Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens

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ABSTRACT A total of 960 male Ross 308 chicks (day-old) were used to investigate the effect of age on the relationship between metabolizable energy (ME) and digestible energy (DE) for broiler chickens. Bird growth variables, nitrogen retention (NR), nitrogen digestibility (ND), as well as the relative weight of liver, pancreas, and the gastrointestinal tract were determined. Practical diets that compared 2 cereals (corn and wheat) and exogenous xylanase (0 or 16,000 BXU/kg) were evaluated at 5 ages (7, 14, 21, 28, and 35 D) in a 2 × 2 × 5 factorial arrangement of treatments with 8 replicates per treatment and started with 30 birds per replicate. A randomized block ANOVA analysis of repeated measures was performed, and a 2 × 2 × 5 factorial structure was used to investigate the 2 dietary treatment factors (cereal type and the presence of xylanase) within the 5 bird ages (7, 14, 21, 28, and 35 D), and their interactions. Apparent metabolizable energy (AME) increased linearly from 7 until 28 D of age, but ($P < 0.05$) decreased at 35 D of age. Digestible energy was high at 7 D of age, then dropped and remained similar ($P > 0.05$) from 14 to 35 D of age. The AME: DE ratio was lowest ($P < 0.05$) at 7 D of age but there were no ($P > 0.05$) differences thereafter. Cereal type and xylanase supplementation did not ($P > 0.05$) change the ME: DE ratio. The results indicate that determining ME before 14 D of age may give absolute values that are lower than would be obtained with older birds. ME values that are determined on older broiler chickens may overestimate the energy availability of practical feeds used in broiler starter feeds.

Key words: age, broiler, metabolizable energy, digestible energy, diet

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INTRODUCTION

Determining the energy availability of a feed ingredient and practical feeds is important in order to evaluate their nutritional and economic value for poultry. The most common method used to measure energy availability is metabolizable energy (**ME**). Metabolizable energy is defined as energy that is available for use by the animal once the energy losses in the feces, urine, and combustible gases have been subtracted. Metabolizable energy is commonly used for poultry because of the simplicity to collect droppings since poultry void feces and urine through a common cloaca, and also because the assay can be carried out on large numbers without sacrificing the birds (Zaefarian et al., 2013).

Metabolizable energy does not measure digestibility but rather energy metabolizability, because urine that contains energy is voided with the feces in the droppings of birds. Poultry ME values may be corrected to a state of nitrogen equilibrium (**MEn**). However, the droppings also include endogenous losses, so the determination is an apparent metabolizable energy (**AME**). The ME values determined include the energy losses due to microbial fermentation in the ceca. However, the chickens do not derive as much as its total AME from fermentation as the other farm animals (JøRgensen et al., 1996; Apajalahti and Vienola, 2016).

Payne et al. (1968) suggested the use of distal ileal contents to measure the digestion of nutrients. This requires the collection of the ileal digesta and the analysis of energy and an inert marker to calculate digestible energy (**DE**). Inert digestibility markers, such as titanium dioxide, chromic oxide, or acid insoluble ash (**AIA**), are used. The determined DE of some poultry feeds is now available in the literature.

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Although both ME and DE are used to estimate energy availability in poultry feeds, the ratio between ME and DE may not always be the same. A major difference between ME and DE is that ME is postcecal and DE is prececal (Ravindran et al., 1999). Therefore, ME values that estimate energy availability incorporate some energy loss that has occurred during fermentation in the ceca. Recently, hatched chicks have relatively small numbers of bacteria in their gastrointestinal tracts and the numbers then increase with age (Geyra et al., 2001; Amit-Romach et al., 2004; Olukosi et al., 2007). The contribution of bacterial fermentation may be affected by age. In addition, the relationship between ME and DE may also vary with the dietary constituents. Practical diets vary in their contents of non-starch polysaccharides (**NSP**), which may be primarily fermented within the gastrointestinal tract (Xie et al., 2017). Furthermore, practical poultry feeds commonly include exogenous enzymes that may hydrolyze a proportion of NSP, reducing viscosity and so reduce the amount of fermentation in the small intestine (Choct et al., 2004; Pirgozliev and Bedford, 2013; Lei et al., 2016; Madsen et al., 2018) but enhance cecal fermentation through provision of oligosaccharides (Choct et al., 1996).

The aim of the present study was to determine and compare the effect of bird age (7, 14, 21, 28, and 35 D), cereal type (corn- and wheat-based diet), and exogenous xylanase supplementation (with or without xylanase) on ME and DE of 2 nutritionally complete, practical broiler chicken feeds. The dietary effects on bird growth variables, as well as the relative weights of liver, pancreas, and the gastrointestinal tract were also determined.

Table 1. Ingredient composition of the experimental diets.

Ingredient	Corn diet %	Wheat diet %
Corn	63.99	0.00
Wheat	0.00	62.58
Soybean meal	30.07	28.50
Wheat bran	1.77	2.00
Soy oil	0.75	3.71
Salt	0.35	0.32
DL-Methionine	0.30	0.28
Lysine HCl	0.25	0.25
Threonine	0.02	0.04
Limestone	0.87	1.01
Monocalcium phosphate	1.12	0.80
Phytase (500 FTU/kg diet)	0.01	0.01
Vitamin mineral premix[1]	0.50	0.50
Total	100.00	100.00
Calculated analysis (as-fed basis)		
ME (kcal/kg)	3,025	3,025
Lysine (%)	1.25	1.25
Methionine + cysteine (%)	0.95	0.95
Calcium (%)	0.95	0.95
Phosphorus (%)	0.77	0.74
Analysed values (as-fed basis)		
Crude protein (%)	19.02	20.53
Crude fat (%)	3.2	4.0
Total NSP (%)	9.0	11.1
Soluble NSP (%)	1.9	2.5
Insoluble NSP (%)	7.1	8.6
Main constituents of NSP		
Arabinose (%)	1.8	2.1
Xylose (%)	2.1	2.9
Mannose (%)	0.6	0.8
Galactose (%)	1.5	2.1
Glucose (%)	2.3	2.5
Pellet quality		
PDI (%)	93.8	90.3
Pellet hardness (Newton)	30.0	27.8

NSP, non-starch polysaccharide; PDI = pellet durability index.

[1]The premix provided (units/kg diet): retinol, 12,000 IU; cholecalciferol, 5,000 IU; α-tocopherol, 34 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg; 80 mg Fe as iron sulfate (30%); 10 mg Cu as a copper sulfate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.2 mg Se as sodium selenite (4.5%); and 0.5 mg Mo as sodium molybdate (40%).

MATERIALS AND METHODS

Ethics Statement

The trial was conducted under the direction of the Harper Adams University Animal Ethics Committee.

Animals and Experimental Design

A total of 960 day-old male Ross 308 chicks were obtained from a commercial hatchery and randomly divided into 32 pens with 30 birds in each. Each of the pens had a solid floor covered with cardboard bedding material. The square cardboard product was corrugated cardboard, which was a material consisting of a fluted corrugated sheet and 2 flat linerboards. Two diets in which the main cereal component was either corn or wheat were formulated and mixed (Table 1). The experimental diets were formulated to meet or exceed the nutritional requirement of broiler chickens as recommended by NRC (1994). Each diet was then split into 2 equal portions, one portion had 100 g/tonne units of xylanase (Econase XT 25, AB Vista, Marlborough, UK) added. The analyzed xylanase activity of the Econase XT 25 was 160,000 BXU/g. This resulted in 4 dietary treatments that included 8 replicates per treatment and started with 30 birds per replicate. Celite (Diatom Retail, Leicester, UK), a source of AIA, was added to all diets at 5 g/kg as an indigestible marker. Exogenous phytase was added to all of the experimental feeds because this is now frequently done with all commercial broiler chicken feeds. The diets were pelleted (Target Feeds Ltd, Whitchurch, UK) with steam-conditioning at 50 to 60°C for 20 s. The pellet diameter was 3 mm. Each of the 4 diets was fed to 8 pens of birds, and the pen of birds was considered to be the experimental unit. During the first 4 D, the diets were provided in crumb form (pelleted feed that was then mechanically broken to small particle sizes). Whole pellets were fed from 4 D until the end of the feeding period. Each pen was equipped with a separate feeder and drinker. Feed and water were offered ad libitum to birds throughout the experiments.

The room temperature was approximately 32°C at day old and was gradually reduced to 20°C at 21 D of age, and was kept the same until the end of the study. A standard lighting program for broilers was followed which decreased from 23 h: 1 h (light: dark) at day old to 18 h: 6 h (light: dark) at 7 D of age that was maintained until the end of the study. The relative humidity was maintained between 50 and 70%.

Sample Collection and Laboratory Analysis

Feed intake (**FI**) by pen was measured on a daily basis and body weight (**BW**) was recorded at 7, 14, 21, 28, and 35 D of age. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) were calculated every week, and mortality was recorded as it occurred.

At days 6, 13, 20, 27, and 34, the solid floor of the pen was removed and replaced by a wire mesh floor. Clean droppings trays were placed under each cage. After 24 h, a clean (free of feed and visible feather contaminants) sample of droppings (the mixture of fecal material and urine) was collected (250 mL specimen jar) and immediately oven-dried (65°C) for 48 h, ground (0.5-mm screen), and stored for analysis. The solid pen floor was then replaced.

At days 7, 14, 21, 28, and 35, 1 bird from each pen was selected randomly and placed separately in a metabolic cage with food deprivation for 12 h. The birds were weighed, and then slaughtered by cervical dislocation. The following variables were weighed: liver, gizzard and proventriculus, pancreas, small intestine (sum of duodenum, jejunum, ileum), and ceca.

In addition, 10 birds at day 7, 5 birds at day 14, 4 birds at day 21, 3 birds at day 28, and 3 birds at day 35 from each pen were selected randomly and killed by cervical dislocation. The intestinal tract was removed, and the contents of the tract from Meckel's diverticulum to the ileal-cecal-colon junction were gently squeezed directly into 250-mL specimen cups. The contents from the individual birds in each pen were pooled to get enough weight of ileal digesta sample for later laboratory analysis. Ileal digesta were immediately oven-dried (65°C) for 24 h, ground, and stored for analysis.

Diets, droppings, and ileal digesta samples were analyzed for dry matter (**DM**), nitrogen, gross energy (**GE**), and AIA concentration. DM was determined by drying of samples in a forced draft oven at 105°C to a constant weight (AOAC, 2000; method 934.01) (NRC, 1994). Nitrogen was determined by the combustion method (AOAC, 2000; method 990.03) using a Leco (FP-528 N, Leco Corp., St. Joseph, MI). GE was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) with benzoic acid used as the standard. The AIA content was measured after ashing the samples and treating the ash with boiling 2 M hydrochloric acid (Scott and Boldaji, 1997).

The content of NSP of the diets was measured using the method proposed by Englyst et al. (1994) (Englyst Fiberzym Kit for Colorimetry, Dunn Nutrition Centre, Cambridge, UK). The procedure included an enzymatic-chemical method to separate the starch from the NSP. The amount of soluble of NSP was obtained as a difference between total NSP and insoluble NSP. All the colorimetric measurements were performed on Beckman DU-640 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA).

The pellet durability index (**PDI**) was determined in duplicates using a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester; TekPro Ltd, Willow Park, North Walsham, Norfolk, UK). Clean pellet samples (100 g), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 s. Fines were removed continuously through the perforations (2 mm in diameter) during the test cycle. After the test cycle, the remaining pellets were ejected and weighed manually. The PDI was calculated as the ratio of the weight of the pellets not passing through the perforations after test to the weight of the whole pellets at the start. Pellet hardness, expressed as the force required to break an individual pellet (Newton), was determined with a force tester (Instron 5543, CAE, Austin) using, for each diet, 10 intact pellets of similar length that did not show any visible deformation.

Calculations

(1) The AME was calculated, using AIA as indigestible marker (Hill and Anderson, 1958), as shown below:

$$\text{Dry matter retention } (\mathbf{DMR}) = \left(\text{AIA}_{\text{droppings}} - \text{AIA}_{\text{feed}}\right)/\text{AIA}_{\text{droppings}}$$

$$\text{AME (MJ/kg)} = \text{GE}_{\text{feed}} - [(1 - \text{DMR}) \times \text{GE}_{\text{droppings}}]$$

where DMR is the dry matter retention, $\text{AIA}_{\text{droppings}}$ is the concentration of AIA in the droppings (g/kg), AIA_{feed} is the concentration of AIA in the feed (g/kg), GE_{feed} is the gross energy in the feed (MJ/kg), $\text{GE}_{\text{droppings}}$ is the gross energy in the droppings (MJ/kg).

(2) The N-corrected apparent metabolizable energy (**AMEn**) value of the experimental diets was determined following the method of Hill and Anderson (1958) calculated as described by Lammers et al. (2008).

$$\text{AMEn} = \text{GE}_{\text{feed}} - (\text{GE}_{\text{droppings}} \times \text{AIA}_{\text{feed}})/\text{AIA}_{\text{droppings}} - (34.39 \times \text{N Retained})/1000$$

where AMEn (MJ/kg) is the N-corrected apparent metabolizable energy content of the diet, GE_{feed} is the gross energy in the feed (MJ/kg), $\text{GE}_{\text{droppings}}$ is the gross energy in the droppings (MJ/kg), AIA_{feed} is the concentration of AIA in the feed (%), $\text{AIA}_{\text{droppings}}$ is

Table 2. The effect of cereal type, xylanase supplementation, and bird age to broiler chickens on average daily gain (g), average daily feed intake, and feed conversion ratio (data based on feeding period from day 1 to 35).[1]

Bird age		ADG (g/b/d)	ADFI (g/b/d)	FCR
Week 1		15.1	17.7	1.17
Week 2		42.0	59.2	1.42
Week 3		62.0	99.7	1.63
Week 4		71.3	130.3	1.86
Week 5		86.6	164.6	1.94
SEM (df = 112)		2.20	5.48	0.068
Treatment				
Cereal	Xylanase			
Corn	No	52.1	87.7	1.63
Corn	Yes	53.2	90.9	1.61
Wheat	No	59.8	106.0	1.62
Wheat	Yes	56.6	92.6	1.55
SEM (df = 21)		2.60	5.26	0.061
Main factor				
Corn		52.6	89.3	1.62
Wheat		58.2	99.3	1.59
SEM (df = 21)		1.84	3.72	0.043
Xylanase				
No		56.0	96.8	1.63
Yes		54.9	91.8	1.58
SEM (df = 21)		1.84	3.72	0.043
Probabilities				
Bird age		<0.001	<0.001	<0.001
Form of response				
Linear		<0.001	<0.001	<0.001
Quadratic		<0.001	0.095	0.085
Cereal		0.006	0.014	0.433
Xylanase		0.562	0.187	0.308
Cereal × xylanase		0.250	0.037	0.545
Bird age × cereal		0.198	0.186	0.728
Bird age × xylanase		0.088	0.138	0.969
Bird age × cereal × xylanase		0.825	0.146	0.194

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SEM, pooled standard error of means.

[1]There were 8 observations per treatment. Week 1 performance data were based on 30 birds; week 2 performance data were based on 20 birds; week 3 performance data were based on 15 birds; week 4 performance data were based on 11 birds; week 5 performance data were based on 8 birds.

the concentration of AIA in the droppings (%), 34.39 (MJ/kg) is the energy value of uric acid; N Retained (g/kg) is the N retained by the birds per kilogram of diet consumed. The N retained was calculated as

$$\text{N Retained} = \text{N}_{\text{feed}} - (\text{N}_{\text{droppings}} \times \text{AIA}_{\text{feed}})/\text{AIA}_{\text{droppings}}$$

where N_{feed} and $\text{N}_{\text{droppings}}$ (g/kg) are N contents of the feed and droppings, respectively.

(3) The nitrogen retention (**NR**) was obtained as described below (Lammers et al., 2008).

$$\text{NR} = (\text{N}_{\text{feed}}/\text{AIA}_{\text{feed}} - \text{N}_{\text{droppings}}/\text{AIA}_{\text{droppings}})/(\text{N}_{\text{feed}}/\text{AIA}_{\text{feed}})$$

where N_{feed} is the nitrogen of the feed (g/kg), AIA_{feed} is the concentration of AIA in the feed (g/kg), $\text{N}_{\text{droppings}}$ is the nitrogen of the droppings (g/kg), and $\text{AIA}_{\text{droppings}}$ is the concentration of AIA in the droppings (g/kg).

(4) The DE was calculated, using AIA as indigestible marker, as shown below (González-Ortiz et al., 2016).

$$\text{DMD} = (\text{AIA}_{\text{digesta}} - \text{AIA}_{\text{feed}})/\text{AIA}_{\text{digesta}}$$

$$\text{DE (MJ/kg)} = \text{GE}_{\text{feed}} - [(1 - \text{DMR}) \times \text{GE}_{\text{digesta}}]$$

where DMD is the dry matter digestibility, $\text{AIA}_{\text{digesta}}$ is the concentration of AIA in the ileal digesta (g/kg), AIA_{feed} is the concentration of AIA in the feed (g/kg), GE_{feed} is the gross energy in the feed (MJ/kg), $\text{GE}_{\text{digesta}}$ is the gross energy in the ileal digesta (MJ/kg).

(1) The nitrogen digestibility (**ND**) was obtained as described below (Lammers et al., 2008).

$$\text{ND} = (\text{N}_{\text{feed}}/\text{AIA}_{\text{feed}} - \text{N}_{\text{digesta}}/\text{AIA}_{\text{digesta}})/(\text{N}_{\text{feed}}/\text{AIA}_{\text{feed}})$$

where N_{feed} is the nitrogen of the feed (g/kg), AIA_{feed} is the concentration of AIA in the feed (g/kg), $\text{N}_{\text{digesta}}$ is

Table 3. The effect of cereal type, xylanase supplementation, and bird age to broiler chickens on postcecal nutrient retention and metabolizable energy determination (data obtained from 7, 14, 21, 28, and 35 day-old birds).[1]

Bird age		DMR (g/g)	AME (MJ/kg)	AMEn (MJ/kg)	NR (g/g)
7 D[1]		0.753	12.61	11.87	0.688
14 D		0.765	12.88	12.15	0.664
21 D		0.776	13.09	12.35	0.679
28 D		0.784	13.17	12.46	0.656
35 D		0.762	12.85	12.19	0.609
SEM (df = 112)		0.0061	0.0950	0.0870	0.0126
Treatment					
Cereal	Xylanase				
Corn	No	0.745	12.59	11.93	0.628
Corn	Yes	0.773	13.07	12.39	0.654
Wheat	No	0.769	12.87	12.11	0.674
Wheat	Yes	0.784	13.16	12.39	0.680
SEM (df = 21)		0.0057	0.091	0.0840	0.0113
Main factor					
Corn		0.759	12.83	12.16	0.641
Wheat		0.776	13.01	12.25	0.677
SEM (df = 21)		0.0040	0.0650	0.0590	0.0080
Xylanase					
No		0.757	12.73	12.02	0.651
Yes		0.779	13.11	12.39	0.667
SEM (df = 21)		0.0040	0.0650	0.0590	0.0080
Probabilities					
Bird age		<0.001	<0.001	<0.001	<0.001
Form of response					
Linear		0.007	<0.001	<0.001	<0.001
Quadratic		<0.001	<0.001	<0.001	0.011
Cereal		<0.001	0.009	0.137	<0.001
Xylanase		<0.001	<0.001	<0.001	0.058
Cereal × xylanase		0.111	0.127	0.135	0.215
Bird age × cereal		0.653	0.756	0.801	0.431
Bird age × xylanase		0.683	0.637	0.630	0.676
Bird age × cereal × xylanase		0.616	0.574	0.541	0.466

DMR, dry matter retention; AME, apparent metabolizable energy; AMEn, N-corrected apparent metabolizable energy; NR, nitrogen retention; SEM, pooled standard error of means.

[1]There were 8 observations per treatment.

the nitrogen of the ileal digesta (g/kg), and $AIA_{digesta}$ is the concentration of AIA in the ileal digesta (g/kg).

Statistical Analysis

Statistical analysis was performed using the GenStat 18 statistical software package (IACR Rothamstead, Hertfordshire, UK). A randomized block ANOVA analysis of repeated measures was performed, and a 2 × 2 × 5 factorial structure was used to investigate the 2 dietary treatment factors (cereal type and the presence of xylanase) within the 5 bird ages (7, 14, 21, 28, and 35 D), and their interactions. Differences were reported as significant at $P < 0.05$.

RESULTS

Bird Growth Performance

Mortality data were transformed before analysis. Mortality was low (<1%), and there were no treatment effects. The mean weights of the birds at 7, 14, 21, 28, and 35 D of age were 175, 480, 916 and 1, 430 and 2, 122 g, respectively, and these were 10 to 15% below to the Ross 308 broiler target weights for commercial flocks. The birds were kept in small groups in research facilities, and the reduced performance compared to large commercial flocks was expected. The ADG, ADFI, and FCR increased with age from week 1 to 5 ($P < 0.05$) (Table 2). ADG and ADFI for the birds fed on wheat-based diets were significantly higher than those receiving corn-based diets ($P < 0.05$). Dietary treatment had no effect on FCR ($P > 0.05$). For ADFI, there was a significant cereal type × xylanase interaction ($P = 0.037$). ADFI was not affected by xylanase addition in the corn-based diets, but was decreased ($P < 0.05$) by 12% with xylanase supplementation in the wheat-based diets.

Postcecal Nutrient Retention and ME Determination

Dry matter retention, AME, and AMEn increased with bird age, and there was a significant quadratic response to age ($P < 0.05$) (Table 3). DMR, AME, and AMEn increased linearly from 7 until 28 D of age but there was a small but significant ($P < 0.05$) decrease at 35 D of age comparing to earlier bird age. NR did

Table 4. The effect of cereal type, xylanase supplementation, and bird age to broiler chickens on prececal nutrient retention and digestible energy determination (data obtained from 7, 14, 21, 28, and 35 day-old birds).[1]

Bird age		DMD (g/g)	DE (MJ/kg)	ND (g/g)
7 D		0.795	13.27	0.841
14 D		0.763	12.55	0.804
21 D		0.778	12.88	0.828
28 D		0.776	12.81	0.825
35 D		0.774	12.73	0.818
SEM (df = 112)		0.0070	0.1370	0.0079
Treatment				
Cereal	Xylanase			
Corn	No	0.750	12.40	0.807
Corn	Yes	0.786	13.01	0.821
Wheat	No	0.776	12.85	0.828
Wheat	Yes	0.797	13.13	0.837
SEM (df = 21)		0.0054	0.1110	0.0071
Main factor				
Corn		0.768	12.70	0.814
Wheat		0.787	12.99	0.832
SEM (df = 21)		0.0038	0.0790	0.0051
Xylanase				
No		0.763	12.62	0.818
Yes		0.791	13.07	0.829
SEM (df = 21)		0.0038	0.079	0.0051
Probabilities				
Bird age		0.003	<0.001	<0.001
Form of response				
Linear		0.066	0.010	0.153
Quadratic Quadratic		0.022	0.017	0.122
Cereal		<0.001	0.001	0.002
Xylanase		<0.001	<0.001	0.041
Cereal type × xylanase		0.051	0.046	0.618
Bird age × cereal		0.223	0.692	0.623
Bird age × xylanase		0.666	0.329	0.135
Bird age × cereal × xylanase		0.174	0.320	0.380

DMD, dry matter digestibility; DE, digestible energy; ND, nitrogen digestibility; SEM, pooled standard error of means.

[1]There were 8 observations per treatment.

not change ($P > 0.05$) from 7 to 28 D of age but also decreased between 28 and ($P < 0.05$) 35 D of age.

Birds fed on wheat-based diets had higher ($P < 0.05$) DMR, AME, and NR compared to those receiving corn-based diets. Xylanase supplementation improved DMR, AME, and AMEn compared to non-supplemented diets in both corn- and wheat-based diets ($P < 0.05$). No 2 or 3-way interactions were observed for these variables ($P > 0.05$).

Prececal Nutrient Retention and DE Determination

There was a significant quadratic response to age ($P < 0.05$) in DMD and DE ($P = 0.003$ and $P < 0.001$, respectively) and a significant effect of age ($P < 0.001$: neither linear nor quadratic) for ND (Table 4). In each of these variables, the greatest value ($P < 0.05$) was observed in the birds at 7 D of age and it was decreased thereafter with few ($P > 0.05$) differences between the later ages.

Birds fed on wheat-based diets had higher DMD, DE, and ND than those receiving corn-based diets ($P < 0.05$). The supplementation of xylanase increased the values of DMD, DE, and ND in both the corn-based and wheat-based diets ($P < 0.05$). There was an interaction ($P = 0.046$) between cereal type and xylanase in DE. Digestible energy was not affected by xylanase addition to the corn-based diets, but was increased ($P < 0.05$) with xylanase supplementation of the wheat-based diets.

The Ratio Between ME and DE

There was a quadratic response ($P < 0.001$) to increasing age in the AME: DE and AMEn: DE ratios (Table 5). The AME: DE ratio was lowest ($P < 0.05$) at 7 D of age but there were no ($P > 0.05$) differences thereafter. In comparison, the NR: ND ratios were similar from 7 to 21 D of age and then decreased ($P < 0.05$).

The AMEn: DE ratio was higher in birds fed on corn-based diets than those fed on wheat-based diets ($P < 0.05$); however, the NR: ND ratio was higher in birds fed the wheat-based diets ($P < 0.05$). There were no ($P > 0.05$) effects of exogenous xylanase addition in any of the variables and no ($P > 0.05$) 2 or 3-way interactions were observed.

Table 5. The effect of cereal type, xylanase supplementation, and bird age to broiler chickens on the relationship between metabolizable energy and digestible energy (data obtained from 7, 14, 21, 28, and 35 day-old birds).[1]

Bird age		AME: DE	AMEn: DE	NR: ND
7 D		0.954	0.897	0.819
14 D		1.027	0.971	0.828
21 D		1.018	0.961	0.820
28 D		1.029	0.973	0.796
35 D		1.011	0.959	0.745
SEM (df = 112)		0.0133	0.0123	0.0177
Treatment				
Cereal	Xylanase			
Corn	No	1.019	0.966	0.780
Corn	Yes	1.005	0.953	0.797
Wheat	No	1.000	0.946	0.816
Wheat	Yes	1.003	0.944	0.814
SEM (df = 21)		0.0086	0.0080	0.0142
Main factor				
Corn		1.012	0.959	0.788
Wheat		1.004	0.945	0.815
SEM (df = 21)		0.0061	0.0057	0.0100
Xylanase				
No		1.012	0.956	0.798
Yes		1.004	0.949	0.806
SEM (df = 21)		0.0061	0.0057	0.0100
Probabilities				
Bird age		<0.001	<0.001	<0.001
Form of response				
Linear		<0.001	<0.001	<0.001
Quadratic		<0.001	<0.001	0.005
Cereal		0.186	0.019	0.015
Xylanase		0.203	0.215	0.457
Cereal type × xylanase		0.346	0.303	0.350
Bird age × cereal		0.843	0.848	0.506
Bird age × xylanase		0.310	0.313	0.369
Bird age × cereal × xylanase		0.601	0.644	0.412

AME: DE, apparent metabolizable energy: digestible energy; AMEn: DE, N-corrected apparent metabolizable energy: digestible energy; NR: ND, nitrogen retention: nitrogen digestibility; SEM, pooled standard error of means.

[1]There were 8 observations per treatment.

Organ Development

There was a quadratic response with age ($P < 0.001$) in the relative weights (to body weight) of liver, gizzard and proventriculus, small intestine, and ceca (Table 6). The relative weights of liver, gizzard, and proventriculus peaked at day 14, followed by a continuous decline from 14 to 35 D of age ($P < 0.05$). Although the absolute weight of small intestine and ceca increased continuously from 7 to 35 D of age ($P < 0.05$), the relative weights of pancreas, small intestine, and ceca decreased in a quadratic form with bird age ($P < 0.05$).

The relative weights of the gizzard and proventriculus in the birds fed the corn-based diets were higher than those fed on wheat-based diets ($P = 0.047$). The absolute and relative weights of the ceca were higher in birds fed the wheat-based diets in comparison to the corn-based diets ($P < 0.05$). Significant interaction was detected among age and cereal on the relative weight of small intestine ($P = 0.049$) (Figure 1). Furthermore, there was a significant interaction between age and xylanase on the relative weight of the ceca ($P = 0.010$) (Figure 2).

DISCUSSION

The experimental diet series were formulated to be typical of a practical, commercial broiler chicken feed using 2 different cereal types. The determined AME for the 2 diets were approximated their predicted values. However, the 2 diets were formulated to have the same AME and, unexpectedly, the results showed that the wheat-based diet had 0.18 MJ/kg higher AME than the corn-based diet. This difference, although statistically significant, was relatively small and understandable because practical feed ingredients were used in the study and it was highly unlikely the predicted AME values used for individual feed ingredients in the 2 formulations would result in exactly the same determined AME value. The addition of exogenous xylanase gave an improvement in the determined AME, and this is consistent with other published data (Munyaka et al., 2015; Pirgozliev et al., 2015). The growth performance of the birds fed the wheat-based diets was superior to those fed the corn-based diets. Other published studies (Abdollahi et al., 2010a; Liu et al., 2014) have commonly observed similar or better growth performance in broilers fed corn-based diets. However, in the present study, the lower expected AME of wheat was balanced by a higher inclusion of soy oil in the wheat-based diet. The evaluation of these diets in the experiment showed that this resulted in the AME of the wheat diet being higher than the corn diet. This may have been a contributory cause of the higher growth performance of the broilers fed the wheat-based diet.

When practical broiler feeds are being formulated generally just one AME is used for each ingredient regardless of the bird age. However, our results showed that AME and AMEn increased linearly with bird age from 7 to 28 D of age for chicks. Batal and Parsons (2002) found that AMEn increased with age, although a regression analysis indicated a plateau after 14 D of age. Scott et al. (1998) also found that determined AME values of a range of cereal ingredients were higher at 16 D of age than those at 8 D. In the present study, there was a significant reduction in AME at 35 D; however, this was only reduced to the same value obtained with the bird age at 14 D of age. The increase in ME with age is probably primarily due to the increasing microbial fermentation of the digesta in the ceca (Shires et al., 1980). Batal and Parsons (2002) compared the effect of age on the determined ME of a practical corn and soybean meal-based diet with the determined ME of a purified dextrose-casein diet. The dextrose-casein diet would have contained very little undigestible yet fermentable material, such as NSP. They found that the determined ME of the dextrose-casein did not change with age (2 to 21 D of age), whereas the determined ME of the practical diets increased up to

Table 6. The effect of cereal type, xylanase supplementation and bird age to broiler chickens on the relative weight (%)[1] and absolute weight of organs and gastrointestinal tract (data obtained from 7, 14, 21, 28, and 35 day-old birds).[2]

Bird age		Relative weight of liver %	Relative weight of gizzard and proventriculus %	Relative weight of pancreas %	Absolute weight of small intestine g	Relative weight of small intestine %	Absolute weight of ceca g	Relative weight of ceca %
7 D (BW = 0.160 kg)		3.414	1.783	0.457	9.83	6.24	1.34	0.842
14 D (BW = 0.466 kg)		4.302	4.350	0.383	24.45	5.25	3.90	0.839
21 D (BW = 0.961 kg)		3.477	2.730	0.316	38.83	4.05	7.83	0.819
28 D (BW = 1.418 kg)		3.318	2.024	0.253	43.34	3.15	11.16	0.785
35 D (BW = 2.256 kg)		3.063	1.473	0.223	65.27	2.96	15.39	0.636
SEM (df = 112)		0.1775	0.1135	0.0425	1.660	0.160	0.573	0.0504
Treatment								
Cereal	Xylanase							
Corn	No	3.688	2.536	0.316	36.03	4.35	7.50	0.758
Corn	Yes	3.546	2.602	0.373	34.63	4.30	7.01	0.677
Wheat	No	3.403	2.271	0.316	37.90	4.26	8.87	0.861
Wheat	Yes	3.421	2.478	0.300	36.80	4.42	8.32	0.841
SEM (df = 21)		0.1546	0.1305	0.0423	1.399	0.174	0.568	0.0525
Main factor								
Corn		3.614	2.569	0.345	35.33	4.32	7.26	0.717
Wheat		3.421	2.375	0.308	37.35	4.34	8.59	0.851
SEM (df = 21)		0.1090	0.0923	0.0299	0.990	0.123	0.401	0.0371
Xylanase								
No		3.546	2.404	0.316	36.97	4.30	8.18	0.809
Yes		3.483	2.540	0.337	35.72	4.36	7.66	0.759
SEM (df = 21)		0.109	0.0923	0.0299	0.990	0.123	0.401	0.0371
Probabilities								
Bird age		<0.001	<0.001	<0.001	<0.001	<.001	<.001	<.001
Form of response								
Linear		<0.001	<0.001	<0.001	<0.001	<.001	<.001	<.001
Quadratic		<0.001	<0.001	0.414	0.280	<.001	0.073	0.024
Cereal		0.075	0.047	0.234	0.054	0.914	0.003	0.002
Xylanase		0.574	0.153	0.492	0.221	0.656	0.208	0.187
Cereal × xylanase		0.471	0.453	0.238	0.883	0.420	0.945	0.429
Week × cereal		0.435	0.508	0.214	0.682	0.049	0.338	0.620
Week × xylanase		0.088	0.099	0.382	0.241	0.424	0.111	0.010
Week × cereal × xylanase		0.822	0.104	0.432	0.101	0.178	0.985	0.837

BW, body weight; SEM, pooled standard error of means.
[1]The relative weights of each organ intestinal segment were calculated as a ratio of live body weight (g/100 g body weigh).
[2]There were 8 observations per treatment.

14 D of age, suggesting fermentation is a component of this effect.

Non-starch polysaccharides are the major part of the DM content of the digesta that would be fermented in the ceca. In the present study, the wheat-based diet had a somewhat higher NSP content than the corn-based diet (11.1% vs. 9.0%). However, there was no interaction detected with bird age and cereal type in AME. Tancharoenrat et al. (2013) also found that there was no interaction ($P > 0.05$) between cereal type and age of broilers for AME.

The addition of supplementary exogenous xylanase would be expected to reduce the amount of fermentable NSP entering the ceca (Vries et al., 2013). Although exogenous xylanase improved dietary AME in the present study, there was no bird age × xylanase interaction. Alamo et al. (2008) and McCracken and Quintin (2000) also found no change in the effect of exogenous xylanase on ME when measured in broiler chicks of different ages.

If part of the age effects on AME were caused by differences in cecal fermentation energy losses, then it follows that the use of digestibility estimates might provide a better comparison of energy availability at different ages. DMD and DE values were both very high at 7 D of age, then dropped and remained similar ($P > 0.05$) from 14 to 35 D of age. The determined DMD and DE were apparent values and included the energy contribution from endogenous losses. One possibility for the unexpected high value at 7 D of age was that there may have been only small amount of endogenous losses within the gastrointestinal tract into the digesta of these relatively newly hatched chicks. In the recently hatched chick, as in neonatal mammals, the small intestinal mucosa is relatively immature with less need for cell replacement and regeneration and so probably has less endogenous loss from this source (Mitjans et al., 1997). The young chicks may also more be able to digest large protein molecular nutrients at this early stage, as is the case with other farm animals (Da Costa et al., 2004), and so these molecules may be more easily digested at this age.

The results of the present study have shown that the determined ME values of feeds increase with age; yet

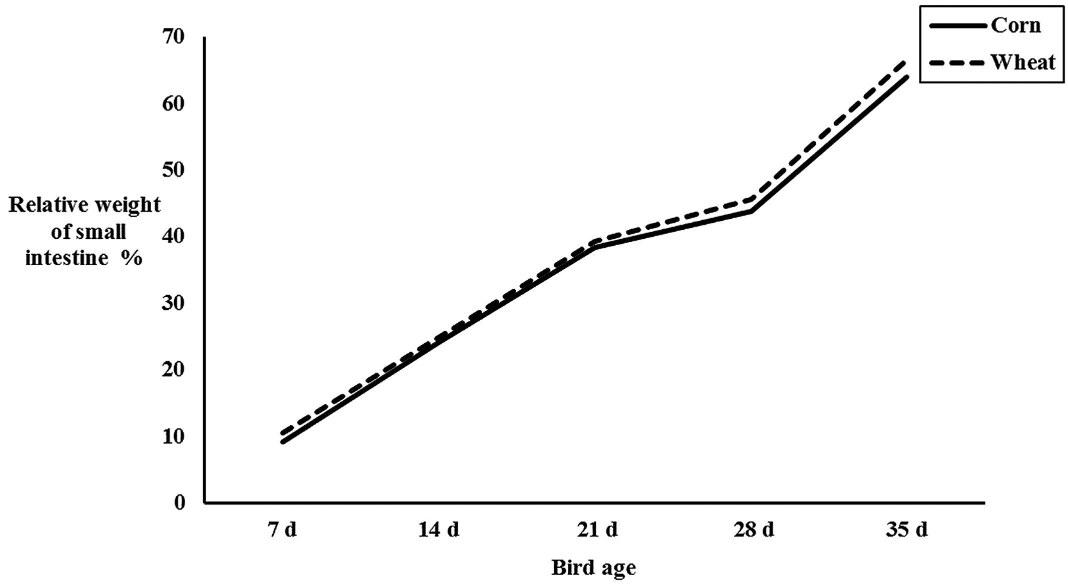

Figure 1. Interactions among age and cereal on the relative weight of small intestine.

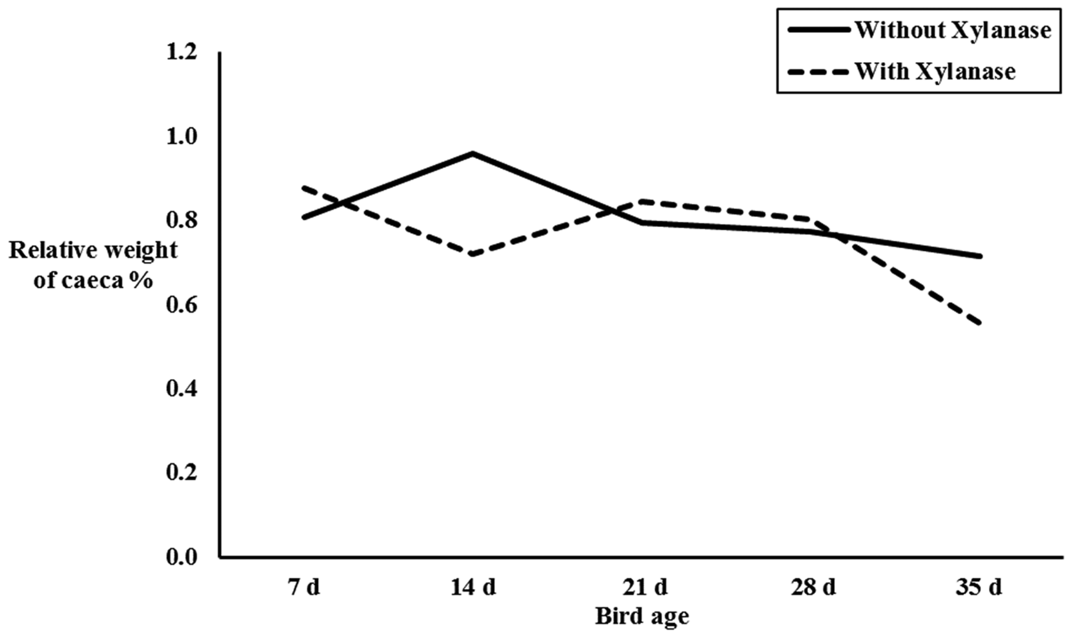

Figure 2. Interactions among age and xylanase on the relative weight of the ceca.

the determined DE values of the same feeds were very high at 7 D and then reduced and remained relatively constant thereafter. The AME: DE ratio was therefore low at 7 D of age, resulting from higher DE and lower ME. Apajalahti et al. (2002) and Wronkowska et al. (2017) have shown that not only do the numbers of microbes in the cecal and ileal digesta increase during the first days post hatching but also the relative dominance of different species within microbiome changes during the first week. These changes involve the gradual increase in numbers of bacterial species that are able to ferment the undigested component of the ileal digesta. It is possible that the cecal microbiome of 7-day-old broiler chicks is not yet effective in fermenting the undigested residues from the intestinal tract.

The AME: DE ratio remained approximately constant after 14 D of age, and the overall ratio for this period was 1.019. O'Neill et al. (2012) determined the ratio of the ME to DE in 18-day-old broilers fed practical feeds comparing a number of different cereals at 18 D in broilers and reported a mean ratio of 1.012. González-Ortiz et al. (2016) also found the AME: DE ratio to be 1.020 in 24 D broilers. The AME: DE ratio was less than 1.0 at 7 D of age. This probably indicates that there is also a high contribution of urinary energy losses at this age. Interestingly, Applegate et al. (2009)

found that the ratio of ME to DE was 0.950 for laying hens at 20 wk of age. These were adult, mature birds that had a relatively low egg production rate, and it is also possible that these birds had a protein intake that was significantly in excess of their requirements and so had high urinary energy losses.

Although the wheat-based diets had a higher NSP content, there was no ($P > 0.05$) difference with the corn-based diets in the AME: DE ratio. The growth rates of the birds fed the wheat-based diets were greater than those fed corn-based diets, and the greater body protein deposition rate probably explains the large difference in the NR: ND ratio between the wheat and corn-based diets.

There was no ($P > 0.05$) change in the AME: DE with the addition of exogenous xylanase although the ratio was numerically lower. The calculated ME: DE ratio from the data of O'Neill et al. (2012) was 1.0206 and 1.0032 for broilers supplemented with 0 and 16,000 BXU/kg xylanase, respectively. If this is a real effect, then it appears from these data that it is likely due to proportionately more energy being recovered from the ileum at the cecal level, suggesting a shift of digestion more caudally with xylanase use (Applegate et al., 2009). Further work is warranted to examine whether addition of exogenous xylanase has a repeatable effect.

In the present study, the relative weights of liver and gizzard and proventriculus peaked at 14 D of age, and then decreased until 35 D of age. The peak of the relative weight of liver and pancreas was in accordance with Ivanovich et al. (2017). The rapid growth of the intestine reaches a maximum between 6 and 10 D and declines thereafter (Sklan, 2001). We also observed a higher relative weight of gizzard and proventriculus in the birds fed on corn-based diets than those fed on wheat-based diets, this was probably due to the lower pellet hardness of the wheat-based diets. In the present study, a higher inclusion of soy oil in the wheat-based diet reduced the PDI of the diets. Hard, particulate feeds have been shown to stimulate the growth of the gizzard and proventriculus (Abdollahi et al., 2010b). The higher weight of ceca in the birds fed the wheat-based diets may relate to the higher fiber content of wheat and the higher NSP content of wheat as compared to corn.

In conclusion, the present study was designed to examine whether the age of broiler chickens had an effect on the determination of energy availability in practical broiler feeds. We examined 2 major variables that frequently differ between commercially available practical feeds—the type of cereal used in the formulation and the addition of exogenous xylanase. Our findings indicate that bird age had significant effect on the relationship between ME and DE for broiler chickens. Determining ME before 14 D of age may give absolute values that are significantly lower than would be obtained with older birds. ME values that are determined on older broiler chickens may overestimate the energy availability of practical feeds used in broiler starter feeds, especially if they contain large amounts of poorly digested but fermentable material. However, our results indicate that 2 major variables in commercial, practical feed formulations—cereal type and exogenous xylanase—do not interact with the relationship between ME and DE.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interest.

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