |
| Solka-Floc[3] | 5.00 | - | - |
| Defluorinated Phosphate | 0.61 | - | - |
| Sodium Bicarbonate | 0.20 | 0.53 | 0.40 |
| Dicalcium Phosphate | 1.10 | 1.92 | 1.69 |
| Sodium Chloride | 0.32 | 0.11 | 0.10 |
| Potassium Sulfate | 1.39 | 1.00 | 0.50 |
| Potassium Chloride | 0.06 | 0.26 | 0.28 |
| Limestone | 1.16 | 1.48 | 1.46 |
| Magnesium Oxide | 0.20 | 0.01 | 0.14 |
| Choline Chloride[4] | 0.38 | 0.33 | 0.33 |
| Vitamin Premix[5] | 0.10 | 0.10 | 0.10 |
| Mineral Premix[6] | 0.10 | 0.10 | 0.10 |
| Titanium Dioxide | 0.50 | 0.50 | 0.50 |
| | | | |
| Nutrient | | | |
| CP (%) | 8.72 | 7.12 | 8.77 |
| AME (kcal/kg) | 3,360 | 3137 | 2903 |
| Ca (%) | 0.88 | 0.88 | 0.88 |
| P (%) | 0.44 | 0.44 | 0.44 |
| K (%) | 0.60 | 0.83 | 0.77 |
| Na (%) | 0.21 | 0.21 | 0.21 |
| Cl (%) | 0.30 | 0.30 | 0.30 |
| Mg (ppm) | 1,285 | 1274 | 1274 |
| Mn (ppm) | 125 | 132 | 144 |
| Choline (ppm) | 1,701 | 1702 | 1702 |
| DEB[7] | 162 | 218 | 216 |
| | | | |
| Analyzed Composition[8] | | | |
| DM[9] | 1.56 | 11.74 | 11.25 |
| GE[10] | 8.76 | 8.29 | 10.85 |
| CP[11] | 3,375 | 4,034 | 4,022 |
| $TiO_2$ Recovery[12] | 0.50 | 0.49 | 0.46 |

[1]CP- Crude protein

[2]Acid casein (Fonterra, Auckland, New Zealand).

[3]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[4]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[5]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[6]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[7]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[8]Corn contained 90.23% DM, 8.59% CP, and 4,326 kcal/kg GE. Wheat contained 91.97% DM, 9.35% crude protein, and 4,229 kcal/kg GE.
[9]Moisture content was calculated utilizing a VirTis 25S freeze dryer.
[10]Crude protein percent was determined using an Elementar Rapid N Cube.
[11]Gross energy was calculated in a Parr 6300 Calorimeter.
[12]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 4.3.** Ingredient composition of experimental diets fed to male Yield Plus × Ross 708 broilers from 18 to 25 D of age (Experiment 2).

| | Semi-purified | Direct | 15 Premix | 30 Premix | 15 Basal | 15 Substit-uted | 30 Basal | 30 Substit-uted |
|---|---|---|---|---|---|---|---|---|
| Ingredient, % | | | | | | | | |
| Corn | - | - | 56.840 | 49.830 | - | - | - | - |
| Soybean Meal | - | - | 34.000 | 40.000 | - | - | - | - |
| Poultry Biproduct Meal | - | - | 7.000 | 7.500 | - | | - | - |
| Soy Oil | - | - | 1.500 | 1.700 | - | - | - | - |
| DL-Met | - | - | 0.370 | 0.470 | - | - | - | - |
| L-Lys HCl | - | - | 0.220 | 0.360 | - | - | - | - |
| L-Thr | - | - | 0.070 | 0.140 | - | - | - | - |
| Corn 15 Premix[1] | - | - | - | - | 96.000 | 81.000 | - | - |
| Corn 30 Premix[1] | - | - | - | - | - | - | 96.000 | 66.000 |
| Added Corn[1] | - | 93.660 | - | - | - | 15.000 | - | 30.000 |
| Magnesium Oxide | 0.200 | 0.010 | - | - | 0.118 | 0.105 | 0.130 | 0.100 |
| Potassium Sulfate | 1.390 | 1.000 | - | - | - | - | - | 0.050 |
| Defluorinated P | 0.610 | - | - | - | 0.600 | 0.600 | 0.600 | 0.800 |
| Sodium Chloride | 0.320 | 0.110 | - | - | 0.320 | 0.320 | 0.320 | 0.320 |
| Dicalcium P | 1.100 | 1.920 | - | - | 0.700 | 0.800 | 0.620 | 0.700 |
| Limestone | 1.160 | 1.480 | - | - | 0.530 | 0.650 | 0.480 | 0.600 |
| Choline Chloride[2] | 0.380 | 0.330 | - | - | 0.250 | 0.260 | 0.240 | 0.270 |
| Vitamin Premix[3] | 0.100 | 0.100 | - | - | 0.100 | 0.100 | 0.100 | 0.100 |
| Mineral Premix[4] | 0.100 | 0.100 | - | - | 0.100 | 0.100 | 0.100 | 0.100 |
| $TiO_2$ | 0.500 | 0.500 | - | - | 0.500 | 0.500 | 0.500 | 0.500 |
| Sand | - | - | - | - | 0.782 | 0.565 | 0.910 | 0.460 |
| Dextrose | 78.880 | - | - | - | - | - | - | - |
| Casein[5] | 10.000 | - | - | - | - | - | - | - |
| Solka-floc[6] | 5.000 | - | - | - | - | - | - | - |
| Sodium Bicarbonate | 0.200 | 0.530 | - | - | - | - | - | - |
| Potassium Chloride | 0.060 | 0.260 | - | - | - | - | - | - |
| | | | | | | | | |
| Nutrient Composition | | | | | | | | |
| CP (%) | 8.72 | 7.12 | - | - | 24.58 | 21.88 | 27.33 | 21.07 |
| AME (kcal) | 3,360 | 3,137 | - | - | 2,945 | 2,987 | 2,897 | 2,997 |
| Ca (%) | 0.88 | 0.88 | - | - | 0.88 | 0.88 | 0.88 | 0.88 |
| P (%) | 0.44 | 0.44 | - | - | 0.44 | 0.44 | 0.44 | 0.44 |
| K (%) | 0.60 | 0.83 | - | - | 0.89 | 0.79 | 0.99 | 0.79 |
| Na (%) | 0.21 | 0.21 | - | - | 0.21 | 0.21 | 0.21 | 0.21 |
| Cl (%) | 0.30 | 0.30 | - | - | 0.30 | 0.30 | 0.30 | 0.30 |
| DEB[7] | 162 | 218 | - | - | 233 | 208 | 261 | 210 |
| Mg (ppm) | 1,285 | 1,274 | - | - | 1,314 | 1,319 | 1,301 | 1,317 |
| Mn (ppm) | 124 | 132 | - | - | 136 | 136 | 137 | 136 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Choline (ppm) | 1,701 | 1,702 | - | - | 1,721 | 1,708 | 1,698 | 1,716 |
| dLys HCl[8] (%) | 0.74 | 0.18 | - | - | 1.33 | 1.15 | 1.58 | 1.15 |
| dMet+Cys[8] (%) | 0.28 | 0.27 | - | - | 0.98 | 0.87 | 1.13 | 0.87 |
| dThr[8] (%) | 0.44 | 0.25 | - | - | 0.86 | 0.77 | 1.01 | 0.77 |
| | | | | | | | | |
| Analyzed Composition | | | | | | | | |
| DM[9] | 96.52 | 88.98 | - | - | 89.36 | 90.67 | 88.38 | 87.63 |
| GE[10] | 3,295 | 3,844 | - | - | 4,136 | 4,099 | 4,164 | 4,123 |
| CP[11] | 8.92 | 8.82 | - | - | 27.02 | 23.55 | 29.02 | 23.25 |
| $TiO_2$ Recovery[12] | 0.49 | 0.52 | - | - | 0.52 | 0.58 | 0.55 | 0.54 |

[1]Corn 15 and Corn 30 premix formulations are shown in this table. The added corn is the corn added in the substitution diets. Corn contained 90.08% DM, 9.28% CP, and 4,144 kcal/kg GE.

[2]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[3]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[4]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[5]Acid casein (Fonterra, Auckland, New Zealand).

[6]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[7]Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.

[8]Digestible Lys, digestible Met+Cys, and digestible Thr

[9]Moisture content was calculated utilizing a VirTis 25S freeze dryer.

[10]Crude protein percent was determined using an Elementar Rapid N Cube.

[11]Gross energy was calculated in a Parr 6300 Calorimeter.

[12]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 4.4.** Ingredient composition, calculated nutrients, and analyzed nutrients in diets fed to male Yield Plus × Ross 708 broilers from 18 to 24 D of age (Experiment 3).

| Diet | Semi-purified | Direct | Premix | Basal | Substituted |
|---|---|---|---|---|---|
| Corn[1] | 0.000 | 93.463 | 54.758 | 0.000 | 30.000 |
| Soybean Meal | 0.000 | 0.000 | 43.889 | 0.000 | 0.000 |
| L-Lys-HCl | 0.000 | 0.000 | 0.569 | 0.000 | 0.000 |
| DL-Met | 0.000 | 0.000 | 0.553 | 0.000 | 0.000 |
| L-Thr | 0.000 | 0.000 | 0.231 | 0.000 | 0.000 |
| Premix | 0.000 | 0.000 | - | 95.422 | 65.014 |
| Dextrose | 78.880 | 0.000 | - | 0.000 | 0.000 |
| Casein | 10.000 | 0.000 | - | 0.000 | 0.000 |
| Solka-Floc[2] | 5.000 | 0.000 | - | 0.000 | 0.000 |
| Sodium Bicarbonate | 0.200 | 0.530 | - | 0.160 | 0.200 |
| Dicalcium Phosphate | 1.100 | 1.920 | - | 1.700 | 1.780 |
| Defluorinated Phosphate | 0.610 | 0.000 | - | 0.000 | 0.000 |
| Sodium Chloride | 0.320 | 0.110 | - | 0.355 | 0.340 |
| Potassium Sulfate | 1.390 | 1.220 | - | 0.000 | 0.240 |
| Potassium Chloride | 0.060 | 0.243 | - | 0.000 | 0.000 |
| Limestone | 1.160 | 1.475 | - | 1.220 | 1.310 |
| Magnesium Oxide | 0.200 | 0.013 | - | 0.117 | 0.090 |
| Choline Chloride-70[3] | 0.380 | 0.326 | - | 0.326 | 0.326 |
| Vitamin Premix[4] | 0.100 | 0.100 | - | 0.100 | 0.100 |
| Mineral Premix[5] | 0.100 | 0.100 | - | 0.100 | 0.100 |
| Titanium Dioxide | 0.500 | 0.500 | - | 0.500 | 0.500 |
| | | | | | |
| Nutrient | | | | | |
| CP (%) | 8.72 | 7.10 | - | 24.41 | 18.91 |
| AME (kcal/kg) | 3,360 | 3130 | - | 2726 | 2862 |
| Ca (%) | 0.88 | 0.879 | - | 0.878 | 0.884 |
| P (%) | 0.44 | 0.441 | - | 0.441 | 0.443 |
| K (%) | 0.60 | 0.906 | - | 1.039 | 0.897 |
| Na (%) | 0.21 | 0.210 | - | 0.206 | 0.210 |
| Cl (%) | 0.30 | 0.295 | - | 0.305 | 0.301 |
| DEB[6] | 162 | 240 | - | 269 | 236 |
| Mg (ppm) | 1,285 | 1285 | - | 1319 | 1336 |
| Choline (ppm) | 1,701 | 1702 | - | 1702 | 1702 |
| | | | | | |
| Analyzed Nutrients[7] | | | | | |
| DM[8], % | 98.06 | 89.62 | - | 91.00 | 90.70 |
| CP[9], % | 8.64 | 7.48 | - | 24.28 | 18.95 |
| GE[10], kcal/kg | 3,431 | 3909 | - | 4151 | 4075 |
| $TiO_2$[11] Recovery, % | 0.47 | 0.44 | - | 0.45 | 0.48 |

[1]Added corn and added soybean meal represent the ingredient substituted into the basal diet in the corn substituted and soy substituted diets, respectively.
[2]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[5]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[6]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[7]Corn contained 89.23% DM, 8.90% CP, and 4,213 kcal/kg GE.
[8]Moisture content was calculated utilizing a VirTis 25S freeze dryer.
[9]Crude protein percent was determined using an Elementar Rapid N Cube.
[10]Gross energy was calculated in a Parr 6300 Calorimeter.
[11]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 4.5.** Growth performance of male Yield Plus × Ross 708 broilers from 18 to 23 D of age (Experiments 1, 2, and 3).[1]

| | BW, g | BW Gain[2,3], g | Feed Disappearance[2], g | Mortality[2,4], % |
|---|---|---|---|---|
| Experiment 1 | | | | |
| Corn | 734 | 37 | 367 | 0.0 |
| Wheat | 741 | 31 | 396 | 0.0 |
| SEM[5] | 8 | 3 | 3 | 0.0 |
| | | | | |
| Experiment 2 | | | | |
| Direct | 822[b] | 66[c] | 436[b] | 0.7 |
| 15 Basal | 1,173[a] | 420[ab] | 525[a] | 3.0 |
| 15 Substituted | 1,162[a] | 423[ab] | 551[a] | 2.2 |
| 30 Basal | 1,193[a] | 449[a] | 544[a] | 0.7 |
| 30 Substituted | 1,169[a] | 413[b] | 564[a] | 1.5 |
| SEM5 | 10 | 8 | 10 | 1.0 |
| | | | | |
| Experiment 3 | | | | |
| Direct | 737[b] | 130[b] | 427[c] | 4.9 |
| Basal | 916[a] | 311[a] | 458[b] | 2.7 |
| Substituted | 913[a] | 299[a] | 492[a] | 3.5 |
| SEM5 | 19 | 14 | 9 | 1.8 |
| | | *Probabilities* | | |
| Experiment 1- ANOVA | 0.39 | 0.18 | 0.0033 | - |
| | | | | |
| Experiment 2- ANOVA | <0.0001 | <0.0001 | <0.0001 | 0.45 |
| Contrasts | | | | |
| Direct vs. 15 Sub. | <0.0001 | <0.0001 | <0.0001 | 0.29 |
| Direct vs. 30 Sub. | <0.0001 | <0.0001 | <0.0001 | 0.58 |
| 15 Sub. vs. 30 Sub. | 0.64 | 0.36 | 0.35 | 0.61 |
| | | | | |
| Experiment 3- ANOVA | <0.0001 | <0.0001 | <0.0001 | 0.91 |

[1]Values within columns within the same experiment with different superscripts are significantly different ($P \leq 0.05$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.
[2]Body weight gain, feed disappearance, and mortality were evaluated from 18 to 23 D of age.
[3]Average BW at 18 D of age was 702, 750, 609 g in Experiments 1, 2, and 3, respectively, and was not different between treatments ($P \leq 0.07$).
[4]Mortality values were arcsine transformed prior to analysis.
[5]Standard error of the mean.

**Table 4.6.** Metabolizable and digestible energy values determined for corn and wheat using male Yield Plus × Ross 708 broilers provided with test diets from 18 to 25 D of age in Experiment 1.[1]

| Method (kcal/kg DM, unless otherwise noted) | Corn[4] | Wheat[4] |
|---|---|---|
| AME[2] | 3,460[b] | 3,036[a] |
| $AME_n$[2] | 3,418[b] | 2,970[a] |
| SME[2] | 3,609[a] | 3,183[a] |
| AIDE[2] | 3,540[ab] | 3,125[ab] |
| SIDE[2] | 3,690[a] | 3,273[b] |
| SEM[3] | 61 | 63 |
| | *Probabilities* | |
| ANOVA | 0.0003 | <0.0001 |

[1]Test ingredients were provided as mash diets containing 94% of the test ingredient for corn and wheat and were corrected for the content of the non-test ingredients. Means within columns with different superscripts are significantly different ($P < 0.05$).
[2]AME= apparent metabolizable energy, $AME_n$ = nitrogen-corrected AME, SME = standardized metabolizable energy, AIDE = apparent ileal digestible energy, SIDE = standardized ileal digestible energy.
[3]Standard error of the mean.
[4]Corn had increased energy than wheat using all determination methods ($P = 0.001$).

**Table 4.7.** Effect of diet and energy determination method on determined energy of diets and ingredients fed to male Yield Plus × Ross 708 broilers from 18 to 25 D of age (Experiment 2).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Diets | | | | | |
| Direct | 3,084[a, x] | 2,950[a, w] | 3,238[a, y] | 3,217[a, y] | 3,371[a, z] |
| 15 Basal | 2,902[c, w] | 2,629[c, v] | 3,056[c, x] | 3,134[a, y] | 3,288[a, z] |
| 15 Substituted | 2,995[b, x] | 2,713[b, w] | 3,149[b, y] | 3,191[a, y] | 3,345[a, z] |
| 30 Basal | 2,759[d, x] | 2,560[c, w] | 2,913[d, y] | 2,931[b, y] | 3,085[b, z] |
| 30 Substituted | 2,987[b, x] | 2,743[b, w] | 3,141[bc, y] | 3,116[a, y] | 3,270[a, z] |
| SEM[3] diets | 29 | 29 | 29 | 31 | 31 |
| | | | | | |
| Methodology | | | | | |
| Direct | 3,290[b, w] | 3,144[x] | 3,455[b, y] | 3,428[y] | 3,592[z] |
| 15 Substituted | 3,635[a, yz] | 3,292[y] | 3,796[a, z] | 3,643[yz] | 3,803[z] |
| 30 Substituted | 3,636[a, y] | 3,281[x] | 3,797[a, yz] | 3,664[y] | 3,825[z] |
| | | | | | |
| SEM[3] | | | | | |
| Direct | 16 | 16 | 16 | 18 | 18 |
| 15 Substituted | 82 | 64 | 82 | 122 | 122 |
| 30 Substituted | 61 | 65 | 61 | 94 | 94 |
| | | | *Probabilities* | | |
| Diets-ANOVA | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | | | | | |
| Method-ANOVA | 0.0004 | 0.46 | 0.0005 | 0.45 | 0.46 |
| Contrasts | | | | | |
| Direct vs. 15 | 0.0005 | 0.25 | 0.0005 | 0.26 | 0.27 |
| Direct vs. 30 | 0.0005 | 0.33 | 0.0006 | 0.29 | 0.30 |
| 15 vs. 30 | 0.89 | 0.86 | 0.89 | 0.92 | 0.92 |

[1]Significant differences caused by method are shown with superscripts A, B, C, and D within a column and within compared treatments. Significant differences of calculation method are shown within rows and differences are shown by superscripts V, W, X, Y, and Z. Effect of calculation was significant ($P \leq 0.0001$) for all diets and methodologies ($P \leq 0.0304$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean. Pooled standard error is presented for diets, while individual SEM is presented for methodology.

**Table 4.8.** Determined energy of diets and corn provided to male Yield Plus × Ross 708 broilers from 18 to 24 D of age (Experiment 3).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Diet | | | | | |
| Corn Direct | 3,213 [a, y] | 3,208 [a, y] | 3,374 [a, z] | 3,229 [a, y] | 3,389 [a, z] |
| Corn Basal | 3,034 [b, y] | 2,830 [c, x] | 3,190 [b, z] | 3,071 [b, y] | 3,227 [b, z] |
| Corn Substituted | 3,170 [ab, y] | 3,011 [b, x] | 3,330 [ab, z] | 3,175 [ab, y] | 3,335 [ab, z] |
| SEM[3] | 19 | 24 | 19 | 26 | 26 |
| Methodology | | | | | |
| Corn Direct | 3,431 [b, y] | 3,421 [b, y] | 3,602 [b, z] | 3,431 [b, y] | 3,602 [b, z] |
| Corn Substituted | 3,675 [a, yz] | 3,604 [a, y] | 3,856 [a, z] | 3,613 [a, yz] | 3,794 [a, z] |
| SEM[3] | | | | | |
| Direct | 19 | 19 | 19 | 26 | 26 |
| Substituted | 62 | 62 | 62 | 69 | 69 |
| | | | *Probabilities* | | |
| ANOVA- Diets | <0.0001 | <0.0001 | <0.0001 | 0.0002 | 0.0001 |
| ANOVA- Method | 0.0025 | 0.0136 | 0.0019 | 0.0403 | 0.0323 |

[1]Significant differences caused by method are shown with superscripts A, B, C, and D within a column and within compared treatments. Significant differences of calculation method are shown within rows and differences are shown by superscripts V, W, X, Y, and Z. Effect of calculation was significant for all diets ($P \leq 0.0001$) and methodologies ($P \leq 0.0267$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean. Pooled standard error is presented for diets, while individual SEM is presented for methodology.

# V. EVALUATION OF ENERGY ASSAY METHODOLOGY FOR BROILER DIETS: 2. SOYBEAN MEAL

## ABSTRACT

Three experiments were conducted to evaluate assay methodology for energy determination of soybean meal (**SBM**) in broiler diets. In Experiment 1, broilers were provided with a direct SBM- and dextrose-based diet where all energy was from SBM or dextrose. In Experiment 2, broilers were provided with the same direct diet as in Experiment 1 or with diets that evaluated SBM energy utilizing substitution at either 10 or 20%. In Experiment 3, SBM energy was evaluated utilizing the direct method and substitution at 20%. In each experiment, chicks were provided with common starter diets from 1 to 17 D of age. Experimental diets were provided from 18 to 25 D of age. A 48-h balance assay was conducted from 21 to 23 D of age to determine ME. Digesta was collected for determination of digestible energy at 24 and 25 D of age. Endogenous losses of energy were determined in each experiment utilizing a dextrose- and casein-based semi-purified diet. Body weight, BW gain, feed disappearance, and mortality were measured during the experimental period. Energy was calculated as AME, $AME_n$, standardized ME, apparent digestible energy, and standardized digestible energy on DM basis. Each experiment had 16 replicate cages (9 chicks/cage) for each diet and were designed as a randomized complete block design. Data were analyzed utilizing one-way ANOVA. In

Experiment 1, SBM AME was determined to be 2,513 kcal/kg. In Experiment 2, AME was higher ($P$ = 0.0024) when determined utilizing substitution at 10 or 20% compared with the direct method (2,496, 2,437, or 2,211 kcal/kg, respectively). In Experiment 3, AME was higher when measured utilizing the substitution method compared with the direct method (2,570 vs. 2,300 kcal/kg, $P$ =0.0032). The caloric difference between methods was largest when measuring $AME_n$ in Experiments 2 and 3. These experiments indicate that assay methodology affects determined energy values for SBM in broiler diets.

## INTRODUCTION

Soybean meal is typically the second highest included ingredient in broiler diets after corn. It is the most commonly utilized protein source in poultry diets (de Coca-Sinova et al., 2008). Soybean meal is a co-product from the production of soybean oil and is generally considered a high-quality protein source due to the favorable amino acid profile that complements the AA profile of corn (Ravindran et al., 2014). However, the carbohydrate composition is primarily comprised of non-starch polysaccharides and oligosaccharides (Choct et al., 2010) that are not highly utilizable by broilers. Furthermore, antinutrients in SBM may lead to increased flow of endogenous losses (Angkanaporn et al., 1994; Cowieson et al., 2009), which could increase variability of determined energy. Despite this, SBM substantially contributes to the energy content of the diet, with Ravindran et al. (2014) reporting AME values ranging from 1,567 to 2,541 kcal/kg on an as-fed basis. Different methodologies are available to measure the energy contribution of SBM. The direct method (Sibbald, 1976) provides broilers with diets where all energy comes from the test ingredient, while the substitution method (Hill and Anderson, 1958)

utilizes a basal diet and a test diet. Furthermore, a variety of calculation methods (Lopez and Leeson, 2008; Khalil et al., 2020) are available including AME, $AME_n$, standardized ME (**SME**), apparent ileal digestible energy (**AIDE**), and standardized ileal digestible energy (**SIDE**). Accurate energy values for SBM are necessary to provide broilers with appropriate energy density for optimal performance objectives and economic returns.

While many studies describe different energy determination methodologies (Wu et al., 2020), limited studies directly compare the results of different methodologies for SBM. The objectives of these experiments were to determine whether assay methodology affects either determined energy or variance of determined energy of SBM when provided to broiler chickens.

## MATERIALS AND METHODS

All procedures involving the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2020-3796, 2021-3895, and 2022-4050).

### *Dietary Treatments*

Broilers were fed a common starter diet in Experiments 1, 2, and 3 (Table 5.1). A dextrose-based semi-purified diet containing 10% casein was utilized to estimate endogenous losses of energy (**EEL**) in Experiments 1, 2, and 3 (Tables 5.2, 5.3, and 5.4). In Experiment 1, energy contribution of SBM was evaluated utilizing the direct method, where all dietary energy was provided by the test ingredient and dextrose (Table 5.2), with the assumption that dextrose has a constant AME of 3,268 kcal/kg (Batal and Parsons, 2004) and is 100% digestible. The diet was formulated to 20% CP to be representative of

typical grower phase diets and thus consisted of 42.97% SBM and 52.27% dextrose. The remainder of the diet consisted of vitamins and minerals to formulate the diets to the specifications of the primary breeder (Aviagen, 2022) and titanium dioxide as an indigestible marker. In Experiment 2, diets were designed to address the low determined energy of SBM in Experiment 1. Diets consisted of a direct diet identical to the diet utilized in Experiment 1, as well as 4 diets (2 basal diets and 2 test diets) evaluated to evaluate the substitution method at two inclusion rates (Table 5.3). Two basal diets were utilized so the substituted diets at 10 or 20% would be formulated to a similar nutrient density of 1.15, 0.87, and 0.77% digestible Lys, Met + Cys, and Thr, respectively. The two substituted diets were formulated to the same digestible Lys, Met + Cys, and Thr density of the corn substituted diets presented in the companion paper reporting assay methodology for cereal grains. The basal premix contained of all energy-contributing ingredients, while the vitamins, minerals, and titanium dioxide were added separately as to not be diluted by substitution. Experiment 3 was designed to further evaluate effects of methodology. Diets consisted of the same SBM direct method described previously, one basal diet, and one substituted diet where 20% additional SBM was substituted into the diet at the expense of the basal. The basal and substituted diets were similar to those utilized in Experiment 2, but without poultry by-product meal or added oil to limit variation.

***Broiler Husbandry***

Three energy balance experiments were conducted in broilers from 18 to 25 D of age. Similar bird management procedures were utilized in each experiment. Cereal grains and ingredient additivity were evaluated concurrently with SBM and data are presented in the two companion manuscripts. Day old Ross YP × 708 (Aviagen Inc., Huntsville, AL)

were procured from a commercial hatchery. All chicks were vaccinated against Marek's Disease, Newcastle Disease, and infectious bronchitis, and received a 1× vaccination against coccidiosis. Nine chicks were placed into each battery cage (68 × 68 × 38 cm; Petersime, Gettysburg, OH) that contained a trough feeder and waterer. Batteries were housed in solid-sided houses equipped with forced-air furnaces, evaporative cooling pads, vent boards, and electronic controllers for temperature control. Temperature was set to 33°C at placement and decreased to a final set point of 25°C at 18 D of age. Photoperiod was set at 23L:1D from 1 to 7 D of age and 20L:4D after 7 D of age.

***Measurements, Sample Collection, and Chemical Analysis***

Birds were weighed at 1, 18, and 23 D of age to calculate BW and BW gain. Feed disappearance was determined from 18 to 23 D of age. Mortality was recorded daily. From 21 to 23 D of age, a 48-h balance assay was conducted to determine ME. Feed disappearance and excreta voided were measured at 24 and 48 h. Multiple excreta sub-samples were collected from the pan below each cage and were pooled into collection bags for analysis. Care was taken to obtain samples free from contamination by feathers or feed. Samples were stored at -20°C until analysis. Samples were homogenized and a 50 g subsample was dried for analysis and calculation of excreta DM. In Experiments 1 and 2, diet, digesta, and excreta samples were freeze dried (VirTis Genesis 25ES, SP Industries Inc., Warminster, PA). In Experiment 3, diet, digesta, and excreta samples were dried in a forced-air oven (Thermo Scientific Heratherm UT 20 P oven, Waltham, MA) at 55°C for 48 h, based on the methodology described by Jacobs et al. (2011). Dried diet, digesta, and excreta samples were ground in a coffee grinder (Capresso Infinity 560 burr grinder, Montvale, NJ) and 0.80 g samples of dried diet and excreta were analyzed for gross energy

(**GE**) utilizing an isoperibol oxygen bomb calorimeter (Model 6300 for Experiments 1 and 2, and Model 6400 for Experiment 3, Parr Instruments, Moline, IA). Nitrogen content of dried excreta was determined in duplicate with 0.25 g samples, while diet samples were analyzed in quadruplicate. In Experiments 1 and 2, N content was analyzed with a combustion analyzer (Rapid N Cube, Elementar, Hanau, Germany; AOAC 968.06, AOAC International, 2006). In Experiment 3, a commercial laboratory analyzed excreta N content (University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO; method 990.03 AOAC International, 2006).

Ileal digesta was collected from 8 chicks per cage to determine digestible energy (**DE**). In Experiments 1 and 2, the collection occurred at D 24 and 25, with half of the replicates being necropsied each day. In Experiment 3, sample collection occurred at D 24. Both AIDE and SIDE were determined. Chicks were euthanized by $CO_2$ asphyxiation. Digesta was collected from the terminal one-third of the ileum to 2 cm proximal to the ileo-cecal junction (Kluth et al., 2005) by flushing the intestinal section with distilled, deionized water (Adedokun et al., 2011). Samples were pooled by pen and were frozen until later analysis. Samples were dried, ground, and analyzed for GE in the same manner described for excreta. Additionally, titanium dioxide concentration was determined in dried digesta and feed samples (Short et al., 1996). Ileal samples (200 mg, duplicate samples) or feed samples (600 mg, quadruplicate samples) were ashed for 12 h at 560°C in a muffle furnace (Thermo Scientific Thermolyne Muffle Ashing Oven F30400, Waltham, MA). Samples were rinsed into beakers with 10 mL 7.4 M HCl and were heated at 200°C until sample was dissolved, and the solution was clear. The solution was then rinsed with 10 mL water into a beaker containing 20 mL 30% hydrogen peroxide and 60 mL water. Color was

allowed to develop for 48 h and then absorbance was analyzed at 410 nm with a spectrophotometer (SpectraMax Plus 384, Molecular Devices LLC., San Jose, CA). Titanium dioxide concentration was determined using simple linear regression and standards with known $TiO_2$ concentrations.

Standardization for EEL was evaluated in each experiment with 16 cages (9 chicks/cage) utilizing the husbandry previously described to determine SME and SIDE. Standardization for both ME and DE was determined based on the EEL determined in the terminal ileum, as excreta. All digesta samples utilized for standardization were evaluated for the presence of exogenous glucose with test strips as a non-destructive method of analysis due to small sample quantity (Quantofix glucose test strips, CTL Scientific Supply Corp., Deer Park, NY) to verify absorption of dietary energy.

***Calculations***

Endogenous losses of energy (kcal/kg DM intake) were determined as follows:

$$EEL\ \left({}^{kcal}/_{kg\ DMI}\right) = GE_{digesta} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}}\right) - (Cellulose\ \%\ \times GE_{cellulose})$$

where $GE_{digesta}$ represents the GE of the dried digesta and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the dried semi-purified diet and digesta, respectively. The diet contained 5% Solka-Floc, an indigestible cellulose source. Thus, the calculation was corrected for cellulose by subtracting GE contributed by Solka-Floc.

Apparent ME, $AME_n$, and SME of diets were calculated using the following three equations derived from Wolynetz and Sibbald (1984):

$$AME = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD\ (kg)}$$

$$AME_n = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)] - \{8.22(kcal/g) \times [N_{in}(g) - N_{ex}(g)]\}}{FD\ (kg)}$$

$$SME = EEL\ (kcal/kg) + \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD\ (kg)}$$

where $GE_{in}$ represents the total GE intake by the birds during the collection period, $GE_{ex}$ represents the GE of voided excreta during the collection period, FD (kg) represents the total feed disappearance during the collection period on a DM basis, and $N_{in}$ and $N_{ex}$ represent the total N intake and excretion during the experimental period. Nitrogen corrected AME was corrected with a factor of 8.22 kcal/g (Hill and Anderson, 1958).

Apparent ileal digestible energy and SIDE of diets were calculated with the following two equations derived from Khalil et al (2020):

$$AIDE\ \left({}^{kcal}/_{kg}\right) = GE_{diet} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)$$

$$SIDE\left({}^{kcal}/_{kg}\right) = GE_{diet} \times \left(\frac{EEL}{GE_{diet}} + \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)\right)$$

where $GE_{diet}$ and $GE_{digesta}$ represent the GE of the diet and digesta, respectively, on a DM basis and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the diet and digesta, respectively.

Apparent ME, $AME_{n,}$ SME, AIDE, and SIDE in SBM on a DM basis was calculated with the following equations.

$$Direct\ ingredient\ energy\ \left({}^{kcal}/_{kg}\right) = \frac{(Diet\ energy - energy\ from\ dextrose)}{soybean\ meal\ \%} \times 100$$

$$Substituted\ ingredient\ energy\ \left({}^{kcal}/_{kg}\right) = \frac{Energy_{td} - (Energy_{bde} \times Inclusion_{td})}{Inclusion_{ti}} \times 100$$

Energy of SBM determined with the direct method was calculated by subtracting the energy contribution from dextrose from the energy of the diet and then dividing by the inclusion rate of SBM and multiplying by 100. Energy utilizing the substitution method was calculated by subtracting the determined energy of the energy contributing ingredients of the basal diet ($Energy_{bde}$, calculated as energy of the basal diet divided by the inclusion percent of energy contributing ingredients) multiplied by the inclusion rate of the test diet ($Inclusion_{td}$) from the determined energy of the substituted diet ($Energy_{td}$) to determine the energy value provided by the test ingredient. The energy provided by the test ingredient was then divided by the inclusion rate of the test ingredient ($Inclusion_{ti}$) and multiplied by 100.

***Statistical Analyses***

Dietary treatments were randomly allocated in each experiment as a randomized complete block design where pen location was the blocking factor. Cage (9 chicks/cage) was considered the experimental unit with each dietary treatment represented with 16 replicate cages. Initial BW at 18 D of age, final BW, BW gain, feed disappearance, and mortality were evaluated in each experiment using a one-way ANOVA with PROC MIXED in SAS (2016) according to the following model:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where $\mu$ is the overall mean, $\tau_i$ are the factor level effects of the $i^{th}$ dietary treatment where the $\Sigma\tau_i = 0$, $\beta_j$ are the random block effects, which are identically and independently normally distributed, and $\varepsilon_{ij}$ represents the random error of the $i^{th}$ treatment and the $j^{th}$ block and are identically and independently normally distributed. Similarly, differences in energy determination method as well as energy calculation method for both diets and test

ingredients were analyzed with one-way ANOVA using the same model as for growth performance characteristics. In Experiment 2, preplanned orthogonal contrasts were utilized to analyze the difference in growth performance and determined energy between SBM direct vs. SBM 10, SBM direct vs. SBM 20, and SBM 10 vs. SBM 20. Mortality was arcsine transformed prior to analysis. Statistical significance was considered at $P \leq 0.05$.

## RESULTS AND DISCUSSION

### *Diet Analysis*

In Experiment 1, SBM contained 46.7% CP. The semi-purified and SBM diet contained 8.8 and 20.3% CP, respectively, compared with formulated values of 8.7% and 20.0%. Titanium recovery was 0.50 and 0.46% for the semi-purified and SBM diets, respectively. In Experiment 2, SBM contained 46.9% CP. The semi-purified and soy diet contained 8.9 and 18.9% CP, respectively, compared with formulated values of 8.7% and 20.00%. All basal and substituted diets had approximately 2% CP percentage points higher analyzed CP than formulated CP. The SBM 10 basal and SBM 20 basal diets had 21.4 and 18.2% analyzed CP compared with formulated CP of 19.2 and 16.0%, respectively. Soybean meal 10 and SBM 20 substituted diets had 23.4 and 23.6% analyzed CP compared with formulated CP of 21.9 and 22.0%, respectively. Titanium dioxide recovery was ranged from 0.49 to 0.55% compared with the formulated 0.50%. The SBM utilized in Experiment 3 contained 47.97% CP. Analyzed CP for all diets was close to formulated values. Titanium dioxide recovery was slightly below formulated values, ranging from 0.45 to 0.48%. Determined energy of diets is presented in Tables 5.7 and 5.8.

***Growth Performance***

In Experiment 1 (Table 5.5), broilers provided with the SBM direct diet gained 228 g from 18 to 23 D of age and had feed disappearance of 473 g. Mortality during the experimental period was 0.69%. In Experiment 2, broilers provided with the SBM 10 basal, the SBM 10 substituted, or SBM 20 substituted diets had the highest BW gain from 18 to 23 D of age of 406, 430, and 425 g, respectively, while broilers provided with the SBM direct diet had the lowest BW gain of 283 g ($P$ <0.0001). Feed disappearance was lowest ($P$ < 0.0001) in broilers provided with the semi-purified diets, intermediate with broilers provided with SBM 10 substituted diets, and higher in all other treatments. In Experiment 3, broilers that received the semi-purified diet had reduced BW ($P$ = 0.0244) compared with broilers that received the SBM substituted diet, with broilers receiving the SBM basal diet having intermediary BW. Broilers that received the SBM substituted diet had the highest ($P$ = 0.0006) BW gain compared with chicks receiving other diets. Feed disappearance and mortality were not different ($P \geq 0.21$) between treatments.

***Effect of Energy Calculation***

In Experiment 1 (Table 5.6), SBM had AME of 2,513 kcal/kg on DM basis, and SME of 2,672 kcal/kg on DM basis. $AME_n$ was reduced to 2,194 kcal/kg. Apparent ileal digestible energy and SIDE were 2,720 and 2,880 kcal/kg, respectively. In Experiment 2 (Table 5.7), SBM had the highest ($P$ = 0.0041) determined kcal/kg when calculated as SIDE and the lowest ($P$ = 0.0041) kcal/kg when calculated as $AME_n$, regardless of methodology utilized. In Experiment 3 (Table 5.8), a similar response was detected as in Experiment 2, where SBM had the highest ($P$ = 0.0084) kcal/kg when calculated as SIDE and the lowest ($P$ = 0.0084) kcal/kg when calculated as $AME_n$ when determined using

either the direct or substituted method. Endogenous losses of energy were estimated to be 150, 134, and 161 kcal/kg DM intake in Experiments 1, 2, and 3, respectively.

There was a pronounced difference between $AME_n$ determined utilizing the direct and substitution methods. Nitrogen-corrected AME was reduced by 694 kcal/kg and 473 kcal/kg when determined utilizing the direct method compared with the substitution method in Experiments 2 and 3, respectively. In Experiment 2, $AME_n$ decreased 26.0, 5.0, and 6.1% compared with AME in the direct, substituted 10, and substituted 20% methods, respectively. In Experiment 3, $AME_n$ decreased 20.2 and 10.2% compared with AME in the direct and substituted methods, respectively. Nitrogen-corrected AME is utilized to correct the determined energy to a neutral N balance for comparative purposes (Hill and Anderson, 1958, Sibbald, 1989). These values align with the reduced BW gain exhibited by broilers provided with the direct diet, indicating a reduction in N retention. Nitrogen-correction penalizes proteinaceous ingredients more than carbonaceous ingredients due to the assumption that all N is utilized for energy, and thus may not be as representative of the energy actually available to the broiler (Lopez and Leeson, 2008).

In Experiments 1, 2, and 3, AIDE was higher ($P \leq 0.0041$) than AME, and SIDE was similarly higher than SME. This is expected based on the model of energy partitioning, where energy values are highest as GE, reduced in DE, and lowest in ME (Latshaw and Moritz, 2009). As a function of the calculation used for SME or SIDE, all determined standardized values were higher than their corresponding apparent value. One limitation of the method utilized for estimating EEL is that it only measures basal endogenous losses and not specific losses. Basal losses are fixed and associated with feed disappearance, while specific losses are caused by diet components (Ravindran, 2021). Components in SBM

such as phytate or trypsin inhibitor could lead to increased specific losses (Cowieson et al., Aderibigbe et al., 2021). As specific losses are not typically determined, the sloughed losses, composed of mucins, epithelial cells, and other components, are attributed to the diet ingredients rather than the birds, thus reducing apparent energy determinations.

***Effect of Assay Methodology***

In Experiment 2, methodology affected differences in energy for all ME values when utilizing ANOVA for analysis ($P \leq 0.0024$), but no differences were detected for DE values ($P \geq 0.29$). Apparent ME was higher ($P = 0.0024$) when determined utilizing substitution at 10 or 20% at 2,496 or 2,437 kcal/kg DM, compared with 2,211 kcal/kg when utilizing the direct method. Standardized ME and $AME_n$ followed similar patterns where the substitution method at either inclusion rate resulted in energy values higher ($P \leq 0.0024$) than the direct method. Apparent and standardized digestible energy values were not different ($P \geq 0.29$) based whether energy was determined utilizing substitution or direct method, but values determined with the substitution method at either inclusion rate were numerically higher than those determined utilizing the direct method. When evaluating the determined energy values utilizing contrasts, the contrasts between the direct method and either substitution method were different for ME values ($P \leq 0.0047$) but not DE values ($P \geq 0.17$). The contrast between substitution at 10 or 20% was not significant ($P \geq 0.32$) for any of the calculation methods. Standard error of the mean was numerically highest when energy was determined by substitution at 10%, ranging from 73 kcal/kg for $AME_n$ to 97 kcal/kg for AIDE and SIDE. Standard error was lowest when utilizing the direct method, ranging from 23 kcal/kg for AIDE to 28 kcal/kg for AME and SME. This is due to the calculation method used to determine energy of the SBM. In all methods, the

variance is attributed only to the SBM and not to the diet as a whole, as evidenced by the increase in standard error ingredient energy but not in dietary energy (Table 5.7). In the substitution method, the variance is attributed only to the substituted portion of the diet, and not to the SBM contained in the basal. This SBM represented 42.97, 10, or 20% of the diet, in the direct, substituted 10, and substituted 20 diets, respectively.

Experiment 3 exhibited similar results to Experiment 2, with the energy in the direct method being numerically lower for all calculation methods than the substituted method. Apparent ME was reduced ($P = 0.0032$) from 2,570 kcal/kg DM when determined utilizing the substitution method to 2,300 kcal/kg when determined utilizing the direct method, with SME values ($P = 0.0029$) having a similar decrease between the two methods. The largest caloric difference observed was between $AME_n$ values, with a 473 kcal/kg ($P < 0.0001$) difference in determined values between methodologies. Apparent and standardized DE for SBM were numerically lower ($P = 0.08$) when determined utilizing the direct method compared with the substitution method. Standard error was larger for AME and SME when utilizing the direct method (72 vs. 46 kcal/kg), and for AIDE and SIDE (59 vs. 120 kcal/kg). However, SEM was slightly lower for $AME_n$ when determined with the substitution method compared with the direct method (38 vs. 42 kcal/kg).

Differences in energy in SBM may be attributable to the assay methodology used, or to SBM itself as the three experiments herein utilized different sources of SBM produced in different years. Factors such as soybean variety (Perryman and Dozier, 2012), origin (de Coca-Sinova et al., 2008; Ravindran et al., 2014), processing techniques (Parsons et al., 1992), and chemical composition (Parsons et al., 2000) affect energy utilization. Furthermore, variability is introduced by genetic development of the chicks utilized

(Sibbald, 1976) and individual flock variability. Thus, energy values determined in the different experiments herein cannot be directly compared with each other.

The different methodologies in the current experiments included differences in nutrient and ingredient composition. Furthermore, the direct method utilized a semi-purified diet, while the substitution method utilized a practical corn- and SBM-based diet. Rochell et al (2012) and Adeola and Ileleji (2009) compared the effects of utilization of a semi-purified diet or practical corn- and SBM-based diets on nutritional value of dried distiller's grains with solubles (**DDGS**). Rochell et al. (2012) evaluated the effect of semi-purified diets or corn- and SBM-based diets on rate of passage of DDGS or meat and bone meal and determined that broilers provided with semi-purified diets had faster rate of passage than broilers provided with practical diets. Rate of passage may influence nutrient utilization by limiting or extending the time for digestion and absorption (Svihus et al., 2002). Furthermore, rate of passage may alter the microflora composition (Choct et al., 1996), which may further alter digestive dynamics.

In the current research, the direct method employed a semi-purified diet to be representative of grower-phase diets. Utilization of a semi-purified diet may have led to an increased rate of passage, which could have in turn resulted in the reduction in determined energy values due to less time for enzyme activity and nutrient absorption. Additionally, Rochell et al. (2012) observed that digestibility coefficients for amino acids were generally higher when determined utilizing practical diets than utilizing semi-purified diets, which could indicate that practical diets facilitate improved nutrient uptake. However, Adeola and Ileliji (2009) observed the opposite results of the current experiments and demonstrated that AME and $AME_n$ were increased when evaluated in semi-purified diets compared with

practical corn- and SBM-based diets. An explanation for this discrepancy could be the varying inclusion rate between the experiments and the resulting differences in nutrient composition.

Another variable between the methods was the inclusion of SBM. The direct diet contained 42.97% SBM. The 10% and 20% substituted diets from Experiment 2 contained a total of 31.50 and 34.44% SBM, respectively, including the SBM from both the basal portion of the diet and the added SBM. The 20% substituted diet from Experiment 3 contained 34.00% total SBM. It has been reported that inclusion rate may affect determined energy (Mitre et al., 2020). Thus, ingredients should be included in energy assays in practical inclusion rates. Additionally, no diets in the current experiments contained phytase. Phytate content of the diets with higher concentrations of SBM could have caused an antinutrient effect by binding other nutrients such as carbohydrates or amino acids, thus limiting their absorption (Selle and Ravindran, 2007).

Despite similar CP values in the different methods, the amino acid profile of the direct diet is substantially different from the substituted diets. Soybean meal has digestible Lys and Thr of 2.57 and 1.57%, respectively (Rostagno et al., 2017). At 42.97% SBM, the direct diet has approximately 1.10 and 0.67% digestible Lys and Thr, respectively. This provides the broilers with a digestible Thr:Lys ratio of 0.61, compared with a requirement of 0.69 digestible Thr:Lys from 1 to 14 D of age (Dozier et al., 2015) or 0.68 from 21 to 35 D of age (Dozier et al., 2016). The reduction in digestible threonine may affect the energy available to the bird. Dozier et al. (2001) demonstrated that male broilers provided with diets low in Thr had reduced recovery of $AME_n$ compared with broilers provided with diets adequate in Thr and suggested that effect may be due to the role of Thr on intestinal

functions. Intestinal mucin has a high concentration of Thr (Carlstedt et al., 1993; Abbasi et al, 2014), as do digestive enzymes (Block et al., 1966). The reduction in digestible Thr could have impaired energy absorption through impacting the mucosa and unstirred water layer, or through reducing digestion through decreased digestive enzyme production.

In addition to the role of amino acids on intestinal function, there are substantial interactions between digestion of amino acids and starch (Truong et al., 2015), which may have further affected the results seen in these experiments. In the direct diet, starch was primarily contributed by dextrose, which is rapidly digestible (Weurding et al., 2001). This may have led to more amino acids being catabolized for energy in enterocytes in lower portion of the small intestine. This could have led to reduced determined energy as amino acids, while being the preferential energy source of enterocytes, are not utilized as efficiently as glucose (Truong et al., 2015). Furthermore, the imbalance of starch and amino acid digestion rate, caused by the rapidly digestible starch in the direct method diet, may have caused competitive inhibition of absorption (Stephens et al., 1984). This inhibition could have further reduced determined energy of SBM when using the direct method.

These experiments indicated that there are associative effects between SBM and the other feed ingredients in the basal and substituted diets that affect the energy utilization of the bird. Additionally, these experiments demonstrated that assay methodology has a pronounced effect on determined energy of SBM, as utilization of semi-purified diets with the inclusion of SBM utilized in these experiments provided consistently lower energy values than utilization of the substitution method. Further research is warranted to develop methodology that is more likely to provide values that are additive but also that minimizes variability.

## REFERENCES


Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. Poult. Sci. 88:579–585.

Abbasi, M. A., A. H. Mahdavi, A. H. Samie, and R. Jahanian. 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. Braz. J. Poult. Sci. 16:35–44.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Aderibigbe, A. S., A. J. Cowieson, K. M. Ajuwon, and O. Adeola. 2021. Contribution of purified soybean trypsin inhibitor and exogenous protease to endogenous amino acid losses and mineral digestibility. Poult. Sci. 100:101486.

Angkanaporn, K., M. Choct, W. L. Bryden, E. F. Annison, and G. Annison. 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. J. Sci Food Agri. 66:399-404.

AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD.

Aviagen. 2022. Ross 708 Broiler: Nutrient Specifications. Aviagen, Huntsville, AL.

Batal, A. B., and C. M. Parsons. 2004. Utilization of various carbohydrate sources as affected by age in the chick. Poult. Sci. 83:1140-1147.

Block, R. J., K. W. Weiss, and D. B. Cornett. 1966. The amino acid composition of proteins. Pages 149–295 in: The Amino Acid Composition of Proteins and Foods. R. J. Block and D. Bolling, ed. Charles C. Thomas Publisher, Springfield, IL.

Carlstedt, I., A. Herrmann, H. Karlson, J. Sheehan, L. A. Fransson, and G. C. Hansson. 1993. Characterization of different glycosylated domains from the insoluble mucin complex of rat small intestine. J. Biol. Chem. 268:18771–18781.

Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609-621.

Choct, M., Y. Dersjant-Li, J. McLeish, and M. Peisker. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australas. J. Anim. Sci. 23:1386–1398.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. World's Poult. Sci. J. 65:401–417.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101–108.

de Coca-Sinova, A., D. G. Valencia, E. Jimenez-Moreno, R. Lazaro, and G. G. Mateos. 2008. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poult. Sci. 87:2613-2623.

Dozier, W. A., III, E. T. Moran, Jr., and M. T. Kidd. 2001. Male and female broiler responses to low and adequate dietary threonine on nitrogen and energy balance. Poult. Sci. 80:926-930.

Dozier, W. A., III, K. J. Meloche, P. B. Tillman, and Z. Jiang. 2015. Growth performance of male broilers fed diets varying in digestible threonine to lysine ratio from 1 to 14 days of age. J. Appl. Poult. Res. 24:475-462.

Dozier, W. A., III, P. B. Tillman, and Z. Jiang. 2016. Growth performance and carcass characteristics of male broilers fed diets varying in digestible threonine to lysine ratio from 21 to 49 days of age. J. Appl. Poult. Res. 25:571-580.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603.

Jacobs, B. M., J. F. Patience, W. A. Dozier, K. J. Stalder, and B. J. Kerr. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. J. Anim. Sci. 89:2624–2630.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, F., and V. Ravindran. 2020. Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poult. Sci. 99:6809-6817.

Kluth, H., K. Mehlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271–279.

Latshaw, J. D., and J. S. Moritz. 2009. The partitioning of metabolizable energy by broiler chickens. Poult. Sci. 88:98–105.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Mitre, K. 2020. Distillers Dried Grains with Solubles: Determination of Metabolizable Energy Values Using Regression-Based Assays and their Correlation with Chemical Composition. Master's Thesis, University of Arkansas.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Parsons, C. M., K. Hashimoto, K. J. Wedekind, Y. Han, and D. H. Baker. 1992. Effect of overprocessing on availability of amino acids and energy in soybean meal. Poult. Sci. 71:133–140.

Parsons, C. M., Y. Zhang, and M. Araba. 2000. Nutritional evaluation of soybean meals varying in oligosaccharide content. Poult. Sci. 79:1127-1131.

Perryman, K. R., and W. A. Dozier. 2012. Apparent metabolizable energy and apparent ileal amino acid digestibility of low and ultra-low oligosaccharide soybean meals fed to broiler chickens. Poult. Sci. 91:2556-2563.

Ravindran, V. 2021. Progress in ileal endogenous amino acid flow research in poultry. J. Anim. Sci. Biotechnol. 12:5.

Ravindran, V., M. R. Abdollahi, and S. M. Bootwalla. 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poult. Sci. 93:2567-2577.

Rochel, S. J., T. J. Applegate, E. J. Kim, and W. A. Dozier, III. 2012. Effects of diet type and ingredient composition on rate of passage and apparent ileal amino acid digestibility in broiler chicks. Poult. Sci. 91:1647-1653.

Rostagno, H. S. 2017. Brazilian Tables for Poultry and Swine, Composition of Feedstuffs and Nutrient Requirements. 4th ed Federal University of Vicosa, Minas Gerais, Brazil.

SAS Institute. 2016. SAS® User's Guide. Statistics. Version 9.4 ed. SAS Inst. Inc., Cary, NC.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Sibbald, I. R. 1989. Metabolizable energy evaluation of poultry diets. Pages 12–26 in Recent Developments in Poultry Nutrition. D. J. A. Cole and W. Haresign, ed. Butterworths, Essex, UK.

Sibbald, I. R. 1976. The true metabolizable energy values of several feedingstuffs measured with roosters, laying hens, turkeys and broiler hens. Poultry Sci. 55:1459-1463.

Stevens, B. R., J. D. Kaunitz, and E. M. Wright. 1984. Intestinal transport of amino acids and sugars: advances using membrane vesicles. Ann. Rev. Physiol., 46:417-433.

Svihus, B., H. Hetland, M. Choct, and F. Sundby. 2002. Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Br. Poult. Sci. 43:662-668.

Truong, H. H., S. Y. Liu, and P. H. Selle. 2015. Starch utilisation in chicken-meat production: the foremost influential factors. Anim. Prod. Sci. 56:797–814.

Weurding, R. E., A. Veldman, W. A. Veen, P. J. van der Aar, and M. W. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2335.

**Table 5.1.** Ingredient and calculated nutrient composition of common starter diet fed to male Yield Plus × Ross 708 broilers from 1 to 17 D of age (Experiments 1, 2, and 3).

| Ingredient | Inclusion, % | Calculated Nutrient | Diet, % |
|---|---|---|---|
| Corn | 50.81 | Crude Protein | 23.26 |
| Soybean Meal | 40.89 | Digestible Lysine | 1.23 |
| Vegetable Oil | 4.38 | Digestible Methionine | 0.64 |
| Defluorinated Phosphate | 1.89 | Digestible Threonine | 0.84 |
| Calcium Carbonate | 0.78 | Digestible TSAA | 0.93 |
| Sodium Chloride | 0.46 | Calcium | 1.01 |
| DL-Methionine | 0.34 | Phosphorus-AV | 0.48 |
| Mineral Premix[1] | 0.10 | Sodium | 0.22 |
| Vitamin Premix[2] | 0.10 | | |
| L-Threonine | 0.09 | $AME_n$ (kcal/kg) | 3,053 |
| L-Lysine | 0.08 | | |
| Choline[3] | 0.08 | | |

[1]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

**Table 5.2.** Ingredient and calculated nutrient composition of diets fed to male Yield Plus × Ross 708 broilers from 18 to 25 D of age (Experiment 1).

| Ingredient, % | Semi-purified | Soybean meal |
|---|---|---|
| Dextrose | 78.88 | 52.27 |
| Soybean Meal[1] | - | 42.97 |
| Casein[2] | 10.00 | - |
| Solka-Floc[3] | 5.00 | - |
| Defluorinated Phosphate | 0.61 | - |
| Sodium Bicarbonate | 0.20 | 0.17 |
| Dicalcium Phosphate | 1.10 | 1.79 |
| Sodium Chloride | 0.32 | 0.38 |
| Potassium Sulfate | 1.39 | - |
| Potassium Chloride | 0.06 | - |
| Limestone | 1.16 | 1.18 |
| Magnesium Oxide | 0.20 | 0.21 |
| Choline Chloride[4] | 0.38 | 0.33 |
| Vitamin Premix[5] | 0.10 | 0.10 |
| Mineral Premix[6] | 0.10 | 0.10 |
| Titanium Dioxide | 0.50 | 0.50 |
| | | |
| Nutrient | | |
| CP (%) | 8.72 | 20.00 |
| AME (kcal/kg) | 3,360 | 2719 |
| Ca (%) | 0.88 | 0.88 |
| P (%) | 0.44 | 0.44 |
| K (%) | 0.60 | 0.90 |
| Na (%) | 0.21 | 0.21 |
| Cl (%) | 0.30 | 0.30 |
| Mg (ppm) | 1,285 | 1274 |
| Mn (ppm) | 125 | 137 |
| Choline (ppm) | 1,701 | 1702 |
| DEB[7] | 162 | 238 |
| | | |
| Analyzed Nutrients | | |
| DM[8], % | 98.44 | 95.50 |
| CP[9], % | 8.76 | 20.28 |
| GE[10], kcal/kg | 3,375 | 3,701 |
| $TiO_2$[11], % | 0.50 | 0.46 |

[1]Soybean meal contained 91.97% DM, 46.68% CP, and 4,623 kcal/kg GE.
[2]Acid casein (Fonterra, Auckland, New Zealand).
[3]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.
[4]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[5]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33

IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[6]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[7]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.

[8]Moisture content was calculated utilizing a VirTis 25S freeze dryer.

[9]Crude protein percent was determined using an Elementar Rapid N Cube.

[10]Gross energy was calculated in a Parr 6300 Calorimeter.

[11]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 5.3.** Ingredient composition of experimental diets fed to male Yield Plus × Ross 708 broilers from 18 to 25 D of age (Experiment 2).

| | Semi-purified | SBM-direct | SBM 10 premix | SBM 20 premix | SBM 10 basal | SBM 20 basal | SBM 10 | SBM 20 |
|---|---|---|---|---|---|---|---|---|
| Ingredient, % | | | | | | | | |
| Corn | - | - | 68.810 | 75.790 | - | - | - | - |
| Soybean Meal | - | 42.970 | 25.000 | 19.000 | - | - | - | - |
| Poultry Biproduct Meal | | - | 3.800 | 2.200 | - | - | - | - |
| Soy Oil | - | - | 1.800 | 2.400 | - | - | - | - |
| DL-Met | - | - | 0.350 | 0.390 | - | - | - | - |
| L-Lys HCl | - | - | 0.180 | 0.170 | - | - | - | - |
| L-Thr | - | - | 0.060 | 0.050 | - | - | - | - |
| Soybean meal 10 premix[1] | - | - | - | - | 96.000 | - | 86.000 | - |
| Soybean meal 20 premix[1] | - | - | - | - | - | 96.000 | - | 76.000 |
| Added Soybean Meal[1] | - | - | - | - | - | - | 10.000 | 20.000 |
| Magnesium Oxide | 0.200 | 0.210 | - | - | 0.090 | 0.080 | 0.105 | 0.110 |
| Potassium Sulfate | 1.390 | - | - | - | - | - | 0.120 | - |
| Defluorinated P | 0.610 | - | - | - | 0.800 | 0.920 | 0.800 | 0.920 |
| Sodium Chloride | 0.320 | 0.380 | - | - | 0.330 | 0.330 | 0.330 | 0.330 |
| Dicalcium P | 1.100 | 1.790 | - | - | 0.800 | 0.850 | 0.800 | 0.800 |
| Limestone | 1.160 | 1.180 | - | - | 0.700 | 0.800 | 0.680 | 0.720 |
| Choline Chloride[2] | 0.380 | 0.330 | - | - | 0.285 | 0.305 | 0.290 | 0.310 |
| Vitamin Premix[3] | 0.100 | 0.100 | - | - | 0.100 | 0.100 | 0.100 | 0.100 |
| Mineral Premix[4] | 0.100 | 0.100 | - | - | 0.100 | 0.100 | 0.100 | 0.100 |
| $TiO_2$ | 0.500 | 0.500 | - | - | 0.500 | 0.500 | 0.500 | 0.500 |
| Sand | - | - | - | - | 0.295 | 0.015 | 0.175 | 0.110 |
| Dextrose | 78.880 | 52.270 | - | - | - | - | - | - |
| Casein[5] | 10.000 | - | - | - | - | - | - | - |
| Solka-Floc[6] | 5.000 | - | - | - | - | - | - | - |
| Bicarbonate | 0.200 | 0.170 | - | - | - | - | - | - |
| Potassium Chloride | 0.060 | - | - | - | - | - | - | - |
| | | | | | | | | |
| Nutrient Composition | | | | | | | | |
| CP (%) | 8.72 | 20.00 | - | - | 19.22 | 15.96 | 21.92 | 22.04 |
| AME (kcal) | 3,360 | 2,719 | - | - | 3,058 | 3,154 | 2,973 | 2,962 |
| Ca (%) | 0.88 | 0.88 | - | - | 0.88 | 0.88 | 0.88 | 0.88 |
| P (%) | 0.44 | 0.44 | - | - | 0.44 | 0.44 | 0.44 | 0.44 |
| K (%) | 0.60 | 0.90 | - | - | 0.72 | 0.62 | 0.91 | 0.91 |
| Na (%) | 0.21 | 0.21 | - | - | 0.21 | 0.21 | 0.21 | 0.21 |
| Cl (%) | 0.30 | 0.31 | - | - | 0.30 | 0.30 | 0.30 | 0.30 |
| DEB[7] | 162 | 238 | - | - | 191 | 163 | 237 | 238 |
| Mg (ppm) | 1,285 | 1,274 | - | - | 1,300 | 1,326 | 1,314 | 1,322 |
| Mn (ppm) | 124 | 137 | - | - | 136 | 135 | 137 | 138 |
| Choline (ppm) | 1,701 | 1,702 | - | - | 1,713 | 1,723 | 1,716 | 1,722 |
| dLys HCl[8] (%) | 0.74 | 1.10 | - | - | 1.00 | 0.80 | 1.15 | 1.15 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| dMet+Cys[8] (%) | 0.28 | 0.49 | - | - | 0.85 | 0.82 | 0.87 | 0.87 |
| dThr[8] (%) | 0.44 | 0.67 | - | - | 0.69 | 0.57 | 0.77 | 0.77 |
| | | | - | - | | | | |
| Analyzed Composition | | | | | | | | |
| DM, % | 96.52 | 94.32 | - | - | 89.33 | 90.17 | 90.59 | 90.59 |
| CP, % | 8.92 | 17.88 | - | - | 21.39 | 18.18 | 23.43 | 23.55 |
| GE, kcal/kg | 3,295 | 3,441 | - | - | 4,113 | 4,121 | 4,119 | 4,121 |
| $TiO_2$, % | 0.49 | 0.51 | - | - | 0.55 | 0.53 | 0.54 | 0.55 |

[1]Soybean meal (**SBM**) 10 and SBM 20 premix formulations are shown in this table. The added SBM is the SBM added in the substitution diets. Soybean meal contained 91.61% DM, 46.88 CP, and 4,292 kcal/kg GE.
[2]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[3]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[4]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[5]Acid casein (Fonterra, Auckland, New Zealand).
[6]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.
[7]Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[8]Digestible Lys, digestible Met+Cys, and digestible Thr
[9]Moisture content was calculated utilizing a VirTis 25S freeze dryer.
[10]Crude protein percent was determined using an Elementar Rapid N Cube.
[11]Gross energy was calculated in a Parr 6300 Calorimeter.
[12]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 5.4.** Ingredient composition, calculated nutrients, and analyzed nutrients in diets fed to male Yield Plus × Ross 708 broilers from 18 to 24 D of age (Experiment 3).

| Ingredient Composition, % | Semi-purified | Direct | Premix | Basal | Substituted |
|---|---|---|---|---|---|
| Corn | - | - | 80.587 | - | - |
| Soybean Meal[1] | - | 42.970 | 18.615 | - | 20 |
| L-Lys-HCl | - | - | 0.266 | - | - |
| DL-Met | - | - | 0.426 | - | - |
| L-Thr | - | - | 0.106 | - | - |
| Soybean Meal Premix | - | - | - | 94.444 | 75.208 |
| Dextrose | 78.880 | 52.250 | - | - | - |
| Casein[2] | 10.00 | - | - | - | - |
| Solka-Floc[3] | 5.000 | - | - | - | - |
| Sodium Bicarbonate | 0.200 | 0.190 | - | 0.200 | 0.300 |
| Dicalcium P | 1.100 | 1.790 | - | 1.820 | 1.780 |
| Defluorinated P | 0.610 | - | - | - | - |
| Sodium Chloride | 0.320 | 0.382 | - | 0.330 | 0.270 |
| Potassium Sulfate | 1.390 | - | - | 0.740 | - |
| Potassium Chloride | 0.060 | - | - | - | - |
| Limestone | 1.160 | 1.180 | - | 1.380 | 1.316 |
| Magnesium Oxide | 0.200 | 0.212 | - | 0.060 | 0.100 |
| Choline chloride-70[4] | 0.380 | 0.326 | - | 0.326 | 0.326 |
| Vitamin Premix[5] | 0.100 | 0.100 | - | 0.100 | 0.100 |
| Mineral Premix[6] | 0.100 | 0.100 | - | 0.100 | 0.100 |
| Titanium Dioxide | 0.500 | 0.500 | - | 0.500 | 0.500 |
| | | | | | |
| Nutrient composition | | | - | | |
| CP (%) | 8.72 | 19.99 | - | 14.50 | 20.85 |
| AME (kcal/kg) | 3,360 | 2,718 | - | 2,959 | 2,822 |
| Ca (%) | 0.88 | 0.879 | - | 0.882 | 0.903 |
| P (%) | 0.44 | 0.440 | - | 0.439 | 0.449 |
| K (%) | 0.60 | 0.905 | - | 0.904 | 0.898 |
| Na (%) | 0.21 | 0.215 | - | 0.206 | 0.210 |
| Cl (%) | 0.30 | 0.300 | - | 0.303 | 0.255 |
| DEB[7] | 162 | 240 | - | 235 | 249 |
| Mg (ppm) | 1,285 | 1,274 | - | 1,319 | 1,322 |
| Mn (ppm) | 124 | 137 | - | 136 | 139 |
| Choline (ppm) | 1,701 | 1,702 | - | 1,702 | 1,702 |
| | | | | | |
| Analyzed Nutrients[8] | | | - | | |
| DM[9], % | 98.38 | 96.36 | - | 91.00 | 88.02 |
| CP[10], % | 8.82 | 20.63 | - | 15.73 | 21.17 |
| GE[11], kcal/kg | 3,338 | 3,573 | - | 3,989 | 4,067 |
| $TiO_2$[12] Recovery, % | 0.48 | 0.48 | - | 0.42 | 0.47 |

[1]Added soybean meal represents the ingredient substituted into the basal diet substituted diets. Soybean meal contained 91.72% DM, 47.97% CP, and 4,532 kcal/kg GE.

[2]Acid casein (Fonterra, Auckland, New Zealand).

[3]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[4]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[5]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[6]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[7]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.

[8]Moisture content was calculated utilizing a VirTis 25S freeze dryer.

[9]Crude protein percent was determined using an Elementar Rapid N Cube.

[10]Gross energy was calculated in a Parr 6300 Calorimeter.

[11]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 5.5.** Growth performance of male Yield Plus × Ross 708 broilers from 18 to 23 D of age (Experiments 1, 2, and 3).[1]

| | BW, g | BW Gain[2,3], g | Feed Disappearance[2], g | Mortality[2,4], % |
|---|---|---|---|---|
| Experiment 1 | 917 | 228 | 473 | 0.69 |
| | | | | |
| Experiment 2 | | | | |
| SBM Direct | 1,034[c] | 283[c] | 530[b] | 2.3 |
| SBM 10 Basal | 1,152[ab] | 406[a] | 563[a] | 2.8 |
| SBM 10 Substituted | 1,177[a] | 430[a] | 540[ab] | 0.7 |
| SBM 20 Basal | 1,118[b] | 358[b] | 560[a] | 0.8 |
| SBM 20 Substituted | 1,167[ab] | 425[a] | 563[a] | 1.5 |
| SEM[5] | 14 | 11 | 9 | 0.9 |
| | | | | |
| Experiment 3 | | | | |
| SBM Direct | 875[b] | 252[b] | 470 | 0.0 |
| SBM Basal | 884[ab] | 270[b] | 484 | 1.5 |
| SBM Substituted | 935[a] | 317[a] | 489 | 2.3 |
| SEM[5] | 14 | 14 | 10 | 0.9 |
| | | *Probabilities* | | |
| Experiment 2- ANOVA | <0.0001 | <0.0001 | <0.0001 | 0.26 |
| Contrasts | | | | |
| Direct vs. 15 Sub. | <0.0001 | <0.0001 | 0.45 | 0.25 |
| Direct vs. 30 Sub. | <0.0001 | <0.0001 | 0.0093 | 0.56 |
| 15 Sub. vs. 30 Sub. | 0.63 | 0.76 | 0.06 | 0.56 |
| | | | | |
| Experiment 3- ANOVA | 0.0244 | 0.0006 | 0.21 | 0.22 |

[1]Values within columns within the same experiment with different superscripts are significantly different ($P \leq 0.05$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage. Data in Experiment 1 was not statistically compared with other treatments.
[2]Body weight gain, feed disappearance, and mortality were evaluated from 18 to 23 D of age.
[3]Average BW at 18 D of age was 702, 749, 617 g in Experiments 1, 2, and 3, respectively, and was not different between treatments ($P \leq 0.46$).
[4]Mortality values were arcsine transformed prior to analysis.
[5]Standard error of the mean.

**Table 5.6.** Metabolizable and digestible energy values determined for soybean meal using male Ross 708 × YP broilers provided with test diets from 18 to 25 D of age (Experiment 1).[1]

| Method (kcal/kg DM, unless otherwise noted) | Soybean meal |
|---|---|
| AME[2] | 2,513[c] |
| $AME_n$[2] | 2,194[d] |
| SME[2] | 2,661[b] |
| AIDE[2] | 2,720[ab] |
| SIDE[2] | 2,880[a] |
| SEM[3] | 49 |
| | *Probability* |
| ANOVA | <0.001 |

[1]Test ingredients were provided as a mash diet containing 43% soybean meal and 52% dextrose. Means with different superscripts are significantly different ($P < 0.05$).
[2]AME= apparent metabolizable energy, $AME_n$ = nitrogen-corrected AME, SME= standardized metabolizable energy, AIDE = apparent ileal digestible energy, SIDE = standardized ileal digestible energy.
[3]Standard error of the mean.

**Table 5.7.** Effect of diet and energy determination method on determined energy of diets and soybean meal fed to Ross YP × 708 broilers from 18 to 25 D of age in kcal/kg on DM basis (Experiment 2).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Diets | | | | | |
| Direct | 2,667 $^{d, w}$ | 2,420 $^{d, v}$ | 2,822 $^{d, y}$ | 2,742 $^{d, x}$ | 2,893 $^{d, z}$ |
| 10 Basal | 3,042 $^{b, w}$ | 2,795 $^{b, v}$ | 3,182 $^{b, y}$ | 3,403 $^{b, x}$ | 3,542 $^{b, z}$ |
| 10 Substituted | 2,837 $^{c, w}$ | 2,644 $^{c, v}$ | 2,972 $^{c, y}$ | 3,195 $^{c, x}$ | 3,329 $^{c, z}$ |
| 20 Basal | 3,193 $^{a, w}$ | 2,930 $^{a, v}$ | 3,333 $^{a, y}$ | 3,534 $^{a, x}$ | 3,674 $^{a, z}$ |
| 20 Substituted | 2,865 $^{c, w}$ | 2,636 $^{c, v}$ | 3,000 $^{c, y}$ | 3,135 $^{c, x}$ | 3,269 $^{c, z}$ |
| SEM[3] | 16 | 16 | 16 | 19 | 19 |
| Methodology | | | | | |
| Direct | 2,211$^{b, x}$ | 1,637$^{b, w}$ | 2,362$^{b, y}$ | 2,385 $^{y}$ | 2,536 $^{z}$ |
| 10 Substituted | 2,496$^{a, xy}$ | 2,371$^{a, x}$ | 2,637$^{a, yz}$ | 2,514 $^{xyz}$ | 2,665 $^{z}$ |
| 20 Substituted | 2,437$^{a, yz}$ | 2,288$^{a, y}$ | 2,593$^{a, z}$ | 2,508 $^{yz}$ | 2,659 $^{z}$ |
| SEM[3] | | | | | |
| Direct | 28 | 27 | 28 | 23 | 31 |
| 10 Substituted | 87 | 73 | 87 | 97 | 97 |
| 20 Substituted | 65 | 60 | 65 | 72 | 81 |
| | | | *Probabilities* | | |
| Diets-ANOVA | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Methodology | | | | | |
| ANOVA *P*-Value | 0.0024 | <0.0001 | 0.0024 | 0.29 | 0.30 |
| Contrasts | | | | | |
| Direct vs. 10 | 0.0012 | <0.0001 | 0.0012 | 0.17 | 0.17 |
| Direct vs. 20 | 0.0047 | <0.0001 | 0.0047 | 0.20 | 0.21 |
| 10 vs. 20 | 0.44 | 0.32 | 0.44 | 0.95 | 0.95 |

[1]Significant differences caused by method are shown with superscripts A, B, C, and D within a column and within compared treatments. Significant differences of calculation method are shown within rows and differences are shown by superscripts V, W, X, Y, and Z. Effect of calculation was significant for all diets ($P \leq 0.0001$) and for all methodologies ($P \leq 0.0041$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean. Pooled standard error is presented for diets, while individual SEM is presented for methodology.

**Table 5.8.** Effect of diet and energy determination method on determined energy of diets and soybean meal fed to Ross YP × 708 broilers from 18 to 24 D of age in kcal/kg on DM basis (Experiment 3).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Diets | | | | | |
| Direct | 2,707 [c, y] | 2,505 [c, x] | 2,857 [c, z] | 2,775 [c, y] | 2,926 [c, z] |
| Basal | 3,191 [b, y] | 3,063 [b, x] | 3,348 [b, z] | 3,183 [b, y] | 3,339 [b, z] |
| Substituted | 3,055 [b, y] | 2,909 [b, x] | 3,216 [b, z] | 3,064 [b, y] | 3,224 [b, z] |
| SEM[3] | 18 | 23 | 18 | 23 | 23 |
| | | | | | |
| Methodology | | | | | |
| Direct | 2,300 [b, y] | 1,835 [b, x] | 2,474 [b, yz] | 2,465 [b, yz] | 2,638 [b, z] |
| Substituted | 2,570 [a, yz] | 2,308 [a, y] | 2,747 [a, yz] | 2,703 [a, yz] | 2,892 [a, z] |
| | | | | | |
| SEM[3] | | | | | |
| Direct | 46 | 42 | 46 | 59 | 59 |
| Substituted | 72 | 38 | 72 | 120 | 120 |
| | | | *Probabilities* | | |
| ANOVA- Diets | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| ANOVA- Method | 0.0032 | < 0.0001 | 0.0029 | 0.08 | 0.08 |

[1]Significant differences caused by method are shown with superscripts A, B, C, and D within a column and within compared treatments. Significant differences of calculation method are shown within rows and differences are shown by superscripts V, W, X, Y, and Z. Effect of calculation was significant for all diets ($P \leq 0.0001$) and methodologies ($P \leq 0.0084$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean. Pooled standard error is presented for diets, while individual SEM is presented for methodology.

# VI. EVALUATION OF ENERGY ASSAY METHODOLOGY FOR BROILER DIETS: 3. ADDITIVITY

## ABSTRACT

Two experiments were conducted to evaluate effects of assay methodology, utilization of ME or digestible energy, and standardization for endogenous losses of energy (**EEL**) on additivity of energy in broiler diets. In Experiment 1, energy of corn, wheat, and soybean meal (**SBM**) was determined concurrently in separate experiments utilizing only the direct method. Additivity was evaluated in high and low CP blends of SBM and either corn or wheat to assess effect of ingredient ratio on additivity. In Experiment 2, energy utilization of corn and SBM was determined concurrently in a separate experiment utilizing both the direct and substation methods. Additivity was evaluated in high and low CP blends of corn and SBM. In each experiment, broilers were provided with common starter diets from 1 to 17 D of age. Experimental diets were provided from 18 to 25 D of age. A 48-h balance experiment was conducted from 21 to 23 D of age to determine ME. Digesta was collected for determination of digestible energy and EEL on D 24 and 25. Endogenous losses of energy were estimated in both experiments utilizing semi-purified diets primarily composed of dextrose, casein, and cellulose. Growth performance characteristics were measured from 18 to 23 D of age. Energy utilization was calculated as AME, $AME_n$, standardized ME, apparent ileal digestible energy, and standardized ileal digestible energy.

In each experiment, dietary treatments were represented by 16 replicate cages with 9 chicks per cage. Additivity was evaluated as a one-sample, two-side T-Test where $H_0 = 0$ and additivity is considered at $P \geq 0.05$, while $P < 0.05$ indicates a lack of additivity. In Experiment 1, additivity was not detected ($P \leq 0.0122$). In Experiment 2, additivity was detected when energy was determined with the substitution method ($P \geq 0.07$). These experiments indicated that assay methodology affects additivity of determined energy values, but that standardization for EEL did not provide ingredient values that were more additive than apparent energy values.

## INTRODUCTION

Least cost formulation for broilers assumes additivity of energy (Sibbald et al., 1960), or that the energy of the diet is equal to the sum of the energy of the ingredients utilized multiplied by the proportion of their inclusion. As energy comprises approximately 50 to 70% of diet costs (Skinner et al., 1992), ensuring that energy values utilized for formulation are additive is necessary to avoid under- or over-supplying dietary energy. A lack of additivity of energy of ingredients would indicate that determined energy values are only accurate in diets similar to the diet utilized to measure ingredient energy (Aaradsma et al., 2017). While additivity is a common assumption in formulation of broiler diets, a variety of interactions occur between diet ingredients and nutrients that can affect digestion of absorption of other ingredients (Dale and Fuller, 1980). These may include extra-caloric effects of fat (Mateos et al., 1980), interactions between starch and protein (Liu and Selle, 2015), or antinutritional effects of fiber (Jorgensen et al., 1996). In addition to dietary factors, endogenous losses of energy (**EEL**) could cause a lack of additivity, as

determined AME is sensitive to feed intake while determined TME, which is corrected for EEL, is independent of feed intake (Jonsson and McNab, 1983). It has been reported that standardization for amino acid flows (Cowieson et al., 2019) leads to amino acid values that are more additive; however, standardization for endogenous P losses did not affect additivity of P (Babatunde et al., 2020).

Two common methods of determining ingredient energy are the direct method, where all dietary energy is supplied by the test ingredient (Carpenter and Clegg, 1958; Sibbald, 1976), and the substitution method, which employs a basal diet and a test diet to calculate the energy of the test ingredient (Sibbald and Kramer, 1978). Limited data have evaluated the effect of assay methodology or energy calculation on additivity of energy. Therefore, two experiments were conducted to evaluate the effect of the direct method and the substitution method on additivity of dietary energy, as well as the effect of utilizing apparent or standardized values and metabolizable or digestible energy values on additivity of energy.

## MATERIALS AND METHODS

All procedures involving the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2020-3796 and 2022-4050).

### *Dietary Treatments*

Energy determination in cereal grains and SBM were evaluated concurrently with additivity and the data are presented in the two companion manuscripts. From 1 to 17 D of age in both experiments, broilers were provided a common starter diet (Table 6.1). In both

experiments, a dextrose-based semi-purified diet containing 10% casein was utilized to estimate EEL (Tables 6.2 and 6.3). In Experiment 1, energy balance of corn, wheat, and SBM was evaluated utilizing the direct method, where all dietary energy comes from the test ingredient or glucose (Table 6.2). Additivity was evaluated utilizing blends of corn and SBM or wheat and SBM. Diets were designed to evaluate the effect of ingredient ratio on additivity rather than the effect of dietary CP content on additivity, but are denotated as high CP and low CP diets. In Experiment 2, additivity was evaluated utilizing energy values determined with either the substitution method or the direct method (Table 6.3). The blended diets utilized Experiment 2 were formulated the same as corn and SBM blended diets in Experiment 1. All diets were formulated to be adequate in vitamins and minerals and contained 0.50% titanium dioxide as an indigestible marker.

***Broiler Husbandry***

Two energy balance experiments (Experiments 1 and 2) were conducted in broilers from 18 to 24 D of age. Male YP × Ross 708 chicks (Aviagen Inc., Huntsville, AL) were obtained from a commercial hatchery at day of hatch. All chicks were vaccinated against Marek's Disease, Newcastle Disease, and infectious bronchitis. Additionally, chicks received a 1× vaccination against coccidiosis. Nine chicks were placed into each battery cage (68 × 68 × 38 cm; Petersime, Gettysburg, OH) that contained trough feeders and waterers. Batteries were housed in solid-sided houses with forced air-furnaces, evaporative cooling pads, vent boards, and electronic controllers for temperature control. Temperature was set to 33°C at placement and decrease to 25°C at 18 D of age. Photoperiod was set at 23L:1D from 1 to 7 D of age and 20L:4D after 7 D of age.

***Measurements, Sample Collection, and Chemical Analysis***

Birds were weighed at 1, 18, and 23 D of age to calculate BW and BW gain. Feed disappearance was measured from 18 to 23 D of age. Mortality was recorded daily. From 21 to 23 D of age, a 48-h balance assay was conducted to measure ME. Feed disappearance and excreta voided were measured at 24 and 48 h. Excreta samples were collected from the pan underneath each cage in areas free from contamination by feed or feathers. Samples were pooled into bags for analysis and stored at -20°C until analysis. Samples were homogenized and a 50 g subsample was dried for analysis. In Experiment 1, samples were freeze dried (VirTis Genesis 25ES, SP Industries Inc., Warminster, PA). In Experiment 2, samples were dried in a forced-air oven (Thermo Scientific Heratherm UT 20 P oven, Waltham, MA) at 55°C for 48 h, as described by Jacobs et al. (2011). Dried samples were ground in a coffee grinder (Capresso Infinity 560 burr grinder, Montvale, NJ) and dried feed and excreta were analyzed (0.80 g samples) in quadruplicate or duplicate, respectively, for gross energy (**GE**) utilizing an isoperibol oxygen bomb calorimeter (Model 6300 for Experiments 1 and Model 6400 for Experiment 2, Parr Instruments, Moline, IA). Nitrogen content of dried feed and excreta was determined in quadruplicate or duplicate, respectively, with 0.25 g samples. In Experiment 1, N content was analyzed with a combustion analyzer (Rapid N Cube, Elementar, Hanau, Germany; AOAC 968.06, AOAC International, 2006). In Experiment 2, excreta N content was measured by a commercial laboratory (University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO; method 990.03 AOAC International, 2006).

At 24 and 25 D of age, ileal digesta was collected from 8 chicks per cage for determination digestible energy (**DE**). In Experiment 1, the collection occurred at D 24 and

25, with half the replicates necropsied each day. In Experiment 2, sample collection occurred at D 24. Apparent DE (**AIDE**) and standardized DE (**SIDE**) were evaluated. Chicks were euthanized by $CO_2$ asphyxiation. Digesta was collected from the terminal one-third of the ileum to 2 cm proximal to the ileo-cecal junction (Kluth et al., 2005) by flushing the intestinal section with distilled, deionized water (Adedokun et al., 2011). Samples were pooled by pen and were stored on ice until transfer to freezer for storage at -20°C until analysis. Samples were dried, ground, and analyzed for GE as described for excreta. Additionally, titanium dioxide concentration was determined in dried digesta in duplicate (200 mg) and in dried feed (600 mg) in quadruplicate as described by Short et al. (1996). Samples were ashed in a muffle furnace (Thermo Scientific Thermolyne Muffle Ashing Ove F30400, Waltham, MA) at 580°C for 12 h. Ashed samples were then dissolved in 10 mL 7.4 M HCl on hotplates set to 200°C until solutions were clear. Dissolved samples were rinsed with 10 mL distilled water into a 100 mL beaker that contained 20 mL 30% hydrogen peroxide and 60 mL water. Color was allowed to develop for 48 h, and then absorbance was measured in a spectrophotometer (SpectraMax Plus 384, Molecular Devices LLC, San Jose, CA) at 410 nm. Titanium dioxide concentration was determined by comparing absorbance of samples with standards of a known $TiO_2$ concentration utilizing simple linear regression.

Standardization for EEL was evaluated in both experiments with 16 cages (9 chicks/cage) to determine standardized ME (**SME**) and SIDE.. Standardization for both ME and DE was calculated based on the EEL determined in the terminal ileum. All digesta samples utilized for standardization were evaluated for the presence of exogenous glucose

with test strips (Quantofix glucose test strips, CTL Scientific Supply Corp., Deer Park, NY) to verify absorption of dietary energy.

***Calculations***

Endogenous losses of energy (kcal/kg DM intake) were calculated as follows:

$$EEL\ \left({}^{kcal}/_{kg\ DMI}\right) = GE_{digesta} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}}\right) - (Cellulose\ \%\ \times GE_{cellulose})$$

where $GE_{digesta}$ represents the GE of the dried digesta and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the dried semi-purified diet and digesta, respectively. The calculation is corrected for the content of indigestible cellulose by subtracting the GE of the cellulose multiplied by the dietary inclusion of cellulose.

Diet AME, $AME_n$, and SME for blended diets and feed ingredients were calculated with the following equations:

$$AME = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD(kg)}$$

$$AME_n = \frac{[GE_{in}(kcal) - GE_{ex}(kcal)] - \{8.22(kcal/g) \times [N_{in}(g) - N_{ex}(g)]\}}{FD(kg)}$$

$$SME = EEL\ (kcal/kg) + \frac{[GE_{in}(kcal) - GE_{ex}(kcal)]}{FD(kg)}$$

where $GE_{in}$ represents the total GE disappearance by the birds during the collection period, $GE_{ex}$ represents the GE of voided excreta during the collection period, FD (kg) represents the total feed disappearance during the collection period on a DM basis, and $N_{in}$ and $N_{ex}$ represent the total N disappearance and excretion during the experimental period, respectively. Nitrogen corrected AME was corrected utilizing a factor of 8.22 kcal/g (Hill and Anderson, 1958).

Diet AIDE and SIDE for blended diets and feed ingredients were calculated with the following equations:

$$AIDE\ \left(kcal/kg\right) = GE_{diet} \times \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)$$

$$SIDE\left(kcal/kg\right) = GE_{diet} \times \left(\frac{EEL}{GE_{diet}} + \left(\frac{TiO_{2_{diet}}}{TiO_{2_{digesta}}} \times \frac{GE_{digesta}}{GE_{diet}}\right)\right)$$

where $GE_{diet}$ and $GE_{digesta}$ represent the GE of the diet and digesta, respectively, on a DM basis and $TiO_{2_{diet}}$ and $TiO_{2_{digesta}}$ represent the titanium dioxide concentration in the diet and digesta, respectively.

Apparent ME, $AME_n$, SME, AIDE, and SIDE in ingredients (DM basis) was calculated with these equations:

$$Direct\ ingredient\ energy\ \left(kcal/kg\right) = \frac{Diet\ energy}{Ingredient\ \%} \times 100$$

$$Substituted\ ingredient\ energy\ \left(kcal/kg\right) = \frac{Energy_{td} - (Energy_{bde} \times Inclusion_{td})}{Inclusion_{ti}} \times 100$$

Energy utilization of cereal grains determined with the direct method was calculated by dividing the determined energy of the test diet by the inclusion rate of the test ingredient and multiplying by 100. Metabolizable energy or DE utilizing the substitution method was calculated by subtracting the determined energy of the energy contributing ingredients of the basal diet ($Energy_{bde}$, calculated as energy of the basal diet divided by the inclusion percent of energy contributing ingredients) multiplied by the inclusion rate of the test diet ($Inclusion_{td}$) from the determined ME or DE of the substituted diet ($Energy_{td}$) to determine the energy value provided by the test ingredient. Metabolizable energy or DE provided by

the test ingredient was then divided by the inclusion rate of the test ingredient ($Inclusion_{ti}$) and multiplied by 100 to determine the ME or DE of the test ingredient in kcal/kg DM.

Soybean meal AME, $AME_{n,}$ SME, AIDE, and SIDE (DM basis) were determined utilizing the direct method was calculated as follows:

$$Direct\ ingredient\ energy\ \left({}^{kcal}/_{kg}\right) = \frac{(Diet\ energy - energy\ from\ dextrose)}{soybean\ meal\ \%} \times 100$$

Energy utilization of SBM determined utilizing direct method was calculated by subtracting the energy contribution of dextrose from the energy of the diet to determine the energy contribution of SBM. The energy contribution of SBM was then divided by the inclusion rate of SBM and multiplied by 100.

Predicted energy for determination of additivity was determined as follows:

$$Predicted\ energy, kcal/kg = \sum\left(Energy_n, kcal/kg \times (Inclusion_n, \%)\right)$$

where the predicted energy is equal to the sum of the energy contribution of individual ingredients ($Energy_n$) multiplied by their respective inclusion rate ($Inclusion_{n).}$ The difference between the determined energy of blends (determined in vivo) and the predicted energy (calculated based on ingredient determined energy) was utilized to determine additivity.

***Statistical Analyses***

Dietary treatments were randomly allocated in both experiments as a randomized complete block design. Pen location was the blocking factor and cage was the experimental unit. Each dietary treatment was provided to 16 replicate cages (9 chicks/cage). Growth performance, ingredient energy, and diet energy were analyzed using a one-way ANOVA with PROC MIXED in SAS (2016) according to the following model:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

where μ is the overall mean, $\tau_i$ are the factor level effects of the $i^{th}$ dietary treatment where the $\Sigma\tau_i = 0$, $\beta_j$ are the random block effects which are identically and independently normally distributed, and $\varepsilon_{ij}$ represents the random error of the $i^{th}$ treatment and the $j^{th}$ block and are identically and independently normally distributed. Mortality was arcsine transformed prior to analysis and statistical significance was considered at $P \leq 0.05$. Additivity was determined as a 1-sample 2-side T-Test where $H_0 = 0$ evaluating the difference between predicted energy and determined energy (Osho et al., 2019). Additivity was detected when $P \geq 0.05$, while a lack of additivity was determined when $P < 0.05$.

## RESULTS AND DISCUSSION

### *Diet Analysis*

Analyzed values for CP (Experiments 1 and 2) were in good agreement with calculated values (Table 6.2 and 6.3). In Experiment 1, $TiO_2$ recovery was close to the formulated value of 0.50%. In Experiment 2, $TiO_2$ recovery was slightly reduced from expected values, ranging from 0.42 to 0.48% compared with formulated value of 0.50%. As analyzed values were utilized for calculation of digestibility, it is not likely this affected the results of the experiment.

### *Growth Performance*

In Experiment 1, broilers receiving the high protein corn and SBM diet had increased BW gain ($P < 0.0001$) compared with chicks receiving the low protein corn and SBM diet (Table 6.4). Similarly, broilers receiving the high protein wheat and SBM diet had increased BW gain ($P < 0.0001$) compared with broilers receiving low protein wheat

and SBM diets. No differences ($P \geq 0.13$) were observed between treatments for feed disappearance or mortality. In Experiment 2, no differences ($P \geq 0.24$) were observed for growth performance characteristics. However, the numeric increase ($P = 0.24$) in BW gain of chicks provided with high protein diets compared with those provided low protein diets was similar to that observed in Experiment 1. The lack of significant increase in BW gain is due to the large variance (SEM = 16 g), that may have been a result of the suboptimal amino acid profile of the diets (Vieira et al., 2004).

***Experiment 1***

***Ingredient and Blended Diet Energy.*** In Experiment 1, AME of corn, wheat, and SBM was 3,460, 3,036, and 2,513 kcal/kg on DM basis, respectively (Table 6.5). These values, as well as $AME_{n,}$ SME, AIDE, and SIDE were lower than expected based on database published values (Rostagno et al., 2017). However, AME of blended diets was close to formulated values (Table 6.2) when energy was converted to an as-fed basis. Possible explanations for the reduction in determined energy values are discussed in the companion manuscripts evaluating assay methodology for determining energy of cereal grains or SBM. Some of these reasons may include a sub-optimal starch:protein ratio (Truong et al., 2015), reduced dietary fat content (Mateos et al., 1980; Golian and Maurice, 1992), dietary amino acid profile (Dozier et al., 2001), high concentration of rapidly digestible starch (Selle et al., 2014), rate of feed passage and diet form (Adeola and Ileleji, 2009; Rochell et al., 2012), or antinutrients (Selle and Ravindran, 2007).

***Additivity.*** In Experiment 1, additivity was not detected for any diet utilizing any calculation method ($P \leq 0.0122$; Table 6.6). This was caused by the underestimation of diet ingredient values when determined utilizing the direct method, which may have been

caused by some of the factors listed above. Differences between actual and predicted energy ranged from an underestimation of 111 kcal/kg in the low protein corn and SBM diet when calculated as SIDE to an underestimation of 252 kcal/kg in the high protein wheat and SBM diets when calculated utilizing $AME_{n.}$ Standardization for EEL did not lead to values ($P \leq 0.0122$) that were more additive, nor did utilization of digestible energy values ($P \leq 0.0122$). This lack of additivity was caused by the underestimation of determined energy in diet ingredients when utilizing the direct method.

***Experiment 2***

***Ingredient and Blended Diet Energy.*** In Experiment 2, energy of corn and SBM was determined utilizing either the direct or the substitution method for estimation of energy (Table 6.7). The direct method underestimated energy for corn compared with the substitution method ($P \leq 0.0216$) for AME, $AME_n$, SME, AIDE, and SIDE. Apparent ME of corn was 3,675 kcal/kg DM basis when determined utilizing the substitution method and was 244 kcal/kg higher ($P = 0.0012$) than AME determined utilizing the direct method. However, SEM ranged from 62 to 69 kcal/kg when utilizing the direct method, compared with 19 to 26 kcal/kg when determined with the direct method. The difference in determined energy of SBM was similar to that of corn. Apparent ME, $AME_{n,}$ and SME were all higher ($P \leq 0.0067$) when determined utilizing the substitution method compared with the direct method. Apparent ileal DE and SIDE had numerically higher energy when determined using the substitution method but was not different ($P \geq 0.40$) from energy determined with the direct method. This is likely due to the high variance (SEM = 120 kcal/kg) of energy in SBM when calculated utilizing the direct method. Determined AME

of the blended diets was close to the formulated values (Table 6.3) when converted to as-fed basis.

The causes of differences between determined energy when utilizing the substitution method compared with the direct method are discussed in the companion manuscripts for cereal grains and soybean meal. The substitution method may have provided dietary nutrient profile that was more favorable for digestion and absorption of energy contributing ingredients than in the direct method. As delineated for the ingredients utilized in Experiment 1, factors with may have affected the different determined energy include the starch:protein ratio or digestibility rate of the dietary starch (Selle et al., 2014; Truong et al., 2015), the dietary amino acid profile (Dozier et al., 2001), or the rate of feed passage and diet form (Adeola and Ileleji, 2009; Rochell et al., 2012). The increase in SME and SIDE compared with AME or AIDE, respectively, was expected due to the calculations used to derive the standardized values (Wolynetz and Sibbald, 1984). Similarly, the numeric increase in DE compared with ME values was expected based on the model for energy partitioning (Latshaw and Moritz, 2009).

***Additivity.*** In Experiment 2, additivity was detected ($P \geq 0.07$) in high CP diets with all calculation methods when energy was determined with the substitution method (Table 6.8). Similarly, additivity was detected ($P \geq 0.09$) in the low CP diet when utilizing the substitution method when calculated as AME, SME, AIDE, and SIDE, but not when calculated as $AME_n$ ($P = 0.0194$). Additivity was not detected ($P \leq 0.0337$) for either diet when energy was determined with the direct method, or when energy was determined utilizing the direct method to determine energy of one ingredient and the substitution method was utilized to determine energy of the other ingredient.

Methodology affected additivity in both experiments, as underestimation of ingredient energy values utilizing the direct method led underestimation of predicted energy of the blended diets. Conversely, utilization of the substitution method determined energy values that were higher than the direct method and more additive in the blended diets. This indicates that there are interactions between feed ingredients in broiler diets that affect determined energy. Additivity was detected when energy was determined with the substitution method regardless of calculation method, with the exception of $AME_n$ in the low CP blended diet. This indicates that assay methodology has a greater effect of determination of additivity than calculation. The lack of additivity of $AME_n$ in the low CP blend was due to overestimation of $AME_n$ in dietary ingredients compared with the blend (Wolynetz and Sibbald, 1984). This could be due to N correction penalizing low CP ingredients, such as corn, less than higher protein ingredients (Lopez and Leeson, 2008), leading to an overestimation of the energy contribution of corn in the blended diet. This indicates that the CP content of the diet may affect the additivity of energy calculated as $AME_n$, but that ingredient ratio that is practical in conventional corn- and SBM-based diets does not affect additivity of ingredients when calculated utilizing other methods.

Standardization has been theorized to lead to values that are more additive than apparent values (Stein et al., 2005; Kong and Adeola, 2013; Cowieson et al., 2019). However, it is worth noting that the nomenclature utilized for correction of endogenous losses is not consistent between papers. Standardized values refer to values which are corrected for only basal losses, or those which are independent of diet (Stein et al., 2007). True values correct for total losses including specific losses, or those that are caused by the diet (Stein et al., 2007). Despite this, energy values that are corrected for only basal losses

are often referred to as true values (i.e., TME), which could lead to variation between studies. Standardization has been reported to improve additivity of some amino acids (Cowieson et al., 2019; Osho et al., 2019). Conversely, standardization did not improve additivity of P, as apparent P digestibility was already additive (Babatunde et al., 2020; Babatunde and Adeola, 2021). Limited data are available in the literature delineating effects of standardization on additivity of energy compared with apparent values. One reason for the similarity in additivity of apparent and standardized energy values in the current study could be explained through the relationship between the energy calculations. Khalil et al. (2020) evaluated energy of a variety of cereal grains and calculated the energy as AME, $AME_n$, AIDE, and true ileal digestible energy (**TIDE**). The authors correlated the AIDE and TIDE and determined a Pearson coefficient of 1 ($P = 0.001$), indicating a total linear relationship between AIDE and TIDE. This relationship indicates that the utilization of TIDE would likely not lead to more additive values than the use of AIDE.

Additionally, the similar response between apparent and standardized ME and DE may be in part due to the feed consumption of the birds. In the current experiments, no differences ($P \geq 0.13$) were observed between feed disappearance of broilers provided with different dietary treatments. Furthermore, daily feed disappearance ranged from 91 to 96 g per bird per day. Wolynetz and Sibbald (1984) demonstrated that AME was not affected by feed consumption when broilers were consuming more than approximately 40 g feed consumption per bird per day. The broilers in the current experiments consumed between 91 to 96 g feed per day, which may have minimized the difference between determined apparent and standardized DE or ME. This would further indicate a parallel relationship between apparent and standardized energy that would lead to similar additivity results.

Despite this lack of effect on additivity, there is value in standardization of energy as it reduces effects of individual bird variation (Cowieson et al., 2020), thus providing more consistent energy values.

Additivity of amino acids or energy in diets provided to broiler chickens has been discussed in previous reports. While many papers report a lack of additivity of amino acids (Angkanaporn et al., 1996; Hong et al., 2001; Hong et al., 2002; Kong and Adeola, 2013), additivity of energy has been reported (Dale and Fuller, 1980; Hong et al., 2001; Hong et al., 2002). Dale and Fuller (1980) reported numerical additivity of TME. These authors calculated that the TME of a diet containing corn, SBM, and corn gluten meal was 110 kcal/kg numerically higher than the determined value when fed to broilers, while the calculated TME of a diet containing corn, SBM, and fish meal was 220 kcal/kg numerically higher than the determined value when fed to broilers. However, these authors did not perform statistical analyses to determine additivity. Hong et al. (2001; 2002) conducted 2 additional studies to assess additivity of AME, TME, and amino acids in diets provided to ducks. These authors determined that while amino acids are not additive in duck diets, AME and TME are additive. However, it is worth noting that both studies utilized six replicate cages per treatment (1 duck per cage), which may have been too small a sample to detect a lack of additivity. The current experiments indicate that energy contribution of feed ingredients may be additive in some cases, but the additivity is affected by methodology utilized to evaluate energy balance of individual feed ingredients. This indicates that there are interactions between dietary ingredients that affect the energy utilization by the broiler. Additional research is warranted to further delineate the

mechanisms affecting interactions between digestion and absorption of feed ingredients and dietary nutrients.

In Experiment 1 and 2, determination of energy in feed ingredients utilizing the direct method provided ME or DE values that underestimated the energy of the blended diets. In Experiment 2, utilization of the substitution method provided ME and DE values that were additive. Standardization for EEL did not provide ingredient values that are more additive than apparent values. Similarly, determination of DE did not provide energy values that were more additive than determined ME. These experiments indicated that assay methodology utilized to determine ingredient energy affects additivity of energy in diets provided to broiler chickens.

## REFERENCES


Aardsma, M. P., R. D. Mitchell, and C. M. Parsons. 2017. Relative metabolizable energy values for fats and oils in young broilers and adult roosters. Poult. Sci. 96:2320–2329.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. Poult. Sci. 88:579–585.

Angkanaporn, K., M. Choct, W. L. Bryden, E. F. Annison, and G. Annison. 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. J. Sci Food Agri. 66:399-404.

AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD.

Babatunde, O. O., and O. Adeola. 2021. Additivity of apparent and standardised ileal digestibility of phosphorus in corn and canola meal mixed diets; basal endogenous loss of phosphorus responses to phytase and age in broiler chickens. Br. Poult. Sci. 62:244-250.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Responses of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. 99:3997-4008.

Carpenter, K. J., and K. M. Clegg. 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Sci. Food Agr. 7: 45-51.

Cowieson, A. J., and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98:745–752.

Cowieson, A. J., M. M. Bhuiyan, J. O. B. Sorbara, G. Pappenberger, M. B. Pedersen, and M. Choct. 2020. Contribution of individual broilers to variation in amino acid digestibility in soybean meal and the efficacy of an exogenous monocomponent protease. Poult. Sci. 99:1075-1083.

Dale, N. M., and H. L. Fuller. 1980. Additivity of true metabolizable energy values as measured with roosters, broiler chicks, and poults. Poult. Sci. 59:1941-1942.

Dozier, W. A., III, E. T. Moran, and M. T. Kidd. 2001. Male and female broiler responses to low and adequate dietary threonine on nitrogen and energy balance. Poult. Sci. 80:926-930.

Golian, A., and D. V. Maurice. 1992. Dietary poultry fat and gastrointestinal transit time of feed and fat utilization in broiler chickens. Poult. Sci. 71:1357–1363.

Hong, D., D. Ragland, and O. Adeola. 2001. Additivity and associative effects of metabolizable energy and amino acid digestibility in barley and canola meal for White Pekin ducks. Poult. Sci. 80:1600–1606

Hong, D., D. Ragland, and O. Adeola. 2002. Additivity and associative effects of metabolizable energy and amino acid digestibility of corn, soybean meal, and wheat red dog for White Pekin ducks. J. Anim. Sci. 80:3222-3229.

Jacobs, B. M., J. F. Patience, W. A. Dozier, III, K. J. Stalder, and B. J. Kerr. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. J. Anim. Sci. 89:2624–2630.

Jonsson, G., and J. M. McNab. 1983. A comparison of methods for estimating the metabolizable energy of a sample of grass meal. Br. Poult. Sci. 49:349-359.

Jorgensen, H., X. Q. Zhao, K. E. Knudsen, and B. O. Eggum. 1996. The influence of dietary fiber source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379–395.

Khalil, M. M., M. R. Abdollahi, F. Zaefarian, F., and V. Ravindran. 2020. Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poult. Sci. 99:6809-6817.

Kluth, H., K. Mehlhorn, and M. Rodehutscord. 2005. Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. Arch. Anim. Nutr. 59:271–279.

Kong, C., and O. Adeola. 2013. Ileal endogenous amino acid flow response to nitrogen-free diets with differing ratios of corn starch to dextrose in broiler chickens. Poult. Sci. 92:1276-1282.

Latshaw, J. D., and J. S. Moritz. 2009. The partitioning of metabolizable energy by broiler chickens. Poult. Sci. 88:98–105.

Liu, S. Y., and P. H. Selle. 2015. A consideration of starch and protein digestive dynamics in chicken-meat production. Worlds Poult. Sci. J. 71:297–310.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Mateos, G. G., and J. L. Sell. 1980. Influence of carbohydrate and supplemental fat source on the apparent metabolizable energy of the diet. Poult. Sci. 59:2129–2135.

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333-1340.

Rochel, S. J., T. J. Applegate, E. J. Kim, and W. A. Dozier, III. 2012. Effects of diet type and ingredient composition on rate of passage and apparent ileal amino acid digestibility in broiler chicks. Poult. Sci. 91:1647-1653.

Rostagno, H. S. 2017. Brazilian Tables for Poultry and Swine, Composition of Feedstuffs and Nutrient Requirements. 4th ed Federal University of Vicosa, Minas Gerais, Brazil.

SAS Institute. 2016. SAS® User's Guide. Statistics. Version 9.4 ed. SAS Inst. Inc., Cary, NC.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Selle, P. H., S. Y. Liu, J. Cai, R. A. Caldwell, and A. J. Cowieson. 2014. Graded inclusions of sodium metabisuphite in sorghum-based diets: 1. Reduction of disulphide cross-linkages in vitro and enhancement of energy utilisation and feed conversion efficiency in broiler chickens. Anim. Feed. Sci. Technol. 190:59-67.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult. Sci. 55:303–308.

Sibbald, I. R., and J. K. G. Kramer. 1978. The effect of the basal diet on the true metabolizable energy value of fat. Poult. Sci. 57:685–691.

Sibbald, I. R., J. D. Summers, and S. J. Slinger. 1960. Factors affecting the metabolizable energy content of poultry feeds. Poult. Sci. 39:544-556.

Skinner, J. T., A. L. Waldroup, and P. W. Waldroup. 1992. Effects of dietary nutrient density on performance and carcass quality of broilers 42 to 49 days of age. J. Appl. Poult. Res. 1:367–372.

Stein, H. H., C. Pedersen, A. R. Wirt, and R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387–2395.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282–285.

Titus, H. W. 1956. Energy values of feedstuffs for poultry. Pages 10–14 in Proc. Semiannual Meet. Nutr. Counc. Am. Feed Manufacturers Assoc., St. Louis, MO.

Truong, H. H., S. Y. Liu, and P. H. Selle. 2015. Starch utilisation in chicken-meat production: the foremost influential factors. Anim. Prod. Sci. 56:797–814.

Vieira, S. L., A. Lemme, D. B. Goldenberg, and I. Brugalli. 2004. Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. Poult. Sci. 83:1307–1313.

Wolynetz, M. S., and I. R. Sibbald. 1984. Relationships between apparent and true metabolizable energy and the effects of a nitrogen correction. Poult. Sci. 63:1386-1399.

**Table 6.1.** Ingredient and calculated nutrient composition of common starter diet fed to male Ross YP × 708 broilers from 1 to 17 D of age (Experiments 1 and 2).

| Ingredient | Inclusion, % | Calculated Nutrient | Diet, % |
|---|---|---|---|
| Corn | 50.81 | Crude Protein | 23.26 |
| Soybean Meal | 40.89 | Digestible Lysine | 1.23 |
| Vegetable Oil | 4.38 | Digestible Methionine | 0.64 |
| Defluorinated Phosphate | 1.89 | Digestible Threonine | 0.84 |
| Calcium Carbonate | 0.78 | Digestible TSAA | 0.93 |
| Sodium Chloride | 0.46 | Calcium | 1.01 |
| DL-Methionine | 0.34 | Phosphorus-AV | 0.48 |
| Mineral Premix[1] | 0.10 | Sodium | 0.22 |
| Vitamin Premix[2] | 0.10 | | |
| L-Threonine | 0.09 | $AME_n$ (kcal/kg)[4] | 3,053 |
| L-Lysine | 0.08 | | |
| Choline[3] | 0.08 | | |

[1]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.
[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.
[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).
[4]$AME_n$- nitrogen-corrected apparent metabolizable energy.

**Table 6.2.** Ingredient and calculated nutrient composition of diets fed to male Ross YP × 708 broilers from 18 to 25 D of age (Experiment 1).

| Ingredient, % | Semi-purified | Corn | Wheat | SBM | High CP corn/SBM | Low CP Corn/SBM | High CP wheat/SBM | Low CP wheat/SBM |
|---|---|---|---|---|---|---|---|---|
| Dextrose | 78.88 | - | - | 52.27 | - | - | - | - |
| Corn | - | 93.66 | - | - | 58.00 | 68.00 | - | - |
| Wheat | - | - | 94.40 | - | - | - | 61.00 | 71.00 |
| Soybean meal (**SBM**) | - | - | - | 42.97 | 36.00 | 26.00 | 33.00 | 23.00 |
| Casein[1] | 10.00 | - | - | - | - | - | - | - |
| Solka-Floc[2] | 5.00 | - | - | - | 1.34 | 1.13 | 1.50 | 1.41 |
| Defluorinated Phosphate | 0.61 | - | - | - | - | - | - | - |
| Sodium Bicarbonate | 0.20 | 0.53 | 0.40 | 0.17 | 0.20 | 0.30 | 0.10 | 0.11 |
| Dicalcium Phosphate | 1.10 | 1.92 | 1.69 | 1.79 | 1.73 | 1.78 | 1.60 | 1.62 |
| Sodium Chloride | 0.32 | 0.11 | 0.10 | 0.38 | 0.35 | 0.27 | 0.34 | 0.34 |
| Potassium Sulfate | 1.39 | 1.00 | 0.50 | - | - | - | - | - |
| Potassium Chloride | 0.06 | 0.26 | 0.28 | - | - | 0.10 | - | - |
| Limestone | 1.16 | 1.48 | 1.46 | 1.18 | 1.26 | 1.32 | 1.26 | 1.33 |
| Magnesium Oxide | 0.20 | 0.01 | 0.14 | 0.21 | 0.10 | 0.08 | 0.17 | 0.16 |
| Choline Chloride[3] | 0.38 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |
| Vitamin Premix[4] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral Premix[5] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Titanium Dioxide | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| | | | | | | | | |
| Nutrient | | | | | | | | |
| CP (%) | 8.72 | 7.12 | 8.77 | 20.00 | 21.16 | 17.27 | 21.03 | 17.30 |
| AME (kcal/kg) | 3,360 | 3,137 | 2,903 | 2,719 | 2,781 | 2,883 | 2,645 | 2,719 |
| Ca (%) | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 |
| P (%) | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 |
| K (%) | 0.60 | 0.83 | 0.77 | 0.90 | 0.93 | 0.80 | 0.97 | 0.81 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Na (%) | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 |
| Cl (%) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mg (ppm) | 1,285 | 1,274 | 1,274 | 1,274 | 1,274 | 1,271 | 1,275 | 1,272 |
| Choline (ppm) | 1,701 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 |
| DEB[6] | 162 | 218 | 216 | 238 | 246 | 211 | 261 | 223 |
| | | | | | | | | |
| Analyzed nutrients[7] | | | | | | | | |
| DM[8], % | 98.44 | 88.26 | 88.75 | 95.5 | 88.72 | 88.36 | 89.17 | 89.06 |
| CP[9], % | 8.76 | 8.29 | 10.85 | 20.28 | 24.97 | 18.95 | 21.29 | 18.35 |
| GE[10], kcal/kg | 3,375 | 4,034 | 4,022 | 3,701 | 4,205 | 4,165 | 4,194 | 4,116 |
| $TiO_2$[11] Recovery, % | 0.50 | 0.49 | 0.46 | 0.46 | 0.50 | 0.49 | 0.49 | 0.48 |

[1]Acid casein (Fonterra, Auckland, New Zealand).

[2]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[5]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[6]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.

[7] Corn contained 90.23% DM, 8.59% CP, and 4,326 kcal/kg GE. Wheat contained 91.97% DM, 9.35% crude protein, and 4,229 kcal/kg GE. Soybean meal contained 91.97% DM, 46.68% CP, and 4,623 kcal/kg GE.

[8]Moisture content was calculated utilizing a VirTis 25S freeze dryer.

[9]Crude protein percent was determined using an Elementar Rapid N Cube.

[10]Gross energy was calculated in a Parr 6300 Calorimeter.

[11]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 6.3.** Ingredient composition, calculated nutrients, and analyzed nutrients in diets fed to male Ross YP × 708 broilers from 18 to 24 D of age (Experiment 2).

| | Semi-Purified | Corn Direct | SBM Direct | Corn Basal | Corn Substituted | SBM Basal | SBM Substituted | High CP Blend | Low CP Blend |
|---|---|---|---|---|---|---|---|---|---|
| Ingredient, % | | | | | | | | | |
| Corn | - | 93.463 | - | 52.251 | 35.600 | 76.110 | 60.608 | 58.000 | 68.000 |
| Soybean meal (**SBM**) | - | - | 42.970 | 41.880 | 28.534 | 17.581 | 14.000 | 36.000 | 26.000 |
| Dextrose | 78.880 | - | 52.250 | - | - | - | - | - | - |
| Casein | 10.000 | - | - | - | - | - | - | - | - |
| Corn-Sdded[1] | - | - | - | - | 30.000 | - | - | - | - |
| SBM-Added[1] | - | - | - | - | - | - | 20.000 | - | - |
| Solka-Floc[2] | 5.000 | - | - | - | - | - | - | 1.387 | 0.882 |
| Sodium Bicarbonate | 0.200 | 0.530 | 0.190 | 0.160 | 0.200 | 0.200 | 0.300 | 0.150 | 0.300 |
| Dicalcium P | 1.100 | 1.920 | 1.790 | 1.700 | 1.780 | 1.820 | 1.780 | 1.730 | 1.780 |
| Defluorinated P | 0.610 | - | - | - | - | - | - | - | - |
| Sodium Chloride | 0.320 | 0.110 | 0.382 | 0.355 | 0.340 | 0.330 | 0.270 | 0.355 | 0.270 |
| Potassium Sulfate | 1.390 | 1.220 | - | - | 0.240 | 0.740 | - | - | 0.300 |
| Potassium Chloride | 0.060 | 0.243 | - | - | - | - | - | - | 0.050 |
| Limestone | 1.160 | 1.475 | 1.180 | 1.220 | 1.310 | 1.380 | 1.316 | 1.255 | 1.316 |
| Magnesium Oxide | 0.200 | 0.013 | 0.212 | 0.117 | 0.090 | 0.060 | 0.100 | 0.097 | 0.076 |
| Choline Chloride-70[3] | 0.380 | 0.326 | 0.326 | 0.326 | 0.326 | 0.326 | 0.326 | 0.326 | 0.326 |
| Vitamin Premix[4] | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 |
| Mineral Premix[5] | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 |
| L-Lys-HCl | - | - | - | 0.543 | 0.370 | 0.251 | 0.200 | - | - |
| DL-Met | - | - | - | 0.528 | 0.360 | 0.402 | 0.320 | - | - |
| L-Thr | - | - | - | 0.220 | 0.150 | 0.100 | 0.080 | - | - |
| Titanium Dioxide | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |

| Nutrient | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CP (%) | 8.72 | 7.10 | 19.99 | 24.41 | 18.91 | 14.50 | 20.85 | 21.16 | 17.27 |
| AME (kcal/kg) | 3,360 | 3,130 | 2,718 | 2,726 | 2,862 | 2,959 | 2,822 | 2,781 | 2,883 |
| Ca (%) | 0.88 | 0.879 | 0.879 | 0.878 | 0.884 | 0.882 | 0.903 | 0.878 | 0.878 |
| P (%) | 0.44 | 0.441 | 0.440 | 0.441 | 0.443 | 0.439 | 0.449 | 0.441 | 0.440 |
| K (%) | 0.60 | 0.906 | 0.905 | 1.039 | 0.897 | 0.904 | 0.898 | 0.933 | 0.901 |
| Na (%) | 0.21 | 0.210 | 0.215 | 0.206 | 0.210 | 0.206 | 0.210 | 0.203 | 0.210 |
| Cl (%) | 0.30 | 0.295 | 0.300 | 0.305 | 0.301 | 0.303 | 0.255 | 0.305 | 0.284 |
| DEB[6] | 162 | 240 | 240 | 269 | 236 | 235 | 249 | 240 | 242 |
| Mg (ppm) | 1,285 | 1,285 | 1,274 | 1,319 | 1,336 | 1,319 | 1,322 | 1,274 | 1,289 |
| Mn (ppm) | 125 | 132 | 137 | 140 | 138 | 136 | 139 | 139 | 137 |
| Choline (ppm) | 1,701 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 | 1,702 |
| Analyzed Nutrients[7] | | | | | | | | | |
| DM[8], % | 98.06 | 89.62 | 96.36 | 91.00 | 90.70 | 91.00 | 88.02 | 90.64 | 90.54 |
| CP[9], % | 8.64 | 7.48 | 20.63 | 24.28 | 18.95 | 15.73 | 21.17 | 21.92 | 17.92 |
| GE[10], kcal/kg | 3,431 | 3,909 | 3,573 | 4,151 | 4,075 | 3,989 | 4,067 | 4,045 | 4,038 |
| $TiO_2$[11] Recovery, % | 0.47 | 0.44 | 0.48 | 0.45 | 0.48 | 0.42 | 0.47 | 0.43 | 0.44 |

[1]Added corn and added SBM represent the ingredient substituted into the basal diet in the corn substituted and SBM substituted diets, respectively.

[2]Solka-Floc (International Fiber Corporation, North Tonawanda, NY) powdered cellulose.

[3]Choline chloride-70 (Balchem Corporation, New Hampton, NY).

[4]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg: D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[5]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4mg; Se (sodium selenite), 0.3 mg.

[6]DEB- Dietary electrolyte balance (DEB) was calculated as DEB (mEq) = Na/0.023 + K/0.039 – Cl/0.035.
[7] Corn contained 89.23% DM, 8.90% CP, and 4,213 kcal/kg GE. Soybean meal contained 91.72% DM, 47.97% CP, and 4,532 kcal/kg GE.
[8]Moisture content was calculated utilizing a VirTis 25S freeze dryer.
[9]Crude protein percent was determined using an Elementar Rapid N Cube.
[10]Gross energy was calculated in a Parr 6300 Calorimeter.
[11]$TiO_2$ concentrations were determined using the protocol described by Short et al., 1996.

**Table 6.4.** Growth performance of male Ross YP × 708 broilers from 18 to 23 D of age (Experiments 1 and 2).[1]

| | BW, g | BW Gain[2,3], g | Feed Disappearance[2], g | Mortality[2,4], % |
|---|---|---|---|---|
| Experiment 1 | | | | |
| HP[6] Corn-SBM | 953[a] | 266[a] | 468 | 0.0 |
| LP[7] Corn-SBM | 935[ab] | 231[b] | 478 | 0.0 |
| HP[6] Wheat-SBM | 926[ab] | 220[b] | 456 | 0.7 |
| LP[7] Wheat-SBM | 897[b] | 194[c] | 460 | 0.7 |
| SEM[5] | 11 | 5 | 7 | 0.7 |
| | | | | |
| Experiment 2 | | | | |
| High CP Blend | 885 | 268 | 482 | 0.7 |
| Low CP Blend | 863 | 240 | 481 | 0.0 |
| SEM[5] | 17 | 16 | 12 | 0.9 |
| | | *Probabilities* | | |
| ANOVA- Experiment 1 | 0.0048 | <0.0001 | 0.13 | 0.40 |
| ANOVA- Experiment 2 | 0.39 | 0.24 | 0.97 | 0.96 |

[1]Values within columns and within experiments with different superscripts are significantly different ($P \leq 0.05$). Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage. SBM = soybean meal.
[2]Body weight gain, feed disappearance, and mortality were evaluated from 18 to 23 D of age.
[3]Average BW at 18 D of age was 702 g and 620 g in Experiment 1 and Experiment 2, respectively, and was not different between treatments ($P \leq 0.07$).
[4]Mortality values were arcsine transformed prior to analysis.
[5]Standard error of the mean.
[6]High crude protein.
[7]Low crude protein.

**Table 6.5.** Determined energy of feed ingredients and blended diets provided to male Ross YP × 708 broilers from 18 to 25 D of age on DM basis (Experiment 1).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Cereal Grains | | | | | |
| Corn | 3,460 | 3,418 | 3,609 | 3,540 | 3,690 |
| Wheat | 3,036 | 2,970 | 3,183 | 3,125 | 3,273 |
| SEM[3] | 62 | 60 | 62 | 62 | 62 |
| | | | | | |
| Soybean Meal (**SBM**) | 2,513 | 2,182 | 2,661 | 2,720 | 2,880 |
| SEM3 | 50 | 46 | 49 | 47 | 48 |
| | | | | | |
| Blended Diets | | | | | |
| HP[4] Corn-SBM | 3,089[b] | 2,920[ab] | 3,224[b] | 3,177[b] | 3,308[ab] |
| LP[4] Corn-SBM | 3,177[a] | 3,048[a] | 3,316[a] | 3,238[a] | 3,367[a] |
| HP[4] Wheat-SBM | 2,909[c] | 2,794[c] | 3,050[c] | 2,948[c] | 3,077[c] |
| LP[4] Wheat-SBM | 2,978[c] | 2,868[bc] | 3,118[c] | 3,074[bc] | 3,200[bc] |
| SEM3 | 29 | 26 | 29 | 42 | 42 |
| | | | *Probabilities* | | |
| Cereal-ANOVA | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Blended Diets-ANOVA | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

[1]Significant differences caused by diet are compared within experiments. Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage. Significant differences when more than two diets were compared are denoted with superscripts. Soybean meal was not directly compared with another diet.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean.

[4]HP = high protein, LP = low protein. The HP and LP corn-SBM diets were composed of 58/36 or 68/26% corn/SBM, respectively, while the HP and LP wheat-SBM diets were composed of 61/33 or 71/23% wheat/SBM, respectively.

**Table 6.6.** Difference in predicted and actual metabolizable and digestible energy values determined for corn and soybean meal or wheat and SBM blends using male Ross 708 × YP broilers provided with test diets from 18 to 25 D of age (Experiment 1).[1]

| Method (kcal/kg DM, unless otherwise noted) | HP[2] Corn-SBM | LP[2] Corn-SBM | HP[2] Wheat-SBM | LP[2] Wheat-SBM |
|---|---|---|---|---|
| AME[3] | 173 | 175 | 229 | 244 |
| $AME_n$[3] | 131 | 147 | 252 | 240 |
| SME | 173 | 176 | 230 | 246 |
| AIDE[3] | 129 | 114 | 135 | 221 |
| SIDE[3] | 124 | 111 | 132 | 219 |
| SEM | 24 | 26 | 46 | 51 |
| ANOVA | | *Probabilities* | | |
| AME | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| $AME_n$ | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| SME | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| AIDE | <0.0001 | 0.0006 | 0.0107 | 0.0006 |
| SIDE | 0.0002 | 0.0008 | 0.0122 | 0.0006 |

[1]Data were compared using a one-sample two-tailed T-test comparing analyzed energy values with predicted energy values computed utilizing analyzed energy values of individual grains. SBM = soybean meal.
[2]HP = high protein, LP = low protein. The HP and LP corn-SBM diets were composed of 58/36 or 68/26% corn/SBM, respectively, while the HP and LP wheat-SBM diets were composed of 61/33 or 71/23% wheat/SBM, respectively.
[3]AME= apparent metabolizable energy, $AME_n$ = nitrogen-corrected AME, SME = standardized metabolizable energy, AIDE = apparent ileal digestible energy, SIDE = standardized ileal digestible energy.

**Table 6.7.** Determined energy of feed ingredients and blended diets provided to male Ross YP × 708 broilers from 18 to 24 D of age (Experiment 2).[1]

| | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
|---|---|---|---|---|---|
| Corn | | | | | |
| Corn Direct | 3,431 | 3,421 | 3,602 | 3,431 | 3,602 |
| Corn Substituted | 3,675 | 3,604 | 3,856 | 3,613 | 3,794 |
| SEM[3]- Direct | 19 | 19 | 19 | 26 | 26 |
| SEM[3]- Substituted | 62 | 62 | 62 | 69 | 69 |
| | | | | | |
| Soybean Meal (**SBM**) | | | | | |
| SBM Direct | 2,300 | 1,835 | 2,474 | 2,465 | 2,638 |
| SBM Substituted | 2,570 | 2,308 | 2,747 | 2,703 | 2,892 |
| SEM[3]- Direct | 46 | 42 | 46 | 59 | 59 |
| SEM[3]- Substituted | 72 | 38 | 72 | 120 | 120 |
| | | | | | |
| Blended Diets | | | | | |
| High CP Blend | 3,034 | 2,867 | 3,195 | 3,071 | 3,231 |
| Low CP Blend | 3,145 | 3,005 | 3,305 | 3,184 | 3,344 |
| SEM[3] | 14 | 37 | 14 | 31 | 31 |
| | | | *Probabilities* | | |
| Corn-ANOVA | <0.0001 | <0.0001 | <0.0001 | 0.0002 | 0.0001 |
| SBM-ANOVA | 0.0032 | < 0.0001 | 0.0029 | 0.08 | 0.08 |
| Blended Diets-ANOVA | <0.0001 | 0.06 | <0.0001 | 0.0030 | 0.0030 |

[1]Significant differences caused by diet are compared treatments. Data represent the mean of 16 replicate cages per treatment with 9 chicks per cage.

[2]AME = apparent metabolizable energy. AIDE = apparent ileal digestible energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. SIDE = standardized digestible energy.

[3]SEM = standard error of the mean. Standard error is shown for individual determination methods due to large differences in variability and is pooled for blended diets due to similarity.

**Table 6.8.** Difference in determined and predicted metabolizable and digestible energy values determined for corn and soybean meal blends using male Ross YP × y08 broilers provided with test diets from 18 to 24 D of age (Experiment 2).[1].

| Methodology and Diet | Energy Calculation | | | | |
|---|---|---|---|---|---|
| High CP[3] | AME[2] | $AME_n$[2] | SME[2] | AIDE[2] | SIDE[2] |
| Corn Direct, SBM Direct | 216 | 190 | 221 | 194 | 192 |
| Corn Substituted, SBM Substituted | -16 | -63 | -19 | 2 | -10 |
| Corn Direct, SBM Substituted | 119 | 20 | 116 | 108 | 101 |
| Corn Substituted, SBM Direct | 74 | 83 | 67 | 88 | 81 |
| SEM[4] | 10 | 33 | 10 | 32 | 32 |
| Low CP[3] | | | | | |
| Corn Direct, SBM Direct | 214 | 201 | 213 | 210 | 209 |
| Corn Substituted, SBM Substituted | -23 | -46 | -31 | 24 | 12 |
| Corn Direct, SBM Substituted | 143 | 78 | 142 | 148 | 143 |
| Corn Substituted, SBM Direct | 47 | 77 | 40 | 86 | 78 |
| SEM[4] | 17 | 18 | 17 | 28 | 28 |
| | | | *Probabilities* | | |
| High CP | | | | | |
| Corn Direct, SBM Direct | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0001 |
| Corn Substituted, SBM Substituted | 0.08 | 0.08 | 0.07 | 0.94 | 0.75 |
| Corn Direct, SBM Substituted | <0.0001 | 0.0044 | <0.0001 | 0.0041 | 0.0065 |
| Corn Substituted, SBM Direct | <0.0001 | 0.0055 | <0.0001 | 0.0149 | 0.0225 |
| Low CP | | | | | |
| Corn Direct, SBM Direct | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0019 |
| Corn Substituted, SBM Substituted | 0.20 | 0.0194 | 0.09 | 0.42 | 0.67 |
| Corn Direct, SBM Substituted | <0.0001 | 0.0004 | <0.0001 | 0.0002 | 0.0002 |
| Corn Substituted, SBM Direct | 0.0143 | 0.0005 | 0.0337 | 0.0099 | 0.0163 |

[1]Additivity was determined as the difference between the predicted energy, calculated using the values determined in the current experiment, minus the determined energy. The determined energy was measured utilizing 16 replicate diets each for the high and low CP diets with 9 chicks per cage. Data were analyzed as a 1-sample, 2-tailed T-test where $H_0$=0. Additivity was detected when $P \geq 0.05$. SBM = soybean meal.

[2]AME = apparent metabolizable energy. $AME_n$ = nitrogen-corrected apparent metabolizable energy. SME = standardized metabolizable energy. AIDE = apparent ileal digestible energy. SIDE = standardized digestible energy.

[3]Each methodology was analyzed individually for all 5 calculation methods.

[4]Standard error of the mean.

# VII. CONCLUSIONS

The cost of providing broilers with dietary energy comprises a large proportion of production costs, necessitating further precision in formulating dietary energy for broilers. However, current methods utilized for energy determination in ingredients are variable, lack sensitivity, and may not be additive in formulation. Standardization for EEL has been proposed as a method of addressing these concerns. Current literature has sparse data describing methods for determining EEL or providing standardized energy values for feed ingredients. Therefore, 2 experiments evaluated suitability for estimation of EEL, while 6 experiments assessed effect of standardization and diet methodology on determined energy of feed ingredients. Two experiments evaluated effect of standardization, utilization of DE or ME, and energy determination methodology on additivity of dietary energy.

Two experiments were designed to assess suitability of semi-purified diets for estimation of EEL based on glucose recovery and broiler growth in Chapter 3. Experiment 1 demonstrated that cornstarch is not an appropriate ingredient in diets for estimation of EEL. Additionally, chicks provided with diets void of cellulose had insufficient digesta for analysis, while broilers provided with diets that contained casein maintained BW through the experimental period. This indicated that diets should be dextrose-based and contain cellulose and casein. Experiment 2 evaluated effect of dietary casein concentration on EEL and demonstrated that CP concentration did not affect EEL but did affect BW gain. While growth performance was not the purpose of these experiments, maintenance of BW may indicate that the broilers provided with higher dietary CP are in a physiological state more

similar to broilers provided with corn- and SBM-based diets. Based on these two experiments, a semi-purified diet primarily composed of dextrose, cellulose, and casein is appropriate for estimation of EEL.

Next, a series of 3 experiments was designed to evaluate effects of assay methodology, standardization, and calculation method on determined energy of either cereal grains or SBM, and 2 experiments were designed to assess additivity of ingredients in blended diets. Experiment 1 (Chapters 4 and 5) was designed to evaluate effect of standardization for EEL on additivity of energy, and energy was therefore only determined utilizing the direct method. The resulting determined energy values were lower than expected based on feed ingredient database values and were determined to not be additive in Experiment 1 for additivity (Chapter 6), regardless of standardization. Based on this, Experiment 2 (Chapters 4 and 5) was conducted to compare the effect of assay methodology on either corn or SBM. This experiment demonstrated that energy values determined utilizing the direct method are lower than those determined with the substitution method, indicating interactions between ingredients and dietary nutrients. Experiment 3 (Chapters 4 and 5) for corn and SBM evaluated the effect of methodology and calculation on determined energy. Experiment 2 for additivity (Chapter 6) utilized the determined energy values from those Experiment 3 for corn and soybean meal (Chapters 4 and 5) to predict energy of blended diets to assess effect of methodology, calculation, and standardization on additivity of ingredients. The energy values determined utilizing the substitution method were more additive than values determined utilizing the direct method. However, standardization for EEL did not lead to increased additivity of energy. These

results support that there are substantial interactions between ingredients utilized in broiler diets.

Collectively, these experiments indicate that determined energy of feed ingredients can vary widely based on assay methodology and calculation method. Standardization for EEL led to notable increases in determined energy values but did not appear to be more additive than apparent energy values. Additionally, the substitution method provided determined energy values that were more additive than the direct method, as the direct method underestimated ingredient energy values. Further research is warranted to further delineate factors that influence EEL and to elucidate methodology for energy determination assays that is more additive and less variable to formulate energy in broiler diets most efficiently.