Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens

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ABSTRACT Two experiments were conducted to determine the ileal endogenous energy losses (**IEEL**) and nitrogen-corrected apparent metabolizable energy (**AMEn**) and true ileal digestible energy (**TIDE**) of 4 cereal grains (corn, sorghum, wheat, and barley) for broilers. In experiment 1, a glucose-based purified diet was used to determine the IEEL for correcting the apparent ileal digestible energy (**AIDE**) values to TIDE. The diet was randomly allocated to 6 replicates (6 birds per replicate) of male broilers and fed from 18 to 21 d after hatch. The jejunal and ileal digesta were collected on day 21. The results showed that glucose absorption continued beyond the jejunum but was complete in the terminal ileum demonstrating that endogenous energy losses can be quantified in the ileal digesta of birds by feeding a glucose-based diet. The IEEL were determined to be 347 ± 29.4 kcal/kg DM intake. In experiment 2, 4 experimental diets with similar inclusion (957 g/kg) of grains were developed to determine the AMEn, AIDE, and TIDE. Titanium dioxide (5.0 g/kg) was added to all diets as an indigestible marker. Each diet was randomly allocated to 6 replicates (8 birds per replicate) and fed from 14 to 21 d after hatch, and the ileal digesta were collected on day 21. The AIDE was corrected to TIDE using the IEEL value determined in experiment 1. The TIDE of corn, sorghum, wheat, and barley were determined to be 3,920, 3,650, 3,138, and 2,885 kcal/kg DM, respectively, and was higher ($P < 0.05$) than the corresponding AMEn values of 3,439, 3,284, 2,576, and 2,371 kcal/kg DM, respectively. No differences were observed between the AMEn and AIDE. The AMEn:TIDE ratio tended ($P = 0.06$) to be lower for viscous cereals (wheat and barley) than that for nonviscous cereals (corn and sorghum). The apparent ileal digestibility of DM, nitrogen, and starch was positively correlated ($P < 0.001$) with TIDE ($r = 0.990$, 0.703, and 0.705, respectively) and the AMEn ($r = 0.873$, 0.483, and 0.656, respectively). Further studies are warranted to determine the TIDE of a range of ingredients and to investigate the application of TIDE as a potential available energy system in poultry feed formulations.

Key words: broiler, cereal, true ileal digestible energy, ileal endogenous energy loss

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INTRODUCTION

Efficient poultry production relies on supplying the birds with the adequate amount of nutrients and energy. Special attention should be given to the dietary energy because of its importance in controlling feed intake, which drives bird growth and diet cost. An accurate evaluation of the available energy content of ingredients is, therefore, critical. Apparent metabolizable energy (**AME**), where available energy is calculated as gross energy (**GE**) ingested minus energy excreted in the feces and urine, has been the system of choice for describing available energy for poultry since the 1950s (Hill and Anderson, 1958; Sibbald, 1982). It is not a perfect system, with a number of limitations (Mateos et al., 2019; Wu et al., 2020). It is an excreta-based measurement containing urine that is voided along with feces and also includes the energy loss or gain due to the presence of microbial mass from cecal fermentation. But it is simple, easy to measure, and accounts for most of the energy losses after digestion and metabolism, and these features have positioned the AME well ahead of other energy measurements. Currently, the general approach for the measurement of AME is by total excreta collection; however, partial collection of excreta with the use of an inert marker has also been used instead of total collection (Scott and Boldaji, 1997; Sales and Janssens, 2003).

An alternative energy system that has received some attention is apparent ileal digestible energy (**AIDE**).

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The AIDE is measured at the ileal level and reflects digestibility rather than metabolizability as in the case of the AME (Gehring et al., 2012). A switch of available energy measurement to AIDE will not only overcome the limitations of AME but will also align energy availability with the current trend of using digestible content of nutrients in feed formulations (Lemme et al., 2004; Mutucumarana et al., 2015). The ileal approach would also eliminate some of the errors associated with the classic AME methodology, including the effect of feed intake, contamination from feathers and scales, and potential loss of some excreta during collection (Wu et al., 2020). The lack of relationship between the AME and growth responses sometimes seen in feed enzyme research (Hong et al., 2002; Wu et al., 2004; González-Ortiz et al., 2016) lends further credence to investigate AIDE as an alternative option.

The digesta collected from the terminal ileum contains both dietary undigested nutrients and endogenous materials that are not derived from the feed, for example, digestive juices, bile, mucin, and sloughed intestinal epithelial cells (Nyachoti et al., 1997; Ravindran et al., 2004). Thus, the available energy measured at the terminal ileum is apparent digestible energy, and correction for the nondietary energy flow, referred to as ileal endogenous energy loss (**IEEL**), is necessary for the calculation of true ileal digestible energy (**TIDE**). Currently, there is no methodology available for the measurement of IEEL in poultry. In the case of nutrients, basal ileal endogenous flows have been usually determined after the feeding of respective nutrient-free purified diets, for example, protein-free diets (Muztar and Slinger, 1980; Ravindran et al., 2004; Adedokun et al., 2007) and calcium- and phosphorus-free diets (Mutucumarana and Ravindran, 2016; Anwar et al., 2017). Development of an energy-free diet, however, is not practical, and other approaches therefore need to be explored. Feeding of almost 100% digestible protein sources, such as casein or enzymatically hydrolyzed casein (Lemme et al., 2004; Ravindran et al., 2008; Ravindran, 2016), has been used to measure endogenous protein losses in poultry. A similar approach using a completely digestible simple sugar, such as glucose (Herman, 1974; Riesenfeld et al., 1980), may be used to quantify the IEEL in poultry.

The aims of the studies reported herein were 2-fold: 1) to investigate whether IEEL in broiler chickens can be quantified after feeding a glucose-based purified diet, and 2) if this methodology proves successful, then to estimate the AIDE and TIDE contents of common cereal grains (corn, sorghum, wheat, and barley) and to compare with AMEn contents. Most published data on ileal digestible energy have estimated the AIDE of complete diets, and there are only limited and scattered data available for the AIDE of individual ingredients (Leslie et al., 2007; Gehring et al., 2012; Woyengo and Wilson, 2019).

MATERIALS AND METHODS

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing, and teaching and approved by the Massey University Animal Ethics Committee.

Ingredients

The 4 cereal grains (corn, sorghum, wheat, and barley) were obtained from a local commercial supplier and ground in a hammer mill to pass through a screen size of 3.0 mm. Representative samples were analyzed, in duplicate, for DM, nitrogen (**N**), starch, crude fat, GE, neutral detergent fiber, ash, calcium, and phosphorus.

Diets, Birds, and Housing

Experiment 1: Determination of IEEL To determine the IEEL, a glucose-based purified diet, containing 900 g/kg glucose, was developed (Table 1). Titanium dioxide (**Ti**) was included in the diet as an indigestible marker at an inclusion rate of 5.0 g/kg.

Day-old male broilers (Ross 308) were obtained from a local hatchery, raised on floor pens, and fed commercial broiler starter pellets (230 g/kg crude protein and 3,010 kcal/kg AME). On day 14, birds were moved to grower cages for acclimatization. Between day 14 and 18, pellets were gradually changed to mash as the purified diet was in mash form. On day 18, birds were individually weighed and allocated to 6 cages (6 birds per cage). The glucose-based purified diet was offered ad libitum for 3 d from 18 to 21 d after hatch.

Experiment 2: Determination of AME and Ileal Digestible Energy of Cereal Grains The AME was determined using the direct method. In this method, 4 basal diets were formulated to contain the same inclusion level (957 g/kg) of each cereal grain (Table 2). Titanium dioxide was included in all diets as an indigestible marker at an inclusion rate of 5.0 g/kg. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co., Pty., Ltd., Merrylands, NSW, Australia), then steam-pelleted at 60°C using a pellet mill (Model Orbit 15; Richard Sizer., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35-mm thickness.

Day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery, raised on a floor pen, and fed the same commercial broiler starter diet as experiment 1 until 14 d of age. On day 14, a total number of 192 birds were individually weighed and randomly allocated to 24 cages with 6 replicates per treatment (8 birds per cage). Birds were fed the experimental diets from 14 to 21 d of age.

In both experiments, the floor pens and cages were housed in environmentally controlled rooms with 20 h of fluorescent illumination per day, and feed and water were offered ad libitum. The temperature was maintained at 31°C on day 1 and was gradually reduced to 22°C by the end of the third week. Central ceiling extraction fans and wall inlet ducts controlled ventilation.

Determination of AME (Experiment 2)

The 4 experimental diets were fed for 7 d (14–21 d of age), with the first 3 d serving as an adaptation period. Excreta for each cage were collected during the last 4 d of the assay. Daily excreta collections were pooled within a cage, mixed in a blender, and subsampled. Subsamples were frozen and then lyophilized (Model 0610; Cuddon Engineering, Blenheim, New Zealand). Dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4°C pending analysis. Diets and excreta samples were analyzed for DM, GE, N, and Ti.

Jejunal and Ileal Digesta Collection

At the end of both experiments (day 21), all birds were euthanized by intravenous injection (1 mL per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty., Ltd., Auckland, New Zealand). The small intestine was isolated, and the digesta from the terminal jejunum and terminal ileum (in experiment 1) and terminal ileum (in experiment 2) were collected. The jejunum was defined as a portion from the distal-most point of insertion of the duodenal mesentery to that descends to Meckel's diverticulum. The jejunal digesta were collected from the lower half of the jejunum. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to ~40 mm proximal to ileocecal junction, and digesta were collected from the lower half toward the ileocecal junction. The digesta were removed by gentle flushing with distilled water, as described by Ravindran et al. (2005). Digesta were pooled within a cage, lyophilized (Model 0610; Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve, and stored at 4°C until laboratory analysis. The digesta samples were analyzed for DM, GE, glucose, and Ti in experiment 1 and for DM, N, starch, GE, and Ti in experiment 2.

Chemical Analysis

Dry matter was determined using a standard procedure (Method 930.15; AOAC, 2016). Ash was determined by a standard procedure (Method 942.05; AOAC, 2016) using a muffle furnace at 550°C for 16 h. N was determined by combustion (Method 968.06; AOAC, 2016) using a carbon nanosphere-200 carbon, N, and sulphur auto analyzer (rapid MAX N exceed; Elementar, Donaustraze, Hanau, Germany). Crude protein content was calculated as N × 6.25. Crude fat was determined by Soxtec extraction procedure (Method 2003.06; AOAC, 2016) using the Soxtec System HT 1043 Extraction Unit (Höganäs, Sweden). Starch was measured using a Megazyme kit (Method 996.11; AOAC, 2016) based on thermostable α-amylase and amyloglucosidase (McCleary et al., 1997). Neutral detergent fiber (Method 2002.04; AOAC, 2016) was determined using Tecator Fibertec (FOSS Analytical AB, Höganäs, Sweden). Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996). For minerals, samples were ashed, and then calcium and phosphorus were determined calorimetrically after digestion with HCl (Method 968.08D; AOAC, 2005). Glucose was determined using an assay kit (Rx Daytona Plus; Randox Laboratories Ltd., Crumlin, UK) after enzymatic oxidation in the presence of glucose oxidase. GE was determined by using an adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardized with benzoic acid.

Calculations

All data were expressed on a DM basis, and the AME content of the test diets was calculated using the following formulas:

$$\text{GE metabolizability} = \left[(\text{GE}/\text{Ti})_{\text{Diet}} - (\text{GE}/\text{Ti})_{\text{Excreta}}\right] / (\text{GE}/\text{Ti})_{\text{Diet}}$$

$$\text{AME}_{\text{Diet}}(\text{kcal}/\text{kg}) = \text{GE}_{\text{Diet}} \times \text{GE metabolizability}$$

The AME of the cereal grains was then calculated as follows:

$$\text{AME}_{\text{Grain}}(\text{kcal}/\text{kg}) = \text{AME of test diet} \times (100/95.7)$$

N-corrected AME was determined by correction for zero N retention by assuming 8.73 kcal per g N retained in the body as described by Titus et al. (1959).

Apparent jejunal and ileal absorption of glucose were calculated using the Ti ratios in the diet and digesta as shown in the following formula. All concentrations were expressed as g per kg DM.

$$\text{Apparent absorption} = 1 - \left[(\text{Ti}_{\text{Diet}}/\text{Ti}_{\text{Digesta}}) \times (\text{Glucose}_{\text{Digesta}}/\text{Glucose}_{\text{Diet}})\right]$$

The coefficient of apparent ileal digestibility (**CAID**) of the nutrients was calculated from the dietary ratio of nutrient to Ti relative to the corresponding ratio in the ileal digesta:

$$\text{CAID of nutrient} = \left[(\text{Nutrient/Ti})_{\text{Diet}} - (\text{Nutrient/Ti})_{\text{Digesta}}\right] \Big/ (\text{Nutrient/Ti})_{\text{Diet}}$$

where, $(\text{Nutrient/Ti})_{\text{Diet}}$ = ratio of nutrient to Ti in diet and $(\text{Nutrient/Ti})_{\text{Digesta}}$ = ratio of nutrient to Ti in ileal digesta.

The CAID of GE and AIDE were calculated using following formulas:

$$\text{CAID of GE} = \left[(\text{GE/Ti})_{\text{Diet}} - (\text{GE/Ti})_{\text{Digesta}}\right] \Big/ (\text{GE/Ti})_{\text{Diet}}$$

$$\text{AIDE (kcal / kg)} = \text{GE}_{\text{Diet}} \times \text{CAID of GE}$$

The flow of jejunal and ileal endogenous energy, as kcal lost per kilogram of DM intake (**DMI**), was calculated by using the following formula:

$$\text{Endogenous energy losses (kcal / kg DMI)} = \text{GE}_{\text{Digesta}}\text{(kcal / kg)} \times \left[\text{Ti}_{\text{Diet}}\text{(g / kg)} / \text{Ti}_{\text{Digesta}}\text{(g / kg)}\right]$$

Apparent ileal digestibility data for GE were then converted to true digestibility values, using IEEL determined from birds fed the glucose-based purified diet.

$$\text{Coefficient of true ileal energy digestibility} = \text{CAID of GE} + [\text{Basal IEEL (kcal / kg DMI)} / \text{GE}_{\text{Diet}}\text{(kcal / kg)}]$$

$$\text{TIDE}_{\text{Diet}} = \text{Coefficient of true ileal energy digestibility} \times \text{GE}_{\text{Diet}}$$

$$\text{TIDE}_{\text{Grain}} = \text{TIDE}_{\text{Diet}} \times (100 / 95.7)$$

Statistical Analysis

The data were analyzed as a one-way ANOVA using the GLM procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The data from experiment 2 were subjected to 2 sets of one-way ANOVA to compare the differences in different energy measurements among grains and within a grain. In both experiments, cage served as the experimental unit. Significant differences between means were separated by least significant difference test. Significance of effects was declared at $P < 0.05$. Linear relationships between measured parameters were evaluated using the Pearson's correlation analysis.

RESULTS

The analyzed nutrient composition of the test cereal grains is summarized in Table 3. Estimates for the EEL and coefficient of apparent glucose absorption in the jejunum and ileum are shown in Table 4. The jejunal EEL was higher ($P < 0.05$) than those measured at the terminal ileum and coincided with lower ($P < 0.05$) glucose absorption in the jejunum. Glucose completely disappeared by the time the digesta reached terminal ileum.

For all cereal grains, the TIDE value was higher ($P < 0.05$) than that of AMEn, while no differences were observed between the AMEn and AIDE values (Table 5). The highest ($P < 0.05$) AIDE and TIDE values were recorded for corn and sorghum, followed by wheat and the lowest ($P < 0.05$) values for barley.

The influence of cereal grain type on the AMEn:AIDE and AMEn:TIDE ratios is shown in Table 6. There was no influence of cereal type on the AMEn:AIDE ratio,

Table 1. Composition of the glucose-based purified diet (g/kg, as fed basis), experiment 1.

Ingredient	Inclusion, g/kg
Glucose[1]	900
Cellulose[2]	50.0
Dicalcium phosphate	20.0
Limestone	13.0
Titanium dioxide	5
Vitamin premix[3]	2.5
Trace mineral premix[3]	2.5
Sodium bicarbonate	3.0
Sodium chloride	3.0
Dipotassium phosphate	1.0

[1]Glucose, Dexmonc, Davis Food Ingredients, Dandenong South, Victoria, Australia.

[2]Solkafloc, Ceolus PH-102, Asahi Kasei Corporation, Tokyo, Japan. Added to maintain uniform passage and consistency of digesta in the digestive tract.

[3]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2,400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11,100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

whereas a tendency ($P = 0.059$) was observed for cereal effect for the relationship between AMEn and TIDE. The AMEn:TIDE ratio tended to be lower for the viscous grains (wheat and barley) than that for nonviscous grains (corn and sorghum).

The influence of cereal grain type on the CAID of DM, N, starch, and GE for broiler chickens at 21 d of age is summarized in Table 7. Among the test cereals, corn showed the highest ($P < 0.05$) CAID of DM, followed by sorghum, wheat, and barley, with similar CAID for wheat and barley. Corn and barley had the highest and lowest N and starch digestibility, respectively, with wheat and sorghum being intermediate. The GE digestibility was affected ($P < 0.05$) by the cereal type, with the highest GE digestibility for corn, followed by sorghum, wheat, and the lowest for barley.

Linear correlations between the CAID of DM, N, and starch and AIDE, TIDE, and AMEn are presented in Table 8. There were positive correlations ($P < 0.001$) among all measured response parameters. The AIDE, TIDE, and AMEn were all highly correlated with the CAID of DM, N, and starch.

Table 2. Composition (g/kg, as fed basis) of test diets, experiment 2.

Ingredient	Inclusion, g/kg
Test cereal grain	957.0
Titanium dioxide	5.0
Dicalcium phosphate	19.0
Limestone	13.0
Sodium chloride	2.0
Sodium bicarbonate	2.0
Vitamin premix[1]	1.0
Trace mineral premix[1]	1.0

[1]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2,400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11,100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

DISCUSSION

Ileal Endogenous Energy Losses

It is well accepted that true digestibility values provide a better measure of true utilization potential than apparent values (Lemme et al., 2004). The difference between these 2 measures is the contribution of nondietary materials of endogenous origin to the undigested matter in the ileal digesta. One objective of the present work was to develop and test a methodology for the measurement of IEEL, which could be used to correct the AIDE to true values. Currently, no methodology exists for the determination of IEEL. The simplest approach, used for the measurement of ileal endogenous losses of other major nutrients (protein, fat, calcium, and phosphorus), had been to feed respective nutrient-free purified diets or diets based on purified nutrient sources with ∼100% digestibility. Energy is not a nutrient but a function of several energy-yielding nutrients, and it is impractical to develop an assay diet devoid of energy. It is evident that the only possible option is to test a diet based on a simple monosaccharide, such as glucose, which is the major end-product and absorbed form of carbohydrate digestion. As the basic absorbable form, glucose requires no enzymatic digestion and is quickly and completely absorbed with most of the absorption taking place in the duodenum and jejunum (Herman, 1974).

In the present work, glucose absorption was measured at the jejunum and terminal ileum to investigate its absorption dynamics. The data clearly showed that the absorption of glucose continues beyond the jejunum and is completed only in the ileum. At the terminal ileal level, 100% of the glucose provided in the assay diet was absorbed, and therefore, the energy determined could be considered to have come only from endogenous sources. These results are in agreement with those of Riesenfeld et al. (1980) who used a glucose-based diet and reported that glucose was absorbed 90% in the lower jejunum and almost completely absorbed in the lower ileum. The complete disappearance of glucose, the sole energy source in the assay diet, in the lower ileum demonstrates the possibility of using a glucose-based purified diet for the measurement of IEEL in broilers. In the present study, the IEEL was estimated to be 347 kcal/kg DMI. As this is the first study reporting the IEEL in poultry, no comparable data are available in the literature.

There have been previous studies estimating the endogenous energy losses in poultry, but all were determined, following fasting with precision feeding, in the excreta, of adult roosters for the calculation of true metabolizable energy (Sibbald, 1982). Average excreta endogenous energy output of fasted adult roosters of 2 kg body weight has been reported to range between 0.04 and 14 kcal/bird/d (Sibbald, 1982). These

Table 3. Proximate, carbohydrate, and mineral composition of the test cereal grains (g/kg, as received basis).[1]

Item	Corn	Sorghum	Wheat	Barley
Dry matter	909	909	899	925
Crude protein (nitrogen × 6.25)	80.6	106.3	123.1	125.0
Crude fat	32.4	32.6	18.5	22.0
Starch	590	606	532	499
Neutral detergent fiber	83.1	62.2	103.0	90.1
Ash	20.5	15.5	18.4	16.1
Calcium	0.17	0.10	0.21	0.19
Phosphorus	2.47	2.89	3.51	2.65
Gross energy (kcal/kg)	3,884	3,987	3,867	3,989

[1]Duplicate analysis.

estimates, however, cannot be compared with the IEEL determined in the present study. First, the correction at the excreta level includes metabolic as well as endogenous energy contained in both the feces and urine. Second, the unit of measurement is a function of time (kcal/bird/d) rather than intake and, therefore, cannot be used for the calculation of TIDE in any of the currently used methods of digestibility measurements. Future research investment is warranted to validate the methodology developed in the present work and to further explore the subject of IEEL. It is recognized, as with endogenous amino acid losses (Adedokun et al., 2011; Adeola et al., 2016; Ravindran, 2016), IEEL will be influenced by a number of factors including genotype, age of birds, diet composition, environmental conditions, methods of euthanasia, or the ileal digesta collection method.

An unavoidable limitation in the composition of assay diet used needs to be acknowledged at this point. Cellulose (50 g/kg) was included in the diet to ensure diet structure and uniform passage and consistency of digesta in the digestive tract. Cellulose, being indigestible, would have contributed to the undigested fraction remaining in the terminal ileum, causing some overestimation of IEEL.

Determination of TIDE

Although the focus of the present work is TIDE, a discussion of AIDE data is relevant, and pertinent comparisons are included because the limited published data available on ileal digestible energy to date relate to AIDE. Most data are for the AIDE of complete diets (Camden et al., 2001; de Coca-Sinova et al., 2008; Romero et al., 2014; Yang et al., 2020), but some studies have also reported the AIDE of individual cereal grains, including corn (Gehring et al., 2012), wheat, and barley (Scott et al., 1998). In the present study, the AIDE of corn, sorghum, wheat, and barley was determined to be 3,544, 3,296, 2,758, and 2,519 kcal/kg, respectively. The differences in AIDE among the 4 cereals closely paralleled those in respective values for the ileal digestibility of DM, N (except in wheat), and starch. The AIDE value of corn was close to the range of 3,248 to 3,406 kcal/kg reported for 12 samples by Gehring et al. (2012). The AIDE values determined for wheat and barley in the present study were lower than those reported by Scott et al. (1998). In their study, the AIDE value of different wheat samples ranged from 3,320 to 3,511 kcal/kg and those for hull-less and hulled barley were 2,820 and 3,040 kcal/kg, respectively.

The notable feature of the present work is that the TIDE was determined for 4 common cereal grains with the hope of initiating an interest in the development of matrix values for individual ingredients, which could then be used in feed formulations if the ileal digestible energy system proves to be more predictive of bird performance than the AME. To the authors' knowledge, no previous study has determined TIDE for ingredients or diets because of the lack of IEEL quantification. The TIDE values were higher than the corresponding AIDE values by 369 kcal/kg (average of all cereal grains). This finding was expected, based on the definition of AIDE and TIDE.

The AMEn for all 4 cereals was similar to their counterpart AIDE values. Similar values for viscous cereals were not anticipated as the hindgut fermentation of undigested feed components would have added microbial

Table 4. Endogenous energy loss (kcal/kg DM intake) and coefficient of apparent glucose absorption in the jejunum and ileum of 21-day-old broilers fed glucose-based purified diet.

Segment	Endogenous energy loss	Apparent glucose absorption coefficient[1]
Jejunum	648[a]	0.945[b]
Ileum	347[b]	1.00[a]
SEM[2]	41.0	0.010

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

[1]Analyzed glucose values: diet, 892 g/kg; jejunal digesta, 255 ± 124 g/kg (mean ± SD; 6 replicates); ileal digesta, 5.2 ± 4.8 g/kg (mean ± SD; 6 replicates).

[2]Pooled standard error of mean.

Table 5. Apparent metabolizable energy (AME; kcal/kg DM), nitrogen-corrected AME (AMEn; kcal/kg DM), apparent ileal digestible energy (AIDE; kcal/kg DM), and true ileal digestible energy (TIDE; kcal/kg DM) of test cereal grains in broilers at 21 d of age.[1]

Method	Corn	Sorghum	Wheat	Barley	SEM[3]
AME	3,499^{b,w}	3,346^{b,w}	2,653^{b,x}	2,447^{b,y}	62
AMEn	3,439^{b,w}	3,284^{b,w}	2,576^{b,x}	2,371^{b,y}	60
AIDE	3,544^{b,w}	3,296^{b,x}	2,758^{b,y}	2,519^{b,z}	68
TIDE[2]	3,920^{a,w}	3,650^{a,x}	3,138^{a,y}	2,885^{a,z}	68
SEM[3]	54	52	76	73	

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$). Means in a row not sharing a common letter (w–z) are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]Apparent ileal digestible energy values were corrected to true ileal digestible energy using the ileal endogenous energy flow value of 347 kcal/kg DM intake, determined by feeding a glucose-based diet.
[3]Pooled standard error of mean.

mass and energy and decreased the AME estimate at the excreta level (Shires et al., 1980). Masood et al. (2011) reported that the AMEn of sunflower meal was higher than the IDE value for broiler chickens of 34 d of age (2,390 vs. 2,261 kcal/kg). Interestingly, correction for IEEL resulted in TIDE values that were higher than their counterpart AMEn values for all cereal grains.

The present study demonstrated that corn and sorghum had the highest AMEn, barley the lowest, and wheat being intermediate. In general, these differences follow the trends in published AMEn ranges for these cereals (Mateos et al., 2019). The fermentation of undigested dietary nutrients and differences in NSP contents in barley and wheat provide a plausible explanation for the observed trends in AMEn values among the cereal grains. Microbial population in the ceca multiply in the presence of NSP and undigested nutrients and ferment these substances with the resultant increase in microbial mass in the excreta (Sugahara et al., 2004).

The AMEn:AIDE and AMEn:TIDE ratios were found to be lower than 1.0 for all cereal grains. A range of AMEn:AIDE ratio (0.980–1.004) was observed for 12 corn samples (Gehring et al., 2012). The AMEn:TIDE ratio tended to be lower for viscous cereals (wheat and barley) than that for nonviscous cereals (corn and sorghum). This observation provides further evidence for the hindgut fermentation in viscous cereals with high

Table 6. Influence of cereal grain type on the relationship between nitrogen-corrected apparent metabolizable energy (AMEn) and apparent ileal digestible energy (AIDE) and true ileal digestible energy (TIDE) in broilers at 21 d of age.[1]

Grain type	AMEn:AIDE	AMEn:TIDE[2]
Corn	0.942	0.878
Sorghum	0.999	0.902
Wheat	0.939	0.825
Barley	0.942	0.822
SEM[3]	0.028	0.023

[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]$P = 0.06$.
[3]Pooled standard error of mean.

Table 7. Influence of cereal grain type on the coefficients of apparent ileal digestibility of dry matter, nitrogen, starch, and gross energy in broilers at 21 d of age.[1]

Grain type	Dry matter	Nitrogen	Starch	Gross energy
Corn	0.787^{a}	0.766^{a}	0.991^{a}	0.814^{a}
Sorghum	0.704^{b}	0.703^{b}	0.967^{b}	0.749^{b}
Wheat	0.609^{c}	0.743^{a,b}	0.973^{b}	0.642^{c}
Barley	0.564^{c}	0.644^{c}	0.943^{c}	0.585^{d}
SEM[2]	0.0170	0.0151	0.0051	0.0156

Means in a column not sharing a common letter (a–d) are significantly different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]Pooled standard error of mean.

contents of NSP, which adds microbial mass to the excreta decreasing the AMEn. These findings also suggest that the differences between AMEn and TIDE will be greater in poorly digested ingredients.

Cereal grain type influenced the CAID of DM, N, starch and GE, and AIDE and TIDE. In general, viscous cereal grains (wheat and barley) had lower CAID of DM, starch, and GE than that of corn. These results agree with those of Romero et al. (2014) who reported that a corn-based diet had higher AIDE than a wheat-based diet (3,293 vs. 3,207 kcal/kg) in 21-day-old broilers. Abdollahi et al. (2013) similarly found that a corn-based diet had higher CAID of N (0.766 vs. 0.676) and starch (0.984 vs. 0.920) than a wheat-based diet. Perera et al. (2019) reported a higher CAID of DM (0.738 vs. 0.624) and starch (0.987 vs. 0.870) for wheat than that of waxy starch hull-less barley. The differences between CAID of nutrients for different cereal grains could be related to the antinutritive characteristics of NSP (Annison and Choct, 1991) which are found in higher concentrations in wheat and barley than in corn and sorghum.

Strong correlations were found between the CAID of N, starch, and GE and TIDE and AMEn. These results are in contrast with those of Gehring et al. (2012) who reported no correlation between CAID of starch and AIDE or AMEn of corn and could be related to the high starch digestibility and consequently limited availability of undigested starch for cecal fermentation in the present work. Positive correlations among AIDE, TIDE, and AMEn were evident in the present study. Scott et al. (1998) similarly observed a positive correlation between the AME and AIDE of wheat and barley.

Finally, a limitation in using just one IEEL estimate for the correction of AIDE of all ingredients, regardless of their available energy contents, must be acknowledged. Such an approach may penalize low-energy ingredients, but this is inevitable. A parallel situation exists in the use of one set of ileal endogenous amino acid flow values, measured after the feeding of a protein-free diet, for standardization of amino acid digestibility values, regardless of varying digestible contents.

In conclusion, the present study proposes a novel approach to quantify IEEL in broiler chickens and provide preliminary data on the TIDE of common cereal grains. To the authors' knowledge, this is the first report

Table 8. Pearson correlation coefficients (r-values) between measured biological parameters.[1]

Biological parameter	CAID of DM	CAID of N	CAID of starch	AIDE	TIDE	AMEn
CAID of DM	1.00					
CAID of N	0.725 (0.001)	1.00				
CAID of starch	0.698 (0.001)	0.669 (0.001)	1.00			
AIDE	0.988 (0.001)	0.690 (0.001)	0.695 (0.001)	1.00		
TIDE	0.990 (0.001)	0.703 (0.001)	0.705 (0.001)	1.00 (0.001)	1.00	
AMEn	0.873 (0.001)	0.483 (0.017)	0.656 (0.001)	0.915 (0.001)	0.912 (0.001)	1.00

Abbreviations: AIDE, apparent ileal digestible energy; CAID, coefficient of apparent ileal digestibility; N, nitrogen; TIDE, true ileal digestible energy.

[1]P values are in parentheses.

of the quantification of endogenous energy flow at the ileal level. The AMEn was determined to be lower than the TIDE for all 4 test cereals, with the AMEn:TIDE ratio being markedly lower for viscous cereals. The findings with viscous cereals suggest that the main difference between endogenous energy measurements at excreta and ileal levels arises from the contribution of hindgut fermentation and that the differences will be greater in poorer quality ingredients. Future research is warranted to establish the TIDE of a range of ingredients and evaluation of TIDE as a potential available energy system, and its suitability to be applied in poultry diet formulations merits further research investment.

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