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METABOLIZABLE ENERGY DETERMINATION IN BROILER CHICKENS

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METABOLIZABLE ENERGY DETERMINATION IN BROILER CHICKENS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
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By

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ABSTRACT OF THESIS

METABOLIZABLE ENERGY DETERMINATION IN BROILER CHICKENS

Feed accounts for the highest cost associated in poultry production, with energy-containing feedstuffs being the most expensive portion of the cost of feeding. The increasing demand for poultry meat gives reason to determine accurate apparent metabolizable energy (AME) values for various feedstuff through measuring energy utilization in the birds. The adaptation length of birds fed an experimental diet may affect the determined AME value due to the diet matrix and physiochemical properties of the feedstuff. Therefore, the objective of this thesis was to evaluate a select group of energy-containing feedstuff with different diets and with factors such as coccidia challenge and exogenous enzyme supplementation that may influence the determined AME values in broiler chickens.

KEYWORDS: apparent metabolizable energy, broiler chickens, coccidiosis, corn, oats, wheat

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CHAPTER 1 – LITERATURE REVIEW

Introduction

Feed accounts for around 60% of the costs associated with broiler production with the energy containing feed ingredients being the majority of those costs (Olukosi et al. 2017). Broilers are birds bred for quick growth and high-meat yield, therefore it is important for producers to provide adequate energy in the diets. The nutrient requirements for poultry provided by the Nutritional Research Council (NRC) is over 2-decades-old (published in 1994), while genetic advancements in the birds, and nutrient and energy composition of different feed ingredients, have changed rapidly over time. New data on energy retention can be utilized by the NRC for subsequent published editions, as well as by commercial poultry nutritionists.

Broiler chickens' nutrient recommendations for energy are generally based on metabolizable energy (ME) due to their exceptional anatomy. The feces and uric acid excreted by the birds are mixed in the cloaca, a single opening at the end of the digestive tract. There are methods for redirecting the ureter through surgical procedures but in large-scale studies this is not feasible. Digestible energy can be determined by collecting ileal digesta, however this requires the birds to be euthanized prior to collection and does not account for most of the microbial digestion occurring in the ceca.

Methods for Determining Metabolizable Energy Values of feed and feed ingredients in Broiler Chickens

Metabolizable energy is a tool for understanding the energy sequestered by an animal. The determination of ME is done simply by subtracting the energy found in the urine and feces (excreta in poultry) and subtracting that value from the gross energy (GE) found in the supplied diet (Figure 1.1). The formula is given as Metabolizability = $(GE_{input} – GE_{output} – GE_{urine}) / GE_{input}$ where GE is the gross energy of the diet (input) and the feces/excreta (output). This value is then multiplied by the GE of the diet to determine the ME (Kong and Adeola 2014). This gives the value of energy that has been utilized by the animal by removing the value of energy lost through feces and metabolized, then excreted through urinary processes. It is especially important to measure ME in birds, due to their somewhat unique anatomy. Birds have a single opening for reproduction and excretion of feces and urine. The result is a combination of feces and urine (in the form of uric acid), which cannot be separated. Surgery can be performed to separate the uric acid from the fecal excretions, but it is costly and comes with its own difficulties (Dixon and Wilkinson 1957).

Energy determination can be taken a step further by placing the animal in a metabolic chamber, which allows a researcher to measure energy losses in the form of heat and gas. This method is labor intensive, costly, and can limit the number of birds that can be used in the study (Kong and Adeola 2014), therefore apparent metabolizable energy (AME) is the most common measurement for energy utilization in poultry. The main methods used to determine AME of a diet are the index method and the total collection method. When determining the AME of a test ingredient, the direct or the indirect (difference) methods may be used (Adeola 2001).

The index method requires the use of an indigestible marker added to the diet. This method uses the proportion of marker in the diet with the proportion found in the excreta for a ratio to determine nutrient utilization. The energy utilization % can then be multiplied by the GE of the diet to determine the AME (Adeola 2001). The total collection method does not require the use of an indigestible marker, but it is a more labor-intensive procedure to determine nutrient and energy retention for AME. The benefit of this method is a better representation of the true value by a uniform measurement. The typical procedure starts with a clean tray for excreta and the initial weight of the bird's diet at a set length of time or by using the marker-to-marker method that uses indigestible markers, like chromic oxide, to provide an indication for when to start the collection. This method is not commonly used in poultry due to the marker's inability to be excreted with the uric acid (NRC 1994).

Over the years, AME values have been evaluated for different feedstuffs when fed to poultry. These values help determine the energy utilized by the birds for diet formulations. In Table 1.1, the AME and AMEn (if available) has been collected from various published resources. The AMEn values of feedstuffs obtained from the NRC's publication on poultry has been included as reference (NRC 1994).

Adaptation to Experimental Diets

In poultry research, there is very little information on the adequate adaptation length for feeding an experimental diet before sampling. Broiler chickens are commonly fed a basal diet for the first 14 d after hatching (Adeola and Ileleji 2009; Adebiyi and Olukosi 2015; Olukosi et al. 2017). The experimental diet is then fed from three to seven d before sampling (Adeola 2001). The physicochemical properties of a given feed may affect the rate at which nutrients may pass through the gastrointestinal tract, as well as its effect on the microbial population (i.e. higher levels of a particular nutrient allows for a bacterium to out compete another).

One study involving broiler chickens and turkeys of at least 11 d of age, and laying hens of at least 32 weeks of age, measured the difference in AMEn in birds fed for 10, 7, or 4 d on the experimental diet, of which the three experimental diets were either wheat-SBM-, wheat-corn-SBM-, or wheat-barley-SBM-based diets (Olukosi et al. 2017). In the broiler chickens, there was only a tendency to be different (P-value = 0.062) for AMEn (4 d: 2 916 kcal/kg; 7 d: 3 059 kcal/kg; 10 d: 3 155 kcal/kg), with a similar effect seen in turkeys (4 d: 3 059 kcal/kg; 7 d: 2 725 kcal/kg; 10 d: 3 035 kcal/kg). However, there were no significant differences in AMEn for the laying hens, although there appeared to be a factor of age playing a role in the adaptation period in which the less developed tracts in turkeys and broilers may have lower endogenous enzyme production (Olukosi et al. 2017). Because little is known on the optimal feeding time before the birds are adapted to the new diet, it is important to understand the factors that influence the digestion and absorption of nutrients, including the effect of the ingredients' physicochemical properties, before proceeding with sample collection.

Non-Starch Polysaccharides

Non-starch polysaccharides (NSP) are structural carbohydrate components found in the dietary fiber of plants along with starch, and depending on the ingredients, the contents of NSP may be a significant portion of the feedstuff (Choct 2015; Bederska-Lojewska et al. 2017). There are two categories that NSP can fall under, which are soluble and insoluble. The role of insoluble fiber is not well understood in poultry nutrition, however there is evidence that the insoluble NSP may have different physicochemical properties than soluble NSP, and provide benefits when supplied in the diet of poultry (Choct 2015). For example, there is some evidence that the addition of oat-hulls in a diet, when set at a level of 10%, may increase starch digestion (Hetland et al. 2003). Insoluble fiber is not well utilized by the bird's microbial population, so the addition acts as a diluent of the feed without negative impacts on the bird's performance (Hetland et al. 2004).

Carbohydrates (starch) make up a large portion of feedstuffs used in poultry nutrition. However, the composition of NSP, fiber, and lignin of various cereal grains used can have unintended consequences when fed. Those components are provided for commonly used cereal grains (NRC 1994) in Table 1.2, and less common cereal grains in Table 1.3, used in poultry production. The soluble portion of NSP has been attributed to the decreased nutrient digestion and absorption in poultry (Choct et al. 2010). Water-soluble NSP has been thoroughly studied for its antinutritive effects in broiler chickens (Annison 1991; Carre et al. 1995; Bedford et al. 1998; Yaghobfar and Kalantar 2017). There are two common forms of soluble NSPs found in broiler chicken feed ingredients that are associated with anti-nutritive effects, β-glucans and arabinoxylan (Knudsen 2014).

The levels of these NSPs differ between ingredients and even season (Bederska-Lojewska et al. 2017). However, corn tends to have the lowest levels of β-glucans and arabinoxylan, whereas ingredients like wheat and barley tend to be higher in arabinoxylan and rye and oats tend to be higher in β-glucans (Bederska-Lojewska et al. 2017). Rye and oats, therefore, would likely have more anti-nutritive impacts on the functionality of the bird's gut than other ingredients (Knudsen 2014; Bederska-Lojewska et al. 2017).

Animals do not possess endogenous enzymes that would enable them to digest NSP but ruminant species harbor microbes in the foregut that can synthesize these enzymes and allow the animals to utilize the nutrients from the fiber portion of a feedstuff. Hindgut fermenters are disadvantaged due to most of the microbial populations utilizing the NSP after the occurrence of most nutrient absorption (Boros et al. 1998). There is also a difference seen in digestibility of soluble versus insoluble NSP. A study measuring the digestibility of soluble and insoluble NSP found that adult broiler chickens were able to degrade water-soluble NSP (upwards of 80%) with the assistance of their microbial population, whereas the degradation of insoluble NSP was more limited (Carre et al. 1995).

When compared to corn, wheat contains higher levels of NSP that contribute to antinutritive effects, such as increased digesta viscosity, decreasing digestion and absorption of nutrients, and decreased performance as a consequence of lower ME (Zyla et al. 1999; Hashemipour et al. 2016). Lower weight gain has been observed in broiler chickens when fed diets with high levels of NSP (Mathlouthi et al. 2002; Yaghobfar and Kalantar 2017; Kermanshahi et al. 2018).

When male broilers were fed a corn-based diet or a wheat/barley-based diet from 4 to 20 d of age, the AMEn significantly decreased from 3 241 kcal/kg (corn-based) to 3 085 kcal/kg (wheat/barley-based). The soluble NSP content (arabinoxylans and β-glucans) were analyzed for the three test ingredients. The total soluble arabinoxylans and β-glucans in corn, wheat, and barley were 0.8, 7.6, and 27.3 g/kg on a dry matter basis, respectively (Mathlouthi et al. 2002). Decreased starch digestion does not appear to be the cause for the decreased ME seen in birds fed ingredients with high levels of NSP. A study measuring the relationship between AME and starch hydrolysis *in vivo* and *in vitro* determined that the differences in AME could not be attributed to the level of energy in the wheat or the variety. Meaning, there are other factors responsible for the energy metabolized by the birds, an example being the endosperm cell walls providing a physical barrier to the starch (Wiseman et al. 2000).

High levels of NSP in the diet has been shown to increase digesta viscosity in chickens (Antoniou and Marquardt 1983; Yaghobfar and Kalantar 2017). This is due to the water-soluble portion of the dietary fiber, which forms a gel known as hydrocolloid (Bederska-Lojewska et al. 2017). The increased viscosity can also lead to sticky droppings that have negative impacts in birds raised on bedded floors leading to increased pathogen growth and decreased animal welfare (Bederska-Lojewska et al. 2017). The intestinal environment created by soluble NSP favors the proliferation of anaerobic and Gram-negative bacteria, which results in the increased production of volatile fatty acids. There is also evidence that lactic acid-producing bacteria and *Bifido* bacteria numbers in the small intestine are decreased as a result of soluble NSP (Choct et al. 2010; Yaghobfar and Kalantar 2017), whereas insoluble NSP does not result in a decrease in the number of these bacterium (Kermanshahi et al. 2018).

There are also morphological changes that may occur from diets with high levels of NSP. Wheat- and barley-based diets have been shown to reduce the size of villi located in the small intestine of broiler chickens (Yaghobfar and Kalantar 2017). The role of villi in the intestine is to aid in the absorption of nutrients by increasing the surface area available for absorption (Peuhkuri et al. 2010).

Coccidiosis in Broiler Chickens

Avian coccidiosis is caused by a protozoan parasite from the genus *Eimeria* (Peek and Landman 2011; Quiroz-Castaneda and Dantan-Gonzalez 2015). There are nine known species that infect avian hosts, which include *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria maxima*, *Eimeria tenella*, *Eimeria mitis*, *Eimeria mivati*, *Eimeria hagani*, and *Eimeria praecox*. These parasites target the epithelial lining in various locations along the hindgut of the birds (Quiroz-Castaneda and Dantan-Gonzalez 2015), in turn the damage occurring from the *Eimeria* infection can lead to serious costs to the producers. The annual costs to poultry production were estimated to be over $2.2 billion on a global scale (Peek and Landman 2011).

The classical clinical signs in coccidia challenged birds are increased mortality and morbidity, as well as watery excreta with blood. The sub-clinical signs may be less apparent but can affect the birds' ability to efficiently perform. Birds may also be infected with lower parasitic numbers and not suffer any apparent adverse effects (Williams 1999). The individual pathogenicity and the locations they are typically found to infect has been detailed by Quiroz-Castaneda and Dantan-Gonzalez (2015) in Table 1.4.

The life cycle of coccidiosis begins with the ingestion of the *Eimeria* oocyst. Once ingested, the excystation of the oocyst begins and sporozoites are produced in the initial 24 h. The sporozoites enter the epithelial cells where they reproduce asexually, releasing merozoites. These develop into zygotes surrounded by a protective barrier, and subsequently, are excreted by the birds to infect another host through ingestion. The complete cycle has been diagramed by Shirley et al. (2005) as shown in Figure 1.2.

Birds challenged with coccidiosis have an immune response to the parasitic infection. Chickens lack lymph nodes but do possess a bursa of Fabricus and the thymus which are components of the birds' immune system (Umar et al. 2015). Part of the immune system's response to coccidia infection are secretions of cytokine proteins by cells intended for immune and inflammatory response to pathogens (Wigley and Kaiser 2003). T-cells have a major role in protective immunity with *Eimeria species*, while B-cells provide a more minor role (Blake et al. 2006). In Table 1.5, the different cytokines released by immune cells in the chicken are described. The levels of interleukin (IL)-1β, IL-6, which are pro-inflammatory cytokines, and TNF, a key regulator for immune response and inflammation, have been shown to be produced through *Eimeria* infection (Wigley and Kaiser 2003). Interleukin-10 mRNA was found to be up-regulated with the challenge of *E. tenella* infection, but when the chickens were administered the coccidiocidal drug Sulfachlorpyrazine, the levels of IL-10 were restored (Haritova and Stanilova 2012).

The infection of coccidiosis of the epithelial cells in the small intestine leads to lesions and shortening of the villi (Assis et al. 2010). This morphological change is partly responsible for reduced performance in chickens infected with *Eimeria* species. Another aspect is the immune response that occurs, which can lead to increased resources used for protection against the infection, such as the release of the cytokine proteins. Gene expression of various nutrient transporters may also be down-regulated, such as the amino acid transporter EAAT3, during the time of peak infection of *E. maxima*, whereas others may be unaffected (Fetterer et al. 2014). Regardless, multiple causes for the reduction in health and performance of birds challenged with coccidiosis act in unison, which may explain the losses that occur in poultry production.

Exogenous Enzyme Use in Poultry Production

Exogenous enzymes have been commonly supplemented in poultry diets to enhance nutrient and energy digestion that otherwise would not be available to the birds. Another facet of supplementation is to attempt to counteract the antinutritive effects seen in poultry from soluble NSP contents of plant-based feed ingredients. Enzymes catalyze the digestion of complex nutrients in the feed that are consumed by the animal prior to absorption. Although animals produce their own enzymes for digestion (i.e. endogenous enzymes), nutrients are not completely digested and absorbed due to the birds' innate inefficacies (Ravindran 2013). It is also the case that some necessary enzymes may not be produced by poultry (e.g. xylanase) and must be supplied through alternative means. The two main routes to acquire exogenous enzymes would be through supplementation to the diet or by microbes housed in the gastrointestinal tract. Ruminants are able to digest carbohydrates like cellulose by the microbial populations located in the foregut (most present in the rumen). This is not the case in poultry because the majority of the microbes are housed after the small intestine (i.e. ceca) where very little absorption of nutrients can occur. That is why supplementation of exogenous enzymes in poultry diets are commonly the topic in research and are used in commercial poultry production (Ravindran 2013). In poultry, the main classes of exogenous enzymes supplied to the animals through the diet are carbohydrases, phytases, and proteases, and as the names suggest, these enzymes catalyze carbohydrates, phytate, and proteins, respectively, into smaller complex or simple forms of the nutrients for absorption.

Carbohydrase

As discussed previously, feed ingredients such as wheat, barley, and rye contain high levels of soluble NSP. The antinutritive effects can lead to increased viscosity of digesta and decreased performance of the birds. Carbohydrases may be supplied in a diet to counteract these effects to improve performance. The major players in soluble NSP comes from arabinoxylan and β-glucan. Depending on the ingredient, the appropriate enzyme and levels in the feed may be different.

A study looking at the effects of exogenous enzyme supplementation of β-glucans in barley- or oats-based diets found that in barley-based diets there were no significant differences in performance at 14 and 35 d. However, in the oats-based diet there were significant increases in body weight and feed intake. The reason for this may be the average levels of soluble NSP of oats is higher than in barley. Further evidence of this would be that in the diets fed without exogenous enzyme supplementation, the oats-based diet resulted in significantly lower body weight gain and feed intake than the barley-based diet (Jozefiak et al. 2006).

In another study looking into the effects of different levels of xylanase supplemented to a rye-wheat-based diet found that only the diet supplemented with 200 mg enzyme/kg significantly increased body weight gain of the birds (Steenfeldt et al. 1998). The diets containing 100 mg enzyme/kg and 300 mg enzyme/kg were not different from the control, however in the 200 mg enzyme/kg and 300 mg enzyme/kg diets, the viscosity was significantly decreased, whereas the diet with 100 mg enzyme/kg was only numerically lower (Steenfeldt et al. 1998). The lowest and highest levels of exogenous enzyme supplementation may eventually show significant differences in body weight gain after supplementation over longer periods of time, as this study only measured body weight gain until three wk of age (Steenfeldt et al. 1998).

Pectinases are typically used in a multi-enzyme supplement in broilers, however pectinase may still have merit when supplemented alone. Broiler chickens from 4 to 18 d were fed a raw pea-based diet that was supplemented with pectinase. The birds had significantly higher body weight gain and feed intake when compared to the control diet (Igbasan and Guenter 1996). The carbohydrases may be effective in diets of various ingredients, but when combined there may or may not be an additive effect seen (Cowieson et al. 2006).

Phytase

Phytase is an important enzyme to poultry production. This enzyme catalyzes the removal of phytate-bound phosphorus (P), making more P available to the animal and reducing the waste and pollution of P in the environment (Munir and Maqsood 2012). Another factor is the reduced cost to the producer as a result of decreases in the amount of inorganic P that must be added to the diet. An estimated two-thirds of P in vegetable-based feed ingredients are phytic-bound (Woyengo and Nyachoti 2011). Phytase supplementation may also be linked to increased amino acid utilization by the release of protein-phytate complexes and an increase of energy acquisition (Selle et al. 2000).

Broilers supplemented with phytase in a wheat-casein-based diet had increased digestibility of lysine and threonine, and when the birds were fed a wheat-based diet there was an increase in AME retention (3 443 kcal/kg) compared to the basal diet (3 239 kcal/kg). However, no difference was seen in AME when the birds were fed a barley-based diet (Ravindran et al. 1999). The possible reason for this may be due to the higher levels and proportions of soluble NSP typically found in barley than found in wheat.

Protease

Proteases are supplied in the diet to catalyze the breakdown of proteins into an absorbable form. There is evidence that supplementing protease to the diet may in fact reduce the environmental impact of broiler production by reducing ammonia pollution through a decrease in N excretion (Leinonen and Williams 2015). Multiple classes of proteolytic enzymes are available and target various stages of protein digestion, such as polypeptides and dipeptides (Garcia-Carreon 1997). When protease was supplied to broiler chickens at 80 and 160 mg/kg in a corn-SBM-based diet for 1 to 42 d, daily gain and feed intake significantly increased (Yuan et al. 2015).

Conclusion

In broiler production, feed costs play a major role as it relates to profitability, as well as environmental sustainability of the industry, which may be reflected in the cost of poultry-based products for the consumers. Reducing costs through better understanding of how well poultry utilizes the nutrients and energy in feed is essential. The antinutritive effects seen in feed ingredients high in soluble NSP can impact the performance of the birds and can lead to an increase in the cost of production. In the same way, coccidiosis is also a major concern in poultry production as it relates to the health and wellbeing of the birds, as well as the costs associated in the profitability of poultry producers. Understanding the energy needs of the birds through research and the use of exogenous enzymes can provide insight into the various factors involved in how birds utilize ME, especially when challenged with a pathogen such as coccidiosis. Through research, appropriate methods to evaluate the energy utilization of a feedstuff may be necessary. When it comes to the adequate length of time an experimental diet must be fed for the birds to adapt, this area is under-researched in poultry. Future studies should evaluate (or revaluate) feedstuffs used in poultry production with a closer look at the effects on the digestion and absorption of nutrients and energy from soluble NSP and coccidiosis.

Table 1.1 Metabolizable energy values of various feedstuffs in poultry[1]

Ingredient	Type	Age	AME, kcal/kg	AMEn, kcal/kg	Source
Barley	Broiler	21 d	2 718	2 543	Saki et al. 2010
Barley	Broiler	21 d	3 059	3 035	Olukosi et al. 2017
Barley	Laying hen	33 wk	3 892	2 868	Olukosi et al. 2017
Barley	NR	NR	NR	2 640	NRC 1994
Canola meal	Broiler	21 d	1 793	1 778	D'Agostini et al. 2004
Canola meal	Broiler	21 d	2 005	1 801	Woyengo et al. 2010
Canola meal	NR	NR	NR	2 000	NRC 1994
Corn	Broiler	21 d	3 246	3 235	D'Agostini et al. 2004
Corn	Laying hen	33 wk	3 155	3 107	Olukosi et al. 2017
Corn	Broiler	48 d	3 650	3 611	Schneiders et al. 2017
Corn	Broiler breeder	52 wk	3 785	NR	Liu, et al. 2017
Corn	Broiler	8 d	3 443	3 220	Schneiders et al. 2017
Corn	NR	NR	NR	3 350	NRC 1994
Corn DDGS	Broiler	21 d	3 013	2 963	Adeola and Ileleji 2009
Corn DDGS	NR	NR	NR	2 480	NRC 1994
Corn, sweet	Broiler breeder	52 wk	3 997	NR	Liu, et al. 2017
Corn, waxy	Broiler breeder	52 wk	3 738	NR	Liu, et al. 2017
Oats	NR		NR	2 550	NRC 1994
Rye	Leghorn	21 d	NR	3 009	Marquardt et al. 1994
Rye	NR		NR	2 626	NRC 1994
SBM	Broiler	28 d	2 629	2 364	Schneiders et al. 2017
SBM	Broiler	48 d	2 442	2 278	Schneiders et al. 2017
SBM	Broiler breeder	52 wk	2 492	NR	Liu, et al. 2017
SBM	Broiler	8 d	2 679	2 203	Schneiders et al., 2017
SBM	NR	NR	NR	2 230	NRC 1994
SBM, dehulled	Broiler breeder	52 wk	2 580	NR	Liu, et al. 2017
SBM, dehulled	NR	NR	NR	2 440	NRC 1994

Table 1.1 (continued) Metabolizable energy values of various feedstuffs in poultry

Sorghum	Broiler	30 d	3 175	3 165	Generoso et al. 2008
Sorghum	Broiler	50 d	3 396	3 374	Generoso et al. 2008
Sorghum	NR	NR	NR	3 288	NRC 1994
Triticale	Broiler	32 d	2 242	2 134	Broch, et al. 2015
Triticale	Broiler	35 d	3 155	NR	Im et al. 1999
Triticale	NR	NR	NR	3 163	NRC 1994
Wheat	Broiler	21 d	2 757	2 577	Saki, et al. 2009
Wheat	Broiler	35 d	3 277	NR	Im et al. 1999
Wheat, hard red	NR	NR	NR	2 900	NRC 1994
Wheat bran	Broiler	28 d	1 944	1 867	Schneiders et al., 2017
Wheat bran	Broiler	48 d	2 433	2 273	Schneiders et al., 2017
Wheat bran	Broiler	8 d	1 980	1 827	Schneiders et al., 2017
Wheat bran	NR	NR	NR	1 300	NRC 1994
Wheat middlings	Broiler	35 d	2 698	NR	Im et al. 1999
Wheat middlings	NR	NR	NR	2 000	NRC 1994

[1]NR = not reported

Table 1.2 Non-starch polysaccharide, lignin, and fiber components of common poultry feedstuffs[1,2]

Ingredient	Corn				Wheat						Barley				Sorghum	
Type[3,4]	Grain		Flour		Grain		Flour		Bran		Grain		Flour		Whole grain	
NSP																
β-glucan	0.1		0.1		1.1		0.4		2.4		4.1		3.2		0.1	
Cellulose	2.0		0.0		1.8		0.3		7.0		4.0		1.2		1.4	
NCP	7.0	(1.2)[2]	2.1	(0.8)	9.5	(2.8)	3.2	(1.6)	29.2	(3.0)	14.6	(5.7)	9.0	(4.1)	4.0	(0.5)
Glucose	0.8	(0.2)	0.5	(<0.1)	1.2	(0.4)	0.5	(0.2)	3.4	(0.7)	5.0	(3.9)	3.9	(2.9)	0.8	(0)
AX	4.7	(0.5)	1.0	(0.5)	7.3	(1.8)	2.3	(1.0)	23.2	(1.8)	8.4	(1.2)	4.2	(0.7)	2.4	(0.2)
Arabinose	2.0	(0.3)	0.5	(0.3)	2.8	(0.8)	0.8	(0.3)	8.5	(0.7)	2.7	(0.5)	1.8	(0.3)	1.3	(0.1)
Xylose	2.7	(0.2)	0.5	(0.2)	4.5	(1.1)	1.5	(0.7)	14.7	(1.1)	5.6	(0.7)	2.4	(0.4)	1.1	(<0.1)
A/X	0.74	1.30	1.06	1.18	0.62	0.74	0.53	0.42	0.58	0.78	0.48	0.90	0.75	0.75	1.23	2.00
Total NSP	9.0		2.1		11.3		3.5		36.4		18.6		10.2		5.4	
Klason lignin	1.1		0.4		1.8		–		7.0		3.2		1.4		2.4	
Fiber	10.1		2.6		13.1		3.5		43.4		21.8		11.6		7.8	
Soluble NSP, %		11.8		31.2		21.7		44.3		7.3		26.1		40.2		11.4

[1]Table modified from (Knudsen 2014)

[2]Values in parenthesis are soluble components

[3]AX, arabinoxylan; A/X, arabinose/xylose ratio; NCP, noncellulosic polysaccharides; NSP, non-starch polysaccharides

[4]% of dry matter

Table 1.3 Non-starch polysaccharide, lignin, and fiber components of less common poultry feedstuffs[1,2]

Ingredient	Rye				Oats				Triticale	
Type[3,4]	Whole grain		Bran		Whole grain		Hull		Whole grain	
NSP										
β-glucan	1.7		4.5		2.8		1.4		0.7	
Cellulose	1.4		3.9		8.2		19.6		2.1	
NCP	13.3	(4.3)	38.4	(6.2)	15.0	(4.0)	30.8	(1.3)	10.9	(3.5)
Glucose	2.5	(0.6)	6.6	(1.3)	3.3	(2.8)	2.0	(0.8)	1.2	(0.5)
AX	9.5	(3.2)	29.2	(4.5)	9.7	(0.5)	24.0	(0.2)	8.5	(2.4)
Arabinose	3.6	(1.2)	7.8	(1.2)	1.8	(0.3)	2.8	(0.2)	3.5	(1.0)
Xylose	5.9	(2.0)	21.4	(3.3)	8.0	(0.2)	21.2	(−)	5.0	(1.4)
A/X	0.61	0.63	0.36	0.33	0.22	0.65	0.13	−	0.71	0.77
Total NSP	14.7		42.2		23.2		50.4		12.1	
Klason lignin	2.1		6.8		6.6		14.8		2.0	
Fiber	16.7		49.0		29.8		65.2		15.1	
Soluble NSP, %		25.6		12.8		13.3		2.0		22.7

[1]Modified from (Knudsen 2014)

[2]Values in parenthesis are soluble components

[3]AX, arabinoxylan; A/X, arabinose/xylose ratio; NCP, noncellulosic polysaccharides; NSP, non-starch polysaccharides

[4]% of dry matter

Table 1.4 Main characteristics of *Eimeria* species[1]

Species	Site of development	Pathogenicity	Gross lesions
E. praecox	Duodenum and jejunum	Least pathogenic	Watery intestinal contents Mucus and mucoid casts
E. hagani	Duodenum, jejunum, and ileum	Least Pathogenic	Petechiae and white opacities in the upper small intestine Intestinal content may be creamy or watery
E. acervulina	Duodenum and ileum	Less pathogenic	Limited enteritis causing fluid loss Malabsorption of nutrients
E. mitis	Ileum	Less pathogenic	Limited enteritis causing fluid loss Malabsorption of nutrients
E. mivati	Duodenum and rectum	Less pathogenic	Red petechiae and round white spots Severe denuding of the mucosa
E. maxima	Jejunum and ileum	Moderately-highly pathogenic	Inflammation of the intestinal wall with pinpointed hemorrhages Sloughing of epithelia
E. brunetti	Ceca and rectum	Highly pathogenic	Inflammation of the intestinal wall with pinpointed hemorrhages Sloughing of epithelia
E. tenella	Ceca	Highly pathogenic	Thickened cecal wall and bloody contents at the proximal end Distension of caecum Villi destruction causing extensive hemorrhage and death

Table 1.4 (continued) Main characteristics of *Eimeria* species

E. necatrix	Jejunum, ileum, and ceca	Highly pathogenic	Intestine may be ballooned Mucosa thickened and the lumen filled with fluid, blood and tissue debris Lesions in dead birds are observable as black and white plaques (salt and pepper appearance)

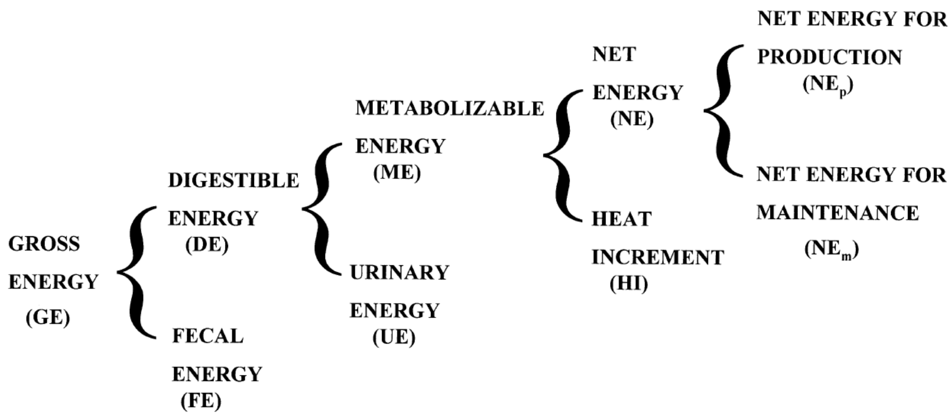
[1]Reproduced from Quiroz-Castaneda and Dantan-Gonzalez (2015)

Table 1.5 Classification and known cytokines in chickens[1]

Functional classification	Described chicken cytokines
Pro-inflammatory	IL-1b, IL-6, IL-8
Th1	IFN-γ, IL-2, IL-18
Th2	None described
Th3/Tr1	TGF-b
Others	IFN-a, IFN-b, IL-15, IL-16, MFG, SCF, chemokines

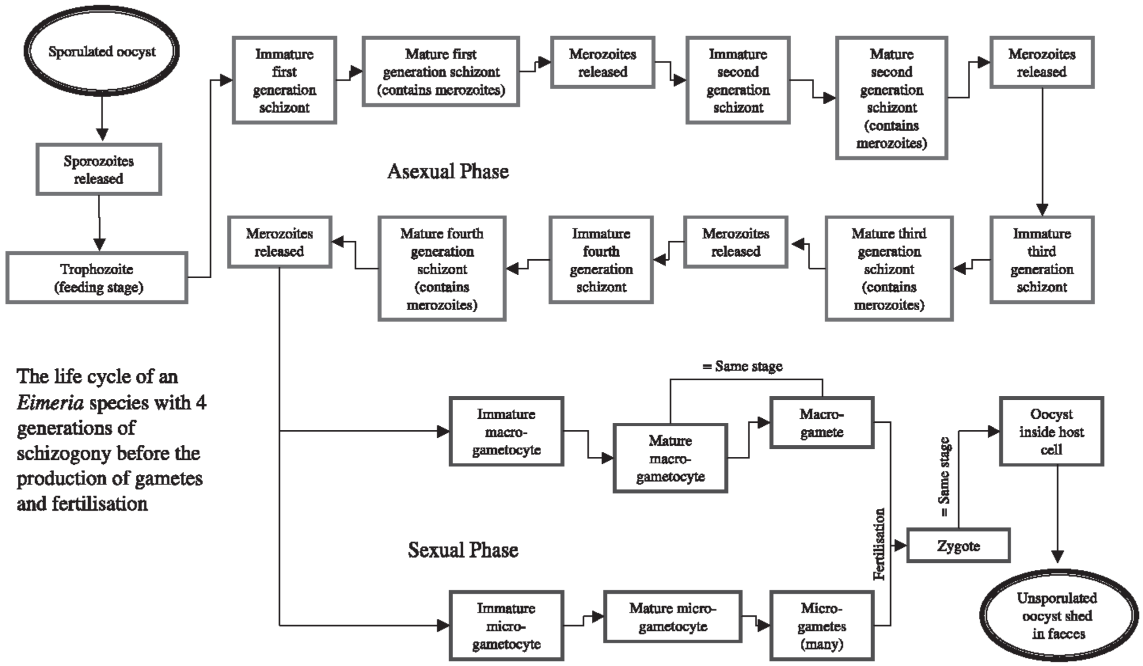
[1]Modified from Wigley and Kaiser (2003) with additional information from Umar et al. (2015)

Figure 1.1 Illustration of energy utilization[1]



[1]Reproduced from the Swine NRC (2012)

Figure 1.2 Diagram of the life cycle for *Eimeria*[1]



[1]Reproduced from Shirley et al. (2005)

CHAPTER 2 – METABOLIZABLE ENERGY VALUES OF CORN AND WHEAT MIDDLINGS IN BROILER CHICKENS[1]

Abstract

Two experiments were conducted to evaluate adaptation length (AL) and composition of reference diets on nitrogen-corrected apparent metabolizable energy (AMEn) in 22-day-old broilers. Birds were allocated to nine treatments (n=6) consisting of wheat-SBM (reference diet), corn-wheat-SBM, and wheat middlings-wheat-SBM (Exp. 1), or oats-SBM (reference diet), corn-oats-SBM, and wheat middlings-oats-SBM (Exp. 2) with three AL (12, 8, and 4 d) in a factorial arrangement of treatments (3 x 3). Dry matter, N, energy (En) utilization and AMEn of corn and wheat middlings were determined using the difference method. In Exp. 1, birds on the wheat middlings-wheat-SBM-based diet had the lowest ($P < 0.05$) dry matter, N, and En utilization, as well as AMEn compared to the other 2 diets. Additionally, AMEn for corn was higher ($P < 0.05$) compared to that of wheat middlings. In Exp. 2, N utilization in birds on the corn-oats-SBM-based diet was lower ($P < 0.05$) compared to birds on the oats-SBM-based diet, however AMEn of corn and wheat middlings were not different. In both experiments, AL was not significantly different. Based on these results, the composition of the reference diet could influence AMEn values of corn and wheat middlings in 22-d-old broilers.

[1]Andrew Dunaway and Sunday A Adedokun; Accepted for publication in the Canadian Journal of Animal Science

Introduction

The demand for animal protein, especially from poultry, continues to increase as a result of an increase in population growth and the demand for meat in developing countries. The increase in demand for animal protein has resulted in an increase in competition for feed ingredients. Corn and wheat are routinely used to supply En in poultry diets (Bourdillon et al. 1990; Amerah et al. 2008; Olukosi and Adeola 2010). The prevailing cost of these feed ingredients is a function of the demand and supply and could also be influenced by the cropping season.

Feed cost constitutes more than 60% of the total cost of poultry production (Olukosi et al. 2017) with significant portions of this cost associated with the cost of meeting the En needs of the birds (Mateos et al. 2007; Amerah et al. 2008; Kong and Adeola 2014; Berrocoso et al. 2017). The acceptability of a feed ingredient in meeting the bird's En need is determined by its metabolizable En value. The metabolizable En of different feed ingredients has been determined and reported (Sibbald and Price 1975; Sibbald 1976; Farrell 1978; Mollah et al. 1983). Most of the available information on feed ingredients were derived using the digestibility and utilization measurements using either the total collection or the index methods (Adeola 2001; Kong and Adeola 2014). In addition, the difference method has been employed in evaluating the metabolizable En of different feed ingredients (Adeola 2001; Olukosi and Adeola 2010; Olukosi et al. 2017). One of the advantages of this method is that it allows the birds to be fed the complete diet with minimal issues in palatability when ingredients with low palatability are being evaluated (Olukosi and Adeola 2010; Adebiyi and Olukosi 2015; Olukosi et al. 2017).

Corn and WM are uniquely different in their levels of non-starch polysaccharides (NSP) and fiber (Rosenfelder et al. 2013; Knudsen 2014), therefore it is important to investigate how these properties would influence how much En is utilized by the birds. In broilers, the NSP concentrations found in wheat has been observed to have an inverse correlation with the values of AME (Annison 1991). The NSP in these cereal grains can affect the viscosity of the digesta, causing anti-nutritive effects (Choct et al. 1996).

Oats tend to be a more soluble cereal due to the higher levels of β-glucan, which has been found to be more easily fermented by gut microflora (Knudsen et al. 1993; Knudsen 2014). This effect could cause an increase in digesta viscosity and proliferation of harmful bacteria leading to a reduction in En and nutrient utilization. In general, the composition of the feed ingredients supplying En in poultry diets would influence the degree to which the birds can effectively utilize the En coming from these feed ingredients (Theander et al. 1989; Jorgensen et al. 1996).

When transitioning from the basal diet to the test diet, there is an adaptation period required before excreta can be collected. Typically, the adaptation length (AL) for poultry diets are three to seven days (Kong and Adeola 2014). However, the optimal length of feeding an experimental diet is not well established (Olukosi et al. 2017) and may vary depending on the age of the birds and composition of the diet. The components of the diet (i.e. high soluble NSP) may affect the extent of En utilization, which is why it is important to investigate the optimal period broilers should be adapted to an experimental diet. Therefore, the objective of these experiments was to investigate the effect of AL and type of reference diets on AME and AMEn of corn and wheat middlings (WM) in 22-d-old broilers.

Materials and Methods

The management of the bird, experimental procedures, and sample collection for the two experiments followed the standard operating procedures for the animal facility as approved by University of Kentucky Animal Care and Use Committee. There were six birds/cage (0.61 x 0.51 x 0.36 m). Birds (male Cobb500 broiler chickens) were raised in battery cages in an environmentally controlled room with 20 h of light and 4 h of dark. All birds had unrestricted access to feed and water throughout the duration of the experiment.

Experimental Diets and General Bird Husbandry

Experiment 1. A total of 324-day-old male broiler chicks (Cobb500) were obtained from a local commercial hatchery and fed a standard corn-SBM based broiler starter diet that met or exceeded nutrient and En requirements (NRC 1994) for birds of this age. All birds were on the broiler starter diet for a minimum of 10 d after which the starter diet was replaced with the experimental diets (Table 2.1). Each of the diets contained five g/kg of titanium dioxide as an index marker. On day 10, all birds were weighed individually and randomized to cages in a completely randomized design with six birds/cage and six replicate cages/treatment. Experimental treatments were arranged as a 3 x 3 factorial with three AL and three diet type resulting in nine dietary treatments. The AL were for 4 (d 18 to 22), 8 (d 14 to 22), and 12 (d 10 to 22) d, whereas the main factor of diet types were wheat-soybean meal (WS; reference diet), corn-wheat-soybean meal (CWS), and wheat middlings-wheat-soybean meal (WWS). Thirty percent of the En yielding portion of the WS diet was replaced with corn or WM to produce the CWS and WWS diets, respectively. Excreta samples were collected on day 21 and 22 for AME and

AMEn determination. Because the AME and AMEn were calculated using the difference method as described by Adeola (2001), similar ratios of the En yielding components (wheat, soybean meal, and soy oil) of the diets were maintained across all the diets (within each experiment).

Excreta samples were dried in a forced-air oven at 55^{o} C. Each of the feed ingredients (oats, wheat, corn, and WM) were analyzed for proximate contents, as well as gross energy (GE) value (Table 2.2). Diets and dried excreta samples were pooled per cage and ground to pass through a 0.5 mm screen using a mill grinder (Wiley Mill Standard Model No. 3, Arthur H. Thomas Co., Philadelphia, USA). Both the diets and excreta samples were analyzed for titanium, dry matter (DM), GE, and N.

Experiment 2. A total of 324-day-old male Cobb500 broiler chicks from the same hatchery as Exp. 1 were used in Exp. 2. The care and treatments of the birds are as describe above for Exp. 1. Experimental treatments were arranged as a 3 x 3 factorial with three AL and three diet types resulting in nine dietary treatments. The AL were for 4 (d 18 to 22), 8 (d 14 to 22), and 12 (d 10 to 22) d, whereas the main factor of diet types were oats-soybean meal (OS; reference diet), corn-oats-soybean meal (COS), and wheat middlings-oats-soybean meal (WOS). Excreta samples were collected on day 21 and 22 for AME and AMEn determination. Collection, treatment, and processing of excreta samples and diets were as described for Exp. 1. Diets and excreta samples were analyzed for titanium, DM, GE, and N.

Chemical Analyses

The DM contents of the six diets and excreta samples were determined by drying the samples at 110 °C for 16 h (method 934.01; AOAC International, 2006). Nitrogen contents of the diets and excreta samples were determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2000; method 990.03), with EDTA as the internal standard. GE of the feed ingredients, diets, and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, parr instruments, Moline, IL, USA) with benzoic acid as a calibration standard. Titanium content of the diets and excreta were determined at University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). Titanium concentrations in diets were determined by flame atomic absorption spectroscopy after the samples were digested using concentrated sulfuric acid and processed as described by (Myers et al. 2004). The crude fat, crude fiber, ash, acid detergent fiber (ADF), and neutral detergent fiber (NDF) of wheat, oats, corn, WM, and soybean meal were determined at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Crude fat was determined by ether extraction (AOAC method 920.39, 2006). Crude fiber analysis content was determined using AOAC Method 978.10 (2006). ADF was determined using AOAC method 973.18 (A-D) (2006) whereas NDF was determined using an Ankom Fiber Analyzer (Ankom Technology, Macedon, NY) Ash contents of the feed ingredients were determined using AOAC Method 942.05 (2006).

Calculations and Statistical Analysis

All the calculations were done using the equations as described by (Olukosi et al. 2017). The coefficient of En and N retention was determined using the index method.

The equation used to calculate retention was En = 1 – [(Ti/To) X (Eo/Ei)]; where Ti is the initial concentration of the titanium marker in the feed, To is the concentration of the titanium marker in the excreta, Eo is the concentration of En or N in the excreta, and Ei is the concentration of En or N in the feed. Apparent metabolizable energy (kcal/kg) was calculated using the following equation AME = GE – [GEo X (Ti/To)] where GEi and GEo En are the GE (kcal/kg) value of the feed and excreta, respectively; Ti and To are the titanium concentrations in the diet and excreta, respectively. The coefficient of energy metabolizability (cME) of the test feed ingredients (corn and WM) were calculated using the indirect method after correcting for the non-En yielding portions of the diets (Olukosi and Adeola 2009). EMti = {EMtd – [EMrd X (1 – FCti/td)]}/FCti/td where EMti is the cEM of the test ingredient, EMtd is the cEM of the test diet, EMrd is the cEM of the reference diet, and FCti/td is the fractional contribution of the test ingredient to the test diet. The caloric value of 8.22 kcal/g was used to correct AME for N to give AMEn (Hill and Anderson 1958).

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc. Cary, NC, 2006). Diets' (Exp. 1: WS, CWS, and WWS; Exp. 2: OS, COS, and WOS) DM, N, En utilization, AME and AMEn were analyzed as a 3 x 3 (diet type x AL) factorial. The respective test feed ingredient (corn or WM), AME, AMEn, and cEM were analyzed as a 2 (corn or WM) x 3 (AL: 12, 8, or 4 d) factorial arrangement of treatments. Cage served as the experimental unit and number of replicates was six per treatment, except when otherwise stated. Outliers (data outside mean ± 3SD) were removed from the data prior to statistical analysis. Where necessary, mean separation was by Tukey's test and the level of significance was set at $P \leq 0.05$. Values for the main effects of diets and AL were reported when interaction was not significant. Both the main and simple effects of AME and AMEn were reported for the ingredients.

Results

The analyzed proximate composition of wheat, oats, corn, and WM used in these studies are reported in Table 2.2. Of all the four feed ingredients used for AME evaluation in this study, either as the reference (wheat and oats) or test feed ingredients (corn and WM), the highest GE was obtained in oats (4 212 kcal/kg, on as-fed basis) whereas wheat had the lowest GE (3 942 kcal/kg, on as-fed basis). Of all the four tested feed ingredients this study (excluding soybean meal), WM had the highest concentration of crude fiber (85.7 g/kg), ADF (123.7 g/kg), NDF (381.7 g/kg), and ash (56.4 g/kg) on an as-fed basis. Oats had the highest crude fat (47.9 g/kg), whereas wheat had the highest level of crude protein (160.0 g/kg) and corn had the lowest level of crude protein (71.2 g/kg) on an as-fed basis (Table 2.2).

Experiment 1

Total tract utilization of DM, N, and En, and AME and AMEn of the diets with WS as the reference diet are presented in Table 2.3. The interactions between diet type and AL were not significant. Birds on the WWS-based diet had the lowest ($P < 0.05$) DM, N, and En utilization, as well as AME and AMEn compared to birds on the WS and CWS-based diets (Table 2.3). The CWS-based diet had the highest ($P < 0.05$) DM, N, and En utilization compared to WS and WWS diets. There was no difference in AME and AMEn for the WS- and CWS-based diets.

The interaction between test feed ingredients (corn and WM) and AL for cEM, AME, and AMEn was not significant in the wheat-based diets (Table 2.4). The cEM (81.3 vs 44.8%), AME (3 671 vs. 2 044 kcal/kg), and AMEn (3 680 vs. 1 913 kcal/kg) values of corn were higher ($P < 0.05$) compared to that of the WM (Table 2.4).

Experiment 2

Total tract retention of DM, N, and En, as well as AME and AMEn of the OS-based diet as the reference diet are presented in Table 2.5. The interactions between diet type and AL were not significant. Total tract N utilization was lower ($P < 0.05$) in the COS-based diet compared to the OS-based diet, whereas there was no difference between OS- and WOS-based diets in N utilization. There was no difference ($P > 0.05$) in DM and En utilization, as well as AME and AMEn between the three diets (Table 2.5).

Similar to Exp. 1, interaction between test feed ingredients (corn and WM) and AL for cEM, AME, and AMEn was not significant in the oats-based diets (Table 2.6). The interaction between the test feed ingredients and AL for cEM, AME, and AMEn was not significant (Table 2.6).

Discussion

Accurately estimating the AME of a feed ingredient is important to poultry nutritionists, as this allows for the formulation of diets that closely meet the bird's requirements for En. Therefore, it is important to evaluate the AME values of feed ingredients. Because of the differences in the physicochemical composition for different sources of En in poultry diets, their interaction with other cereal grains, or alternative sources of En in the diets within the gastrointestinal tracts of the bird could influence the digestibility, as well as utilization of En in different feed ingredients. Secondly, today's feed ingredients and birds are different (improved) from what they were in the last few decades, hence, the need to re-evaluate the AME of diets and feed ingredients. Likewise, the length of time required for complete adaptation to these feed ingredients may be influenced by physicochemical properties of the different feed ingredients in the diet.

Different AL have been used when evaluating different feed ingredients in poultry. This ranges from 3 d (Steenfeldt et al. 1998), 7 d (Hew et al. 1998), 4 to 10 d (Olukosi et al. 2017) to 10 d (Cowieson and Ravindran 2008). Therefore, these experiments were conducted to determine whether different feed ingredients that supply En in broiler diets require different AL for accurate AME determination. In addition to the AL, we examined the effect of different reference diets on the AME of corn and WM. A good understanding on the magnitude of the influence that the reference diet has on a specific En yielding feed ingredient would be important for formulating a diet that adequately meets the bird's En needs.

In Exp. 1, where the reference diet was WS-based, none of the variables evaluated in this study increased with increasing AL to the diet. Likewise, there was no significant interaction between the AL and diet type. This is in line with what was reported by Olukosi et al. (2017) where, unlike in turkeys where AME was significantly influenced by the AL, the increasing AL did not result in significant changes in AME and AMEn values of the diets in the current study (Exp. 1). The high dietary fiber could also result in a decrease in N retention (Janssen and Carré 1989; Mateos et al. 2012; Olukosi et al. 2017) which was obvious in this study. High dietary fiber (as a result of WM substitution to the reference diet) also resulted in a significant decrease in DM and En retention. Unlike for the corn, WM substitution resulted in a depression in all the variables evaluated (e.g. AMEn: WWS 2 872 vs. CWS 3 417 kcal/kg). This decrease in DM, N, and En utilization, as well as AME and AMEn, with WM substitution could be explained in part from the perspective of crude fiber. The WM used in this study contained about 85.7 g/kg of crude fiber compared to corn (15.1 g/kg) with a higher proportion of ADF and NDF in the WM. Broiler chickens have limited ability to handle the soluble NSP due to the antinutritive effects, lack of the appropriate endogenous enzymes, and from the majority of the microbiome located after the small intestine, thus the relatively low En utilization and AME values seen may be a function of said diet (Hughes and Choct 1999).

In Exp. 2, replacing 30% of the reference diet with corn resulted in a significant decrease in N retention. This observation is difficult to explain; however, by replacing 30% of the energy yielding components of the reference diet resulted in similar proportion of oats and soybean meal being replaced. The combination of corn, oats, and soybean meal might have resulted in changes in the dynamics of digesta in terms of interaction, passage rate, and viscosity. Similar observation was seen when 30% of the reference diet was replaced with wheat middlings. When compared to Exp. 1 (WS-reference diet), the DM, En, AME, and AMEn values were higher than what was obtained for the same variable in Exp. 2 (OS-based reference diet). One of the reasons that could be responsible for this is that the crude fiber contents of oats used in this study was higher than that of the wheat. This relatively higher level of fiber, which is also high in NSP content (namely β-glucans), can increase digesta viscosity and have other anti-nutritive effects in the gut. Thus, there could be a reduction in the ability of the digestive enzymes to have access to the digesta with a resultant decrease in nutrients and En digestibility (Burnett 1966; Bedford 1995; Masey O'Neill et al. 2014).

The coefficient of metabolizability of corn (81.3%) and WM (44.8%) were different (Exp. 1). This could be attributed to the composition of the respective feed ingredients with corn having more starch (62.5 vs. 21.8%; NRC 2012) and less crude fiber (1.51 vs. 8.57%) compared to WM. The higher levels of NSP found in WM has the potential to increase digesta viscosity and lead to proliferation of gram-negative bacteria and a reduction of gram-positive bacteria that may lead to inflammation of the intestinal wall (Yaghobfar and Kalantar 2017). This could lead to a reduction in the interaction between the digestive enzymes and the digesta and less absorption of nutrients. Furthermore, the low content of starch in the WM meant less substrate for the digestive enzymes to work on, thus a lower AME value. This could partly explain the relatively low AME and AMEn values for the WM when compared to corn (AMEn: 1 913 vs. 3 680 kcal/kg).

Although no statistical comparison was made between the two studies, the cEM for corn and WM in Exp. 2 was numerically higher than the values obtained from Exp. 1 and were similar for both corn and WM (75 vs. 74%). Likewise, the AMEn for corn and WM were similar (3 216 vs. 3 194 kcal/kg). The amount of En that the birds in Exp. 2 were able to extract from WM when the reference diet was oats was numerically higher (3 194 vs. 1 913 kcal/kg). This observation could be explained in part by the fact that the level of NDF would be higher in the wheat and WM diet compared to the oats and WM diet. However, oats tend to be higher in soluble NSP than wheat, therefore there may be other factors that may have influenced the AMEn, such as increased viscosity, proliferation of gram-negative bacteria, and increased fermentation in the small intestine (Choct et al. 1996).

A numerical decrease in AMEn was observed at 8 d of adaptation. A similar pattern was reported by Olukosi et al. (2017) for barley in laying hens and turkeys. The cause of this slight depression in AME may be due to the relatively higher level of fiber in barley (Olukosi et al. 2017) and WM (current study) affecting the microbiota population in the hindgut, which are adapting to the change in diet and eventually adjusting to the higher level of fiber. The oats-based diet did not reflect this observation.

No conclusive evidence was seen that the main effect of the AL of 4, 8, or 12 d had a significant effect on the AME or the AMEn of the diets in both experiments (Exp 1 and 2). Although there was no statistical comparison made between the two studies, corn AMEn in Exp. 1 was 10% higher in the wheat-soybean meal-based reference diet. One limitation of these studies was that a direct statistical analysis between the two studies could not be done. Future studies in which both reference diets are used within the same study will provide more information regarding the effect of the composition of the reference diet on feed ingredient’s energy values, as well as an in-depth look into the role of NSP may be warranted.

Table 2.1 Ingredient composition and analyzed dry matter, gross energy, and crude protein values of the experimental diets

	Exp. 1			Exp. 2		
Ingredients, g/kg	Reference diet[1]	Corn	Wheat middlings	Reference diet[2]	Corn	Wheat middlings
Wheat	527.9	361.9	361.9	0	0	0
Oats	0	0	0	527.9	361.9	361.9
Soybean meal, 48%	378	259	259	378	259	259
Corn	0	300	0	0	300	0
Wheat middlings	0	0	300	0	0	300
Soy oil	47	32	32	47	32	32
L-lysine HCl	3.2	3.2	3.2	3.2	3.2	3.2
DL-Met	2.2	2.2	2.2	2.2	2.2	2.2
L-Threonine	1.3	1.3	1.3	1.3	1.3	1.3
Dicalcium phosphate	16.8	16.8	16.8	16.8	16.8	16.8
Salt	3.1	3.1	3.1	3.1	3.1	3.1
Limestone	13	13	13	13	13	13
Vitamin-mineral premix[3]	2.5	2.5	2.5	2.5	2.5	2.5
Titanium dioxide	5	5	5	5	5	5
Total	1 000	1 000	1 000	1 000	1 000	1 000
Analyzed nutrient and energy composition[4]						
Dry matter, g/kg	905.9	904.1	912.0	909.2	905.2	913.7
Gross energy, kcal/kg	4 225	4 107	4 149	4 307	4 174	4 225
Crude protein, (N x 6.25), g/kg	247.5	193.4	221.6	255.2	202.4	230.6

[1]Reference diet is wheat (hard red)-soybean meal-based

[2]Reference diet is oats-soybean meal-based

[3]Vitamin-mineral premix was formulated to supply the following at 2.5 grams per kilogram of diet: 11 025 IU of vitamin A; 3 528 IU of vitamin D; 33 IU of vitamin E; 0.91 mg of vitamin K; 2.21 mg of thiamin; 7.72 mg of riboflavin; 55 mg of niacin; 18 mg of pantothenate; 5 mg of vitamin B-6; 0.22 mg d-biotin; 1.10 mg of folic acid; 478 mg of choline; 0.03 of vitamin B-12; 75 mg of Zn; 40 mg of Fe; 64 mg of Mn; 10 mg of Cu; 1.85 mg of I; and 0.30 mg of Se

[4]Values are means of duplicate analyses

Table 2.2 Analyzed proximate composition of the major energy yielding feed ingredients contained in the experimental diets[1]

	Wheat	Oats	Corn	Wheat middlings	Soybean meal
Moisture	105.0	91.1	111.8	93.4	94.5
Crude protein (N x 6.25)	160.0	130.9	71.2	153.2	486.6
Crude fat	14.3	47.9	34.1	28.4	9.6
Crude fiber	25.4	20.8	15.1	85.7	36.2
Acid detergent fiber	41.1	28.4	29.5	123.7	69.6
Neutral detergent fiber	273.1	179.0	82.5	381.7	85.9
Ash	13.9	20.2	12.0	56.4	61.5
Gross energy, kcal/kg	3 942	4 212	4 023	4 145	4 338

[1]g/kg on as-is basis

Table 2.3 Main effect of diet type and adaptation length of total tract retention of dry matter, nitrogen, and energy and metabolizable energy values of diets containing different types of energy yielding feed ingredients fed to broilers for different adaptation length (Exp. 1)[1]

Diet Type[2]	AL, d	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
		Means for main effect of diet type				
Wheat-soybean meal		70.2b	62.4b	75.4b	3 518a	3 383a
Corn-wheat-soybean meal		73.7a	68.1a	77.2a	3 507a	3 417a
Wheat mid-wheat-soybean meal		61.4c	58.8c	66.1c	3 003b	2 872b
		Means for main effect of adaptation length				
	12	68.6	63.7	73.1	3 351	3 234
	8	68.3	62.4	72.7	3 338	3 217
	4	68.5	63.2	72.8	3 338	3 220
Pooled SEM[3]		0.22	0.48	0.22	9.90	10.81
		Probability				
Diet type		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
AL		0.561	0.180	0.447	0.585	0.527
Diet type x AL		0.106	0.059	0.123	0.126	0.094

[1]Number of replicates were 18

[2]DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = nitrogen-corrected apparent metabolizable energy; AL = adaptation length

[3]SEM = standard error of the mean

Table 2.4 Main and simple effects of reference diet and adaptation length on apparent energy metabolizability, metabolizable energy, and metabolizable energy corrected for nitrogen of corn and wheat middlings in broiler chickens[1]

			Exp. 1[2]	
Feed ingredient[3]	AL, d	cEM, %	AME, kcal/kg	AMEn, kcal/kg
			Mean for main effect of ingredient	
Corn		81.3	3 671	3 680
Wheat middlings		44.8	2 044	1 913
			Mean for main effect of AL	
	12	63.7	2 892	2 842
	8	62.4	2 845	2 772
	4	62.7	2 835	2 776
			Simple effect of means	
Corn	12	80.8	3 656	3 672
	8	82.3	3 723	3 732
	4	80.3	3 632	3 636
Wheat middlings	12	46.6	2 129	2 013
	8	43.0	1 966	1 813
	4	44.6	2 037	1 915
Standard deviation		2.36	107.44	114.82
			Probability	
Ingredient		< 0.001	< 0.001	< 0.001
AL		0.482	0.479	0.352
Ingredient x AL		0.075	0.075	0.052

[1]Reference diet: Wheat-soybean meal-based (Exp. 1)

[2]Number of replicate was 5 for simple effects, excluding d 12 WM and d 8 corn where the number of replicates were 4

[3]cEM = coefficient of energy metabolizability; AME = apparent metabolizable energy; AMEn = nitrogen corrected AME; AL = adaptation length

Table 2.5 Main effect of diet type and adaptation length of total tract retention of dry matter, nitrogen, and energy and metabolizable energy values of diets containing different types of energy yielding feed ingredients fed to broilers for different adaptation length (Exp. 2)[1]

Diet Type[2]	AL, d	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
				Means for main effect of diet type		
Oats-soybean meal		64.4	63.0a	67.6	3 198	3 062
Corn-oats-soybean meal		67.2	54.6b	70.7	3 239	3 106
Wheat mid-oats-soybean meal		65.4	57.5ab	69.7	3 219	3 078
				Means for main effect of AL		
	12	67.2	59.1	70.6	3 291	3 157
	8	64.4	55.9	68.2	3 163	3 017
	4	65.5	60.1	69.1	3 202	3 072
Pooled SEM[3]		1.05	2.18	0.99	45.01	50.17
				Probability		
Diet type		0.167	0.029	0.084	0.812	0.817
AL		0.181	0.379	0.234	0.130	0.151
Diet type x AL		0.650	0.544	0.742	0.874	0.858

[1]Number of replicates were 18

[2]DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = nitrogen-corrected apparent metabolizable energy; AL = adaptation length

[3]SEM = standard error of the mean

Table 2.6 Main and simple effects of reference diet and adaptation length on apparent energy metabolizability, metabolizable energy, and metabolizable energy corrected for nitrogen of corn and wheat middlings in broiler chickens[1]

		Exp. 2[2]		
Feed ingredient[3]	AL, d	cEM, %	AME, kcal/kg	AMEn, kcal/kg
		Mean for main effect of ingredient		
Corn		74.6	3 373	3 216
Wheat middlings		74.0	3 381	3 194
		Mean for main effect of AL		
	12	73.4	3 337	3 181
	8	75.7	3 440	3 250
	4	73.9	3 355	3 184
		Simple effect of means		
Corn	12	68.9	3 120	2 946
	8	78.8	3 528	3 407
	4	76.8	3 472	3 294
Wheat middlings	12	77.8	3 554	3 417
	8	73.4	3 351	3 093
	4	70.9	3 237	3 073
Standard deviation		11.95	480.81	541.17
		Probability		
Ingredient		0.881	0.968	0.917
AL		0.881	0.887	0.954
Ingredient x AL		0.249	0.252	0.238

[1]Reference diet: Oats-soybean meal-based (Exp. 2)

[2]Number of replicate was 5 for simple effects, excluding d 8 WM where the number of replicate was 4

[3]cEM = coefficient of energy metabolizability; AME = apparent metabolizable energy; AMEn = nitrogen corrected AME; AL = adaptation length

CHAPTER 3 – THE EFFECT OF DIET TYPE, COCCIDIA VACCINE CHALLENGE, AND EXOGENOUS ENZYME SUPPLEMENTATION ON PERFORMANCE AND APPARENT METABOLIZABLE ENERGY IN BROILER CHICKENS 7 AND 14 DAYS POST CHALLENGE

Abstract

Coccidiosis contributes to excessive global costs to the poultry industry through increased mortality and decreased performance of the birds. The purpose of this study was to examine the effect of exogenous mixed-enzyme supplementation (xylanase, β-glucanase, and pectinase) to a corn-SBM (CS) and a wheat-CS-based (WCS) diet in birds challenged with coccidia vaccine (Coccivac B-52™). On day 14, a total of 448 (n=7) Cobb500 male broilers were placed in a completely randomized design with a 2x2x2 factorial arrangement of treatments. The treatments consisted of two diets (CS or WCS), two levels of enzyme (0 or 10%), and two levels of coccidian vaccine challenge (CVC, 0 or 20x). Apparent metabolizable energy corrected for nitrogen (AMEn) of excreta was determined using the total collection method for the diets and the difference method for individual ingredients (2 x 2) on days 21 (eight birds/cage) and 28 (four birds/cage). Individual bird and feed weights were recorded on days 14, 21, and 28 for determination of performance, and viscosity was determined using jejunal digesta (two birds/cage). Feed intake (FI) of birds from day 14 to 21 had a significant three-way interaction showing that FI decreased ($P < 0.05$) with CVC in most cases. On days 14 to 21, CVC reduced ($P < 0.05$) body weight gain (BWG), FI, and feed efficiency (FE). However, the interaction between diet and CVC for BWG and FE of the CVC birds fed the WCS diet was higher ($P < 0.05$) than the non-CVC birds on days 21 to 28. On day 21, there were

significant interactions seen in AMEn between diet, CVC, and enzyme supplementation with a decrease in CVC birds. Viscosity was higher (P < 0.05) in WCS but decreased (P < 0.05) with the addition of enzymes, whereas viscosity decreased (P < 0.05) with CVC (day 21). By day 28, viscosity was higher (P < 0.05) in birds fed the WCS diet but decreased (P < 0.05) with enzyme supplementation. The AMEn of wheat on day 21 was significantly lower in CVC birds, whereas there was no difference on day 28. This study showed that CVC birds have decreased performance and AMEn seven d post challenge but were able to compensate for the losses in performance and regain similar levels of AMEn in a CS-or CWS-based diet, without the aid of exogenous enzymes.

Introduction

Over the last decade, broiler meat production has increased by 600 million pounds, and in 2018 the total amount was over 4.5 billion pounds (USDA 2019). Due to the demand for broiler-meat production, the amount of feed needed for production will continue to increase. Feed costs account for more than 60% of the costs involved in poultry production (Olukosi et al. 2017). The majority of feed costs come from the energy-containing ingredients. Because of this, it is important to have access to updated energy values of various feed ingredients used in poultry feed to better meet the requirements of the birds and reduce feed wastage through overfeeding.

In addition to feed costs, infection from coccidiosis has had major impacts on commercial poultry production. Broiler chickens are affected by the *Eimeria* family of parasitic protozoan pathogen, which can increase mortality and morbidity in the birds with the clinical form of infection. In both the clinical and sub-clinical forms of coccidiosis, birds may show reduced performance, such as reduced feed intake and body weight gain. They may also show reduced nutrient and energy retention, leading to reduced apparent metabolizable energy (AME) from the diet. In both cases, there are major economic losses, in which the annual global costs to poultry production has been estimated to be over $2.2 billion (Peek and Landman 2011).

Soluble non-starch polysaccharides (NSP) are found in plant-based feed ingredients and are known to possess antinutritive effects, such as increased digesta viscosity, decreased performance, reduced AME retention, reduced villi size in the small intestine, and sticky droppings (Antoniou and Marquardt 1983; Zyla et al. 1999; Mathlouthi et al. 2002; Assis et al. 2010; Bederska-Lojewska et al. 2017; Yaghobfar and Kalantar 2017; Kermanshahi et al. 2018). Corn and wheat are two common energy-containing feed ingredients used in broiler production. Wheat tends to have higher levels of soluble NSP and may negatively affect the bird's ability to utilize nutrients and energy in the diets. Carbohydrase enzymes, specifically NSPase, may be supplemented to the diets to counteract some of the antinutritive effects of NSP. There is evidence that soluble NSP can increase gram-negative bacteria (i.e. *E. coli*) and decrease gram-positive bacteria (i.e. lactic acid-producing bacteria), but by reducing the viscosity through enzyme supplementation it may promote an environment less suited for gram-negative bacterial proliferation (Yaghobfar and Kalantar 2017).

The fact that coccidia vaccine challenge (CVC) and soluble NSP can both impact the birds' ability to sequester energy, thereby reducing the AME retention value of the diet or ingredient. Exogenous enzyme supplementation may improve the nutrient and energy utilization of the diet. Thus, the objective of this study was to compare the effect of feed ingredient types, coccidia vaccine challenge, and exogenous enzyme supplementation in broiler chickens 7- (day 21; peak-CVC) and 14- (day 28; recovery phase) d post-CVC.

Materials and Methods

The management of the bird, experimental procedures, and sample collections for the experiment followed the standard operating procedures for the animal facility as approved by University of Kentucky Animal Care and Use Committee.

Birds and Diets

A total of 448 male Cobb500 broilers were used in this study. On day zero, birds were individually tagged and fed a standard corn-SBM-based starter diet that met or exceeded nutrient and energy requirements from day 0 to 14. Birds were raised in battery cages in an environmentally controlled room with 20 h of light and 4 h of dark. All birds had unrestricted access to feed and water throughout the duration of the experiment. Birds were individually weighed and randomized to treatments on day 14 in a completely randomized design. Four birds/cage were sampled on days 21 and 28, where between days 14 and 21 there were eight birds/cage and days 21 to 28 there were four birds/cage. All birds were weighed prior to sampling on day 21 and the two heaviest and two lightest birds were selected for sampling. Experimental treatments were arranged in a 2 x 2 x 2 factorial for a total of eight treatments and seven replicates/treatment. The reference diet used was a corn-SBM-based diet (CS) in which 30% of the energy yielding portion of the diet (corn, SMB, and soy oil) was replaced with wheat to produce the wheat-corn-SBM-based diet (WCS). The exogenous enzyme containing diets were produced by supplementing with a multi-carbohydrase enzyme added to both the CS and WCS diets.

Ronozyme® WX2 (xylanase) was added at 0.1 g/kg of feed and Ronozyme® VP (glucanase + pectinase) was added at the rate of 0.25 g/kg of feed per the manufacturer's recommendation (DSM, Parsippany, NJ). Birds in the non-CVC treatments were orally gavaged on day 14 with 0.6 ml of distilled water, whereas CVC birds were orally gavaged with 0.6 ml mixture of distilled water and Coccivac®-B52 containing live *Eimeria* occysts (*E. acervulina*, *E. maxima*, *E. mivati*, and *E. tenella*.). The product bulletin has been included in Figure 3.1 (Merck Animal Health). This dose is the equivalent of 20x of what is normally given to broiler chicks on day of hatch.

The total collection method was used to determine energy and nitrogen retention, as well as the AME and AME corrected for nitrogen (AMEn). Seventy-two h before each sampling on day 21 and 28, the excreta collection trays were cleaned, the feed was removed from the feeders, and the feed was weighed at 0 and 72 h. On days 19, 20, 21, and 26, 27, 28 excreta was quantitatively collected and weighed each morning at the same time before storing at −20° C prior to drying in a forced-air oven at 55° C for six days. Dried excreta samples were weighed and pooled by cage. Dried excreta samples, ingredients (corn, wheat, and SBM), and diets were ground to pass through a 0.5 mm screen using a mill grinder (Wiley Mill Standard Model No. 3, Arthur H. Thomas Co., Philadelphia, USA).

Diets and excreta samples were analyzed for dry matter (DM), GE, and N. The DM contents of the samples were determined by drying the samples at 110° C for 16 h (method 934.01; AOAC International, 2006). Nitrogen contents of the diets and samples were determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2000; method 990.03), with EDTA as the internal standard. The GE of the feed ingredients, diets, and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, parr instruments, Moline, IL, USA) with benzoic acid as a calibration standard. Feed ingredients were sent to the University of Missouri for proximate composition value determination as shown in Table 3.1.

Performance

The measured performance parameters were body weight (BW), BW gain (BWG), and feed intake (FI). The weight of the birds and feed were recorded on days 14, 21, and 28 to calculate BWG and FI, which were then used to calculate the feed efficiency (FE).

Histological Analysis

The middle portions of the duodenum, jejunum, and ileum were taken on day 21 for histological analysis. These segments were selected due to the locational specificity in the small intestine of the mixed *Eimeria* species. Samples were processed (stained with haematoxylin and eosin) at the University of Kentucky's Animal Diagnostics Lab (ADL). Villi height and crypt depth were measured at 10x (upright clinical microscope, Model Eclipse Ci-E, Nikon Corporation, Tokyo, Japan) for calculating the villi to crypt depth ratio (VHCD).

Viscosity

Jejunal digesta was taken from the two heaviest birds/cage on days 21 and 28, to have adequate sample quantities, and stored at −20° C prior to determination of the digesta viscosity. Approximately 2 g of thawed digesta were centrifuged (11 500 g for 15 min at 20° C) and the viscosity was determined on 0.5 ml of supernatant using an A&D Company, Limited SV-1A Model viscometer at 40° C (body temperature of chickens).

Chemical Analysis

The DM contents of the two diets, feed ingredients, and excreta samples were determined by drying the samples at 110° C for 16 h (method 934.01; AOAC International, 2006). Nitrogen contents of the diets and excreta samples were determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2000; method 990.03), with EDTA as the internal standard. GE of the feed ingredients, diets, and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr instruments, Moline, IL, USA) with benzoic acid as a calibration standard. The moisture, crude fat, crude fiber, and ash of corn, wheat, and soybean meal were determined at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Crude fat was determined by ether extraction (AOAC method 920.39, 2006). Crude fiber analysis content was determined using AOAC Method 978.10 (2006). Ash contents of the feed ingredients were determined using AOAC Method 942.05 (2006).

Calculations and Statistical Analysis

The coefficient of energy and N retention was determined using the equation: Retention (%) = $[(C_{input} - C_{output})/ C_{input}] \times 100$ where C is the component being measured (i.e. energy and N). Apparent metabolizable energy was calculated using the following equation: AME = (GE × cEM) where GE is the gross energy of the diet and cEM is the coefficient of energy metabolizability (cEM). The cEM of the test feed ingredient (wheat) was calculated using the indirect method after correcting for the non-energy yielding portions of the diets (Olukosi and Adeola 2009). $EMti = EMtd – [EMrd \times (1 – FCti/td)] / FCti/td$ where EMti is the cEM of the test ingredient, EMtd is the cEM of the test diet, EMrd is the cEM of the reference diet, and FCti/td is the fractional contribution of the test ingredient to the test diet. The caloric value of 8.22 kcal/g was used to correct AME for N to give AMEn (Hill and Anderson 1958).

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc. Cary, NC, 2006). The DM, N, energy utilization, AME, and AMEn of the diets were analyzed as a 2 (CS or WCS) x 2 (non-CVC or CVC) x 2 (with or without exogenous enzyme supplementation) factorial arrangement of treatments. The respective test feed ingredient's (wheat) AME, AMEn, and cEM were analyzed as a 2 (non-CVC or CVC) x 2 (with or without exogenous enzyme supplementation) factorial arrangement of treatments. Cage served as the experimental unit, except for jejunal viscosity (two birds/cage) and for histology (one bird/cage), and number of replicates was seven/treatment, unless otherwise stated. Outliers (data outside mean ± 3SD) were removed from the data prior to statistical analysis. Where necessary, mean separation was by Tukey's test and the level of significance was set at $P \leq 0.05$. All values for the main effects and simple effects of diet type, CVC, and exogenous enzyme supplementation are reported regardless of statistical significance.

Results

The analyzed (enzyme analyses were done by DSM) levels of the individual enzyme activities in the control diets were not greater than 5.0 FBG/kg for glucanase while xylanase level was below the detection limit. The level of glucanase (from

Ronozyme® VP) was 18.4 FBG/kg while the level of xylanase (from Ronozyme® WX2) was 259 FXU/kg for the corn-SBM-based diet. The corresponding level for glucanase and xylanase in the wheat-corn-SBM-based diet were 24.5 FBG/kg and 311 FXU/kg, respectively.

Performance

The 21 d BW of the birds fed the WCS diet were lower ($P < 0.05$) compared to the birds fed CS. Additionally, CVC birds had lower ($P < 0.05$) BW than the non-CVC birds, whereas the birds on diets supplemented with exogenous enzymes showed no difference from birds not supplemented. The birds' performance in BWG and FE from 14 to 21 d followed similar trends as the 21 d BW. There was three-way interaction ($P < 0.05$) in the 14 to 21 d FI of the birds in which FI decreased ($P < 0.05$) by CVC in most cases (Table 3.3).

There was a three-way interaction ($P < 0.05$) for the birds' 28 d BW with non-CVC birds that were fed the CS diet which was significantly higher than all treatments with the exception of non-CVC birds fed the CS diet with enzyme supplementation. A two-way interaction for BWG between diet and CVC showed that non-CVC birds fed the WCS diet was lower ($P < 0.05$) than the non-CVC birds fed the CS diet. However, there was no difference between CS and WCS diets in the CVC birds. The two-way interaction for FE between diet and CVC showed no difference between the CS diets in non-CVC and CVC birds. Non-CVC birds fed WCS diet was significantly the lowest in FE. No significant differences were seen in FI from 21 to 28 d (Table 3.4).

Nutrient and Energy Retention

Significant three-way interactions were seen in N and energy retention on day 21. The CVC birds had the lowest ($P < 0.05$) N retention with the exception of the CS birds supplemented with enzymes. Energy retention was lower ($P < 0.05$) in the CVC birds regardless of diet type or enzyme supplementation. The main effect of diet showed that the DM retention was lower ($P < 0.05$) for birds fed the CS diet, and for the main effect of CVC, the CVC birds were lower ($P < 0.05$) than non-CVC DM retention. The main effect of enzyme was not significant for day 21 (Table 3.5). On day 28, N retention was significantly lower for the main effect of diet in the birds fed WCS. All other measured nutrient retention values were non-significant for day 28 (Table 3.6).

AME Contents of Diets and Wheat

There were significant three-way interactions seen in AME and AMEn on day 21. In both AME and AMEn, the CVC birds had lower values when compared to non-CVC, regardless of diet and enzyme supplementation (Table 3.5). By day 28, no significant differences were seen in AME and AMEn for all treatments (Table 3.6).

The main effect of CVC for the test ingredient (wheat) AMEn on day 21 was around 21% lower ($P < 0.05$) in the CVC birds (CVC AMEn: 3 296.6 kcal/kg; non-CVC AMEn; 2 609.6 kcal/kg). There was no difference in the main effect of enzyme for birds supplemented with enzyme (AMEn: 2 951.2 kcal/kg vs 2 953.8 kcal/kg) when compared to birds not supplemented with enzymes (Table 3.7). On day 28, no differences were seen in AMEn for the main effects of CVC or enzyme (Table 3.8).

Viscosity and Ileal Histology

Multiple two-way interactions were seen for jejunal digesta viscosity on day 21. Interaction between diet and CVC showed non-CVC birds fed WCS had the highest ($P < 0.05$) viscosity, whereas viscosity of CVC birds fed CS was the lowest ($P < 0.05$). No difference was seen in non-CVC birds fed CS and CVC birds fed WCS. The interaction between diet and enzyme showed birds fed WCS without enzyme supplementation had the highest ($P < 0.05$) jejunal digesta viscosity, whereas there was no difference between the other three treatments. The interaction between CVC and enzyme showed that non-CVC birds without enzyme supplementation had the highest ($P < 0.05$) viscosity, whereas CVC birds with enzyme supplementation had the lowest ($P < 0.05$) viscosity (Table 3.9).

On day 28, a significant two-way interaction between diet and enzyme was seen for jejunal digesta viscosity. Birds fed the WCS diet without enzyme supplementation had the highest ($P < 0.05$) viscosity with no differences seen between the other treatments. The main effect of CVC for jejunal viscosity was again significantly lower in the CVC birds (Table 3.10).

The ileum villi height was lower ($P < 0.05$) in CVC birds, whereas the crypt depth was higher ($P < 0.05$). No other differences were observed by diet or enzyme supplementation (Table 3.11). Significant two-way interaction between CVC and enzyme for ileal VHCD was seen on day 21. Regardless of enzyme supplementation, CVC birds had the lowest ($P < 0.05$) VHCD in the ileum, whereas non-CVC birds supplemented with enzymes had the highest ($P < 0.05$) VHCD (Table 3.11).

Discussion

The demand for chicken protein will continue to grow and the need for updated and accurate AMEn of feed ingredient values will be necessary for poultry producers. While coccidiosis infection still plagues poultry producers with increased bird mortality and decreased performance, determining the nutrient and energy retention of different feed ingredients in coccidia challenged birds can further our understanding of how individual feed ingredients may affect the birds' ability to perform. Energy-containing feed ingredients fed to broiler chickens can have different inherent properties in each ingredient. There are obvious differences in nutrient and energy values, however there are also physicochemical properties that may change how the birds utilize the nutrients and energy provided by the diet. Wheat contains higher levels of soluble NSP than corn, which has been shown to decrease AMEn and have other antinutritive effects along with other common feed ingredients (e.g. barley, rye, and triticale) used in poultry production (Amerah 2015; Bederska-Lojewska et al. 2017). Enzyme supplementation has been shown to reduce some of the antinutritive effects from soluble NSP (Mathlouthi et al. 2002; Munyaka et al. 2016), which may improve the birds' ability to utilize ingredients high in soluble NSP. Through the various ways AMEn can be reduced or improved, a deeper look into individual ingredients could prove beneficial to the costs associated when feeding broiler chickens.

The measured performance parameters used in this study were BW, BWG, FI, and FE. Both the main effects of diet and CVC significantly decreased the 21 d BW, BWG, and FE (14 to 21 d) in birds fed the WCS diet and CVC birds. The decreased performance from WCS may partially be explained by the higher levels of soluble NSP found in wheat, whereas the effect of CVC to the birds were as expected. In the three-way interaction of FI, non-CVC birds fed WCS had higher FI than the CVC birds fed CS with no difference with exogenous enzyme supplementation.

Day 28 performance showed a three-way interaction for BW with non-CVC birds fed CS generally had higher BW than the WCS and CVC birds. BWG and FE showed two-way interaction between diet and CVC. In the CVC birds fed WCS, the BWG was not different than those fed CS but was significantly higher than non-CVC birds fed WCS. This is an indication that the addition of wheat may be providing some benefit in the CVC birds. One study found that wheat bran derived arabinoxylan provided a stimulatory effect on the birds' immune system (Akhtar et al. 2012), whereas another study could not connect the increase of viscosity directly to a decrease in fecal oocyst output (Banfield et al. 2002). In a similar way, FE of CVC birds fed WCS was significantly higher than non-CVC birds also on WCS, although both WCS fed birds were lower than both CS fed birds. In all cases, FI was not different on day 28.

During peak of CVC infection (day 21), the three-way interaction of N retention was significantly lower in CVC birds, although the CVC birds fed CS with exogenous enzyme supplementation was not different from the non-CVC birds fed WCS. This observation for CVC birds is an expected result of the challenge and follows in line with the energy retention on day 21. The determined energy retention, AME, and AMEn values were all significantly lower in each CVC treatment, meaning that the CVC was negatively affecting the birds' ability to obtain energy from the diets which is reflected in their performance from 14 to 21 d. By day 28, the birds determined energy retention, AME, and AMEn values were no longer different by CVC, therefore the pathogenicity of the coccidia infection had decreased. The only significant difference in nutrient retention was between the N of CS and WCS where N retention decreased in the WCS diet (CS: 70.4%; WCS: 67.1%).

The determined values of wheat through the difference method on day 21 for CVC birds led to a ~20% decrease in AME (3 379.8 kcal/kg vs 2 718.3 kcal/kg) and AMEn (3 296.6 kcal/kg vs 2 609.6 kcal/kg) when compared to non-CVC birds. This observation confirms the effect CVC has on the birds' absorptive capabilities by the infection of the epithelial lining of the small intestine. There were no differences in the AME and AMEn of wheat by day 28, similarly to the diets. The addition of exogenous enzymes to the diet did not improve the AME and AMEn values, although diets were not deficient in energy. The AME and AMEn of wheat determined in the non-CVC birds without exogenous enzyme supplementation was 3 368.9 kcal/kg and 3 290.4 kcal/kg, respectively. The same treatment group on day 28 were similar for AME and AMEn with a slight increase of around 60 kcal/kg. The NRC's Nutrient Requirement of Poultry states that the AMEn of hard red wheat is 2 900 kcal/kg (NRC 1994). The determined AMEn values for 21 and 28 d are around 400 kcal/kg than what is reported by the NRC, however a study using birds of similar age determined the AMEn of wheat to be 3 372 kcal/kg using the regression method (Bolarinwa and Adeola 2012).

There were multiple two-way interactions for jejunal viscosity on day 21. The interaction between diet and CVC for non-CVC birds fed CS was not different from CVC birds fed WCS. This observation may partially explain the improved performance seen in the day 28 birds when fed WCS without exogenous enzyme. Interaction between diet and enzyme showed that the supplementation of exogenous enzyme significantly lowered the viscosity when added to WCS but no difference when CS, which was observed to be the case at day 28 as well. This is evidence for the efficacy of the Ronozyme® enzyme premix in reducing viscosity in diets high in NSP. In the interaction between CVC and enzyme, the addition of exogenous enzyme significantly lowered viscosity in both non-CVC and CVC birds, although there was no evidence of benefits to performance. There was evidence of lingering effects on performance of day 28 CVC birds, but none was observed for AME and AMEn. The day 28 main effect of CVC on jejunal viscosity was significantly lower in the CVC birds, which might explain some of the delays in recovering from CVC seen in performance.

The CVC birds were observed to have changes to their villi and crypt depth in the ileum on day 21. It is clear that the *Eimeria* infection led to damage of the villi leading to deceased surface area for absorption. The two *Eimeria* species that target the ileum of chickens contained in the Coccivac®-B52 used in this study are *E. acervulina* and *E. maxima*, with *E. maxima* being the most pathogenic of the two (Quiroz-Castaneda and Dantan-Gonzalez 2015). Therefore, nutrient transport (i.e. protein) may be reduced as a result of the infection in the ileum. The 21 d ileal VHCD showed two-way interaction between CVC and enzyme. In CVC birds there was no difference between birds supplemented with exogenous enzymes and those that were not. In the cases of the non-CVC birds however, the ratio was improved with the addition of exogenous enzymes, which would suggest that the enzymes have increased the ileum's surface area for absorption in the small intestine. Despite this observation, performance parameters, nutrient and energy retention, AME, and AMEn did not significantly increase with the increased absorptive capabilities in the ileum during the two wk period of this study.

In most cases, the birds met the expectations of this study. The coccidia infection clearly affected the birds' nutrient and energy retention, AME, and AMEn, and hindered their performance, but 14 d post-CVC the birds recovered and made up the difference in performance in most cases. The addition of wheat reduced birds' performance at both 21 and 28 d, likely from the antinutritive effects of soluble NSP and was confirmed in the increased jejunal viscosity. The supplementation of glucanase, xylanase, and pectinase did not provide evidence of improving the health of the CVC birds, and may have decreased the performance of birds fed WCS. The AME and AMEn value of wheat during CVC and with, or without, exogenous enzyme supplementation was successfully determined in this study. However, evidence suggests that a CS-based diet may be better suited for CVC birds than a WCS-based diet. Future studies may look into long-term effects of CVC on performance when birds are fed a WCS-based diet without mixed carbohydrase enzyme supplementation.

Table 3.1 Ingredient composition and analyzed dry matter, gross energy, and crude protein values of the experimental diets[1]

	Without enzymes		With enzymes	
Ingredients, g/kg (as-fed)	CS	WCS	CS	WCS
Corn	639.6	438.6	619.6	418.6
Soybean meal, 48% CP	285.0	195.5	285.0	195.5
Soy oil	30.0	20.5	30.0	20.5
Wheat (hard red)	0.0	300.0	0.0	300.0
Dicalcium phosphate	17.6	17.6	17.6	17.6
Limestone	10.5	10.5	10.5	10.5
Vitamin-mineral premix[2]	2.5	2.5	2.5	2.5
Salt	4.1	4.1	4.1	4.1
DL-methionine	3.0	3.0	3.0	3.0
L-lysine HCl	2.0	2.0	2.0	2.0
L-threonine	0.7	0.7	0.7	0.7
Titanium dioxide	5.0	5.0	5.0	5.0
Ronozyme® WX2 premix[3]	0.0	0.0	10.0	10.0
Ronozyme® VP premix[4]	0.0	0.0	10.0	10.0
Total	1 000	1 000	1 000	1 000
Analyzed nutrient and energy composition				
Gross energy, kcal/kg	4 021.0	3 909.1	4 011.5	3 927.6
Dry matter, g/kg	895.0	888.0	895.0	891.0
Crude protein (N × 6.25), g/kg	196.9	173.1	197.5	176.3
Calcium, g/kg	9.7	10.2	9	8.2
Phosphorus, g/kg	7.2	7.2	6.9	6.5

[1]CS = corn-SBM; WCS = wheat-corn-SBM

[2]Vitamin-mineral premix was formulated to supply the following at 2.5 g per kilogram of diet: 11 025 IU of vitamin A; 3 528 IU of vitamin D; 33 IU of vitamin E; 0.91 mg of vitamin K; 2.21 mg of thiamin; 7.72 mg of riboflavin; 55 mg of niacin; 18 mg of pantothenate; 5 mg of vitamin B-6; 0.22 mg d-biotin; 1.10 mg of folic acid; 478 mg of choline; 0.03 of vitamin B-12; 75 mg of Zn; 40 mg of Fe; 64 mg of Mn; 10 mg of Cu; 1.85 mg of I; and 0.30 mg of Se

[3]Added to the diet at the rate of 0.1 g/kg

[4]Added to the diet at the rate of 0.25 g/kg

Table 3.2 Analyzed proximate composition of the major energy yielding feed ingredients contained in the experimental diets (on as-is basis)

Component, g/kg	Corn	Wheat	Soybean meal
Moisture	116.9	120.6	91.7
Gross energy, kcal/kg	3 890.3	3 873.3	4 223.7
Crude protein (N × 6.25)	75.5	138.6	480.1
Crude fat	22.2	6.0	11.7
Crude fiber	16.5	21.8	31.2
Ash	13.3	16.1	62.2

Table 3.3 Main and simple effects of performance (day 21)[1,2]

			Day 21[3]	Day 14 to 21		
Diet	CVC	Enzyme	BW, g	BWG, g/bird	FI, g/bird	FE, g/kg
				Means for main effect of diet		
Corn-SBM			777.9	383.9	559.2	685.1
Wheat-corn-SBM			746.9	354.6	589.6	599.6
				Means for main effect of CVC		
	-		804.2	411.0	597.5	689.3
	+		720.6	327.5	551.2	595.4
				Means for main effect of enzyme		
		-	757.3	366.4	573.2	639.0
		+	767.5	372.1	575.5	645.7
Standard deviation			25.5	17.7	20.8	26.4
				Diet x CVC		
Corn-SBM	-		820.0	425.9	582.5	732.1
Wheat-corn-SBM	-		788.4	396.1	612.6	646.5
Corn-SBM	+		735.9	341.8	535.9	638.2
Wheat-corn-SBM	+		705.3	313.1	566.6	552.7
				Diet x enzyme		
Corn-SBM		-	768.9	380.7	554.6	684.6
Corn-SBM		+	745.6	352.1	591.8	593.4
Wheat-corn-SBM		-	786.9	387.0	563.7	685.7
Wheat-corn-SBM		+	748.1	357.1	587.3	605.7
				CVC x enzyme		
	-	-	800.2	407.3	597.8	682.3
	-	+	808.2	414.7	597.3	696.3
	+	-	714.3	325.6	548.7	595.7
	+	+	726.8	329.4	553.8	595.1

Table 3.3 (continued) Main and simple effects of performance (day 21)

			Diet x CVC x enzyme			
Corn-SBM	-	-	818.0	423.9	587.9abc	721.7
Corn-SBM	-	+	822.0	428.0	577.0bcd	742.6
Wheat-corn-SBM	-	-	782.4	390.7	607.6ab	643.0
Wheat-corn-SBM	-	+	794.4	401.4	617.6a	650.0
Corn-SBM	+	-	719.9	337.5	521.3e	647.6
Corn-SBM	+	+	751.8	346.1	550.4de	628.8
Wheat-corn-SBM	+	-	720.0	317.7	576.1bcd	545.9
Wheat-corn-SBM	+	+	701.8	312.7	557.1cd	561.4
			Probability			
Diet			<0.0001	<0.0001	<0.0001	<0.0001
CVC			<0.0001	<0.0001	<0.0001	<0.0001
Enzyme			0.143	0.244	0.682	0.356
Diet x CVC			0.940	0.906	0.960	0.990
Diet x enzyme			0.264	0.884	0.232	0.437
Enzyme x CVC			0.744	0.712	0.624	0.312
Diet x CVC x enzyme			0.095	0.402	0.004	0.085

[1]n for main effects: CS = 28, WCS = 27, CVC- = 28, CVC+ = 27, Enzyme- = 27, Enzyme+ = 28
[2]CVC = coccidia vaccine challenge; BW = body weight; BWG = body weight gain; FI = feed intake; FE = feed efficiency
[3]Body weight is the average of all 8 birds in cage

Table 3.4 Main and simple effects of performance (day 28)[1,2]

			Day 21[3]	Day 28	Day 21 to 28		
Diet	CVC	Enzyme	BW, g	BW, g	BWG, g/bird	FI, g/bird	FE, g/kg
					Means for main effect of diet		
Corn-SBM			780.9	1 452.9	671.9	924.5	727.1
Wheat-corn-SBM			746.9	1 368.0	621.1	942.4	659.0
					Means for main effect of CVC		
	-		810.7	1 448.9	638.0	932.9	684.2
	+		717.0	1 372.0	655.0	934.0	701.9
					Means for main effect of enzyme		
		-	759.2	1 407.5	648.3	931.6	696.5
		+	768.5	1 413.4	644.7	935.3	689.5
Standard deviation			27.7	46.6	31.0	43.9	17.4
					Diet x CVC		
Corn-SBM	-		829.9	1 507.6a	677.4a	933.1	726.1a
Wheat-corn-SBM	-		791.6	1 390.3bc	598.7c	932.6	642.2c
Corn-SBM	+		731.8	1 398.3b	666.4ab	915.9	728.0a
Wheat-corn-SBM	+		702.2	1 345.8c	643.5b	952.2	675.7b
					Diet x enzyme		
Corn-SBM		-	772.3	1 507.6	677.4	916.0	730.6
Corn-SBM		+	746.2	1 390.3	598.7	947.1	662.4
Wheat-corn-SBM		-	789.4	1 398.3	666.4	932.9	723.5
Wheat-corn-SBM		+	747.6	1 345.8	643.5	937.7	655.5
					CVC x enzyme		
	-	-	808.7	1 507.6	677.4	939.0	687.4
	-	+	812.8	1 390.3	598.7	926.7	680.9
	+	-	709.8	1 398.3	666.4	924.1	705.6
	+	+	724.3	1 345.8	643.5	943.9	698.2

Table 3.4 (continued) Main and simple effects of performance (day 28)

					Diet x CVC x enzyme		
Corn-SBM	-	-	830.6	1 518.4a	687.8	943.4	729.4
Corn-SBM	-	+	829.2	1 496.7ab	667.0	922.7	722.8
Wheat-corn-SBM	-	-	786.8	1 390.0cd	603.2	934.6	645.5
Wheat-corn-SBM	-	+	796.4	1 390.5cd	594.1	930.7	639.0
Corn-SBM	+	-	714.0	1 363.8cd	649.9	888.6	731.7
Corn-SBM	+	+	750.0	1 432.7bc	683.0	943.1	724.3
Wheat-corn-SBM	+	-	705.5	1 357.7cd	646.9	959.1	674.2
Wheat-corn-SBM	+	+	699.0	1 333.8d	634.9	944.8	672.0
					Probability		
Diet			<0.0001	<0.0001	<0.0001	0.137	<0.0001
CVC			<0.0001	<0.0001	0.049	0.921	<0.001
Enzyme			0.218	0.640	0.674	0.753	0.144
Diet x CVC			0.563	0.013	0.002	0.127	0.002
Diet x enzyme			0.299	0.167	0.254	0.274	0.993
Enzyme x CVC			0.485	0.195	0.179	0.182	0.930
Diet x CVC x enzyme			0.080	0.027	0.070	0.076	0.992

[1]n for main effects: CS = 28, WCS = 27, CVC- = 28, CVC+ = 27, Enzyme- = 27, Enzyme+ = 28

[2]CVC = coccidia vaccine challenge; BW = body weight; BWG = body weight gain; FI = feed intake; FE = feed efficiency

[3]Body weight is the average of the four remaining birds in cage

Table 3.5 Main and simple effects of diet type, coccidia vaccine challenge, and exogenous enzyme supplementation on nutrient and energy retention, apparent metabolizable energy, and apparent metabolizable energy corrected for nitrogen (day 21)[1,2]

Diet	CVC	Enzyme	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
					Means for main effect of diet		
Corn-SBM			67.7	66.4	69.2	3 105.7	3 009.0
Wheat-corn-SBM			69.2	64.0	70.0	3 081.2	2 988.3
					Means for main effect of CVC		
	-		73.8	69.3	76.2	3 387.5	3 303.4
	+		63.1	61.1	63.0	2 799.4	2 694.0
					Means for main effect of enzyme		
		-	68.2	64.9	69.4	3 089.9	2 994.9
		+	68.7	65.5	69.7	3 097.0	3 002.4
Standard deviation			2.4	2.4	2.1	93.1	97.4
					Diet x CVC		
Corn-SBM	-		73.0	70.2	75.9	3 404.3	3 317.6
Wheat-corn-SBM	-		74.6	68.4	76.5	3 370.6	3 289.1
Corn-SBM	+		62.4	62.6	62.6	2 807.1	2 700.4
Wheat-corn-SBM	+		63.8	59.7	63.4	2 791.8	2 687.6
					Diet x enzyme		
Corn-SBM		-	67.2	65.5	68.6	3 085.0	2 986.5
Corn-SBM		+	69.2	64.4	70.2	3 094.8	3 003.4
Wheat-corn-SBM		-	68.2	67.3	69.8	3 126.4	3 031.5
Wheat-corn-SBM		+	69.2	63.7	69.7	3 067.5	2 973.3
					CVC x enzyme		
	-	-	73.7	69.6	76.2	3 390.1	3 306.9
	-	+	73.9	69.0	76.2	3 384.9	3 299.8
	+	-	62.6	60.2	62.7	2 789.8	2 682.9

Table 3.5 (continued) Main and simple effects of diet type, coccidia vaccine challenge, and exogenous enzyme supplementation on nutrient and energy retention, apparent metabolizable energy, and apparent metabolizable energy corrected for nitrogen (day 21)

	+	+	63.5	62.0	63.3	2 809.0	2 705.0
					Diet x CVC x enzyme		
Corn-SBM	-	-	73.2	70.8a	76.0a	3 418.0a	3 332.6a
Corn-SBM	-	+	72.8	69.7a	75.7a	3 390.6a	3 302.6a
Wheat-corn-SBM	-	-	74.2	68.5ab	76.3a	3 362.1a	3 281.2a
Wheat-corn-SBM	-	+	75.0	68.4ab	76.8a	3 379.1a	3 297.0a
Corn-SBM	+	-	61.2	60.2c	61.2b	2 752.1b	2 640.3b
Corn-SBM	+	+	63.6	64.9b	63.9b	2 862.2b	2 760.4b
Wheat-corn-SBM	+	-	64.1	60.2c	64.2b	2 827.6b	2 725.5b
Wheat-corn-SBM	+	+	63.4	59.1c	62.6b	2 755.9b	2 649.6b
					Probability		
Diet			0.035	0.001	0.197	0.349	0.450
CVC			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Enzyme			0.441	0.377	0.593	0.788	0.784
Diet x CVC			0.898	0.404	0.655	0.725	0.773
Diet x enzyme			0.306	0.064	0.087	0.192	0.173
Enzyme x CVC			0.905	0.067	0.929	0.640	0.592
Diet x CVC x enzyme			0.072	0.010	0.021	0.035	0.031

[1]n for main effects – DM: CS = 25, WCS = 26, CVC- = 26, CVC+ = 25, Enzyme- = 25, Enzyme+ = 26; N: CS = 27, WCS = 27, CVC- = 28, CVC+ = 26, Enzyme- = 27, Enzyme+ = 27; En/AME/AMEn: CS = 25, WCS = 27, CVC- = 27, CVC+ = 25, Enzyme- = 25, Enzyme+ = 27;

[2]CVC = coccidia vaccine challenge; DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = apparent metabolizable energy corrected for nitrogen

Table 3.6 Main and simple effects of diet type, coccidia vaccine challenge, and exogenous enzyme supplementation on nutrient and energy retention, apparent metabolizable energy, and apparent metabolizable energy corrected for nitrogen (day 28)[1,2]

Diet	CVC	Enzyme	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
					Means for main effect of diet		
Corn-SBM			73.7	70.4	76.9	3 450.7	3 364.9
Wheat-corn-SBM			73.6	67.1	77.1	3 392.8	3 307.9
					Means for main effect of CVC		
	-		74.1	69.0	77.5	3 447.1	3 362.5
	+		73.3	68.5	76.4	3 396.5	3 310.3
					Means for main effect of enzyme		
		-	73.2	67.7	76.3	3 394.1	3 306.2
		+	74.2	69.8	77.7	3 449.5	3 366.6
Standard deviation			3.4	3.4	2.9	128.0	138.0
					Diet x CVC		
Corn-SBM	-		73.0	70.2	75.9	3 404.3	3 317.6
Wheat-corn-SBM	-		74.6	68.4	76.5	3 370.6	3 289.1
Corn-SBM	+		62.4	62.6	62.6	2 807.1	2 700.4
Wheat-corn-SBM	+		63.8	59.7	63.4	2 791.8	2 687.6
					Diet x enzyme		
Corn-SBM		-	67.2	65.5	68.6	3 085.0	2 986.5
Corn-SBM		+	69.2	64.4	70.2	3 094.8	3 003.4
Wheat-corn-SBM		-	68.2	67.3	69.8	3 126.4	3 031.5
Wheat-corn-SBM		+	69.2	63.7	69.7	3 067.5	2 973.3
					CVC x enzyme		
	-	-	73.7	69.6	76.2	3 390.1	3 306.9
	-	+	73.9	69.0	76.2	3 384.9	3 299.8
	+	-	62.6	60.2	62.7	2 789.8	2 682.9

Table 3.6 Main and simple effects of diet type, coccidia vaccine challenge, and exogenous enzyme supplementation on nutrient and energy retention, apparent metabolizable energy, and apparent metabolizable energy corrected for nitrogen (day 28)

	+	+	63.5	62.0	63.3	2 809.0	2 705.0
					Diet x CVC x enzyme		
Corn-SBM	-	-	74.3	71.2	77.4	3 481.4	3 398.0
Corn-SBM	-	+	73.9	70.8	77.7	3 479.7	3 394.8
Wheat-corn-SBM	-	-	73.9	66.9	77.3	3 406.8	3 322.0
Wheat-corn-SBM	-	+	74.2	67.1	77.7	3 420.7	3 335.3
Corn-SBM	+	-	72.2	67.9	75.0	3 369.9	3 277.0
Corn-SBM	+	+	74.5	71.7	77.5	3 472.0	3 390.0
Wheat-corn-SBM	+	-	72.4	64.9	75.3	3 318.1	3 228.0
Wheat-corn-SBM	+	+	74.1	69.5	77.8	3 425.8	3 346.4
					Probability		
Diet			0.931	0.004	0.848	0.111	0.144
CVC			0.423	0.630	0.164	0.162	0.181
Enzyme			0.303	0.064	0.082	0.127	0.123
Diet x CVC			0.898	0.404	0.655	0.725	0.773
Diet x enzyme			0.306	0.064	0.087	0.192	0.173
Enzyme x CVC			0.905	0.067	0.929	0.640	0.592
Diet x CVC x enzyme			0.748	0.975	0.954	0.944	0.943

[1]n for main effects – DM: CS = 25, WCS = 26, CVC- = 26, CVC+ = 25, Enzyme- = 25, Enzyme+ = 26; N: CS = 26, WCS = 26, CVC- = 27, CVC+ = 25, Enzyme- = 26, Enzyme+ = 26; En/AME/AMEn: CS = 26, WCS = 26, CVC- = 27, CVC+ = 25, Enzyme- = 26, Enzyme+ = 26

[2]CVC = coccidia vaccine challenge; DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = apparent metabolizable energy corrected for nitrogen

Table 3.7 Main and simple effects of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen of the test ingredient (wheat; day 21)[1,2]

CVC	Enzyme	AME, kcal/kg	AMEn, kcal/kg
		Means for main effect of CVC	
-		3 379.8	3 296.6
+		2 718.3	2 609.6
		Means for main effect of enzyme	
	-	3 063.4	2 972.5
	+	3 034.7	2 933.8
Standard deviation		100.0	105.0
		CVC x enzyme	
-	-	3 368.9	3 290.4
-	+	3 390.6	3 302.9
+	-	2 757.9	2 654.5
+	+	2 678.7	2 564.6
		Probability	
CVC		<0.0001	<0.0001
Enzyme		0.465	0.349
Diet x enzyme		0.204	0.219

[1]n for main effects – AME: CVC- = 14, CVC+ = 13, Enzyme- = 13, Enzyme+ = 14; AMEn: CVC- = 14, CVC+ = 12, Enzyme- = 13, Enzyme+ = 13
[2]CVC = coccidia vaccine challenge; AME = apparent metabolizable energy; AMEn = apparent metabolizable energy corrected for nitrogen

Table 3.8 Main and simple effects of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen of the test ingredient (wheat; day 28)[1,2]

CVC	Enzyme	AME, kcal/kg	AMEn, kcal/kg
		Means for main effect of CVC	
-		3 460.2	3 370.3
+		3 391.7	3 302.7
		Means for main effect of enzyme	
	-	3 365.7	3 277.2
	+	3 486.1	3 395.8
Standard deviation		261.3	281.7
		CVC x enzyme	
-	-	3 437.5	3 353.3
-	+	3 482.8	3 387.2
+	-	3 294.0	3 201.0
+	+	3 489.3	3 404.4
		Probability	
CVC		0.512	0.548
Enzyme		0.254	0.296
Diet x enzyme		0.473	0.453

[1]n for main effects – AME: CVC- = 14, CVC+ = 12, Enzyme- = 13, Enzyme+ = 13; AMEn: CVC- = 14, CVC+ = 12, Enzyme- = 13, Enzyme+ = 13

[2]CVC = coccidia vaccine challenge; AME = apparent metabolizable energy; AMEn = apparent metabolizable energy corrected for nitrogen

Table 3.9 Main and simple effects of jejunal viscosity (day 21)[1,2]

Diet	CVC	Enzyme	Viscosity, cP
			Means for main effect of diet
Corn-SBM			2.49
Wheat-corn-SBM			2.85
			Means for main effect of CVC
	-		2.87
	+		2.47
			Means for main effect of enzyme
		-	2.83
		+	2.50
Standard deviation			0.17
			Diet x CVC
Corn-SBM	-		2.64b
Wheat-corn-SBM	-		3.09a
Corn-SBM	+		2.34c
Wheat-corn-SBM	+		2.60b
			Diet x enzyme
Corn-SBM		-	2.55b
Corn-SBM		+	2.43b
Wheat-corn-SBM		-	3.12a
Wheat-corn-SBM		+	2.57b
			CVC x enzyme
	-	-	3.11a
	-	+	2.63b
	+	-	2.56b
	+	+	2.38c
			Diet x CVC x enzyme

Corn-SBM	-	-	2.75
Corn-SBM	-	+	2.54
Wheat-corn-SBM	-	-	3.46
Wheat-corn-SBM	-	+	2.72
Corn-SBM	+	-	2.34
Corn-SBM	+	+	2.33
Wheat-corn-SBM	+	-	2.77
Wheat-corn-SBM	+	+	2.43
			Probability
Diet			<0.0001
CVC			<0.0001
Enzyme			<0.0001
Diet x CVC			0.042
Diet x enzyme			<0.0001
Enzyme x CVC			0.002
Diet x CVC x enzyme			0.265

[1]n for main effects: CS = 28, WCS = 27, CVC- = 28, CVC+ = 27, Enzyme- = 27, Enzyme+ = 28;
[2]CVC = coccidia vaccine challenge

Table 3.10 Main and simple effects of jejunal viscosity (day 28)[1,2]

Diet	CVC	Enzyme	Viscosity, cP
			Means for main effect of diet
Corn-SBM			2.75
Wheat-Corn-SBM			3.04
			Means for main effect of CVC
	-		2.98
	+		2.81
			Means for main effect of enzyme
		-	3.01
		+	2.78
Standard deviation			0.17
			Diet x CVC
Corn-SBM	-		2.80
Wheat-corn-SBM	-		3.16
Corn-SBM	+		2.70
Wheat-corn-SBM	+		2.92
			Diet x enzyme
Corn-SBM		-	2.72b
Corn-SBM		+	2.78b
Wheat-corn-SBM		-	3.30a
Wheat-corn-SBM		+	2.78b
			CVC x enzyme
	-	-	3.10
	-	+	2.86
	+	-	2.93
	+	+	2.70
			Diet x CVC x enzyme

Corn-SBM	-	-	2.78
Corn-SBM	-	+	2.82
Wheat-corn-SBM	-	-	3.41
Wheat-corn-SBM	-	+	2.90
Corn-SBM	+	-	2.66
Corn-SBM	+	+	2.74
Wheat-corn-SBM	+	-	3.19
Wheat-corn-SBM	+	+	2.66
			Probability
Diet			<0.0001
CVC			<0.001
Enzyme			<0.0001
Diet x CVC			0.149
Diet x enzyme			<0.0001
Enzyme x CVC			0.950
Diet x CVC x enzyme			0.730

[1]n for main effects: CS = 28, WCS = 27, CVC- = 28, CVC+ = 27, Enzyme- = 27, Enzyme+ = 28;
[2]CVC = coccidia vaccine challenge

Table 3.11 Main and simple effects of the ileal villi height, crypt depth, and height-to-crypt-depth ratio in the small intestine (day 21)[1,2]

Diet	CVC	Enzyme	Villi height, µm	Crypt depth, µm	VHCD
			Means for main effect of diet		
Corn-SBM			711.4	155.8	4.87
Wheat-corn-SBM			727.4	155.8	4.92
			Means for main effect of CVC		
	-		764.2	134.9	5.84
	+		674.6	176.6	3.95
			Means for main effect of enzyme		
		-	710.2	159.9	4.64
		+	728.7	151.7	5.15
Standard deviation			84.3	30.0	0.87
			Diet x CVC		
Corn-SBM	-		755.5	132.0	5.86
Wheat-corn-SBM	-		772.8	137.8	5.81
Corn-SBM	+		667.2	179.6	3.88
Wheat-corn-SBM	+		682.0	173.7	4.03
			Diet x enzyme		
Corn-SBM		-	712.9	154.7	4.87
Corn-SBM		+	709.8	156.9	4.41
Wheat-corn-SBM		-	707.4	165.0	4.86
Wheat-corn-SBM		+	747.5	146.5	5.43
			CVC x enzyme		
	-	-	747.2	146.8	5.17b
	-	+	781.2	123.0	6.50a
	+	-	673.1	173.0	4.11c
	+	+	676.1	180.3	3.79c

			Diet x CVC x enzyme		
Corn-SBM	-	-	745.4	136.4	5.54
Corn-SBM	-	+	765.7	126.0	6.17
Wheat-corn-SBM	-	-	749.0	128.8	4.80
Wheat-corn-SBM	-	+	796.7	118.5	6.83
Corn-SBM	+	-	680.5	194.6	4.21
Corn-SBM	+	+	654.0	172.2	3.55
Wheat-corn-SBM	+	-	665.8	173.0	4.02
Wheat-corn-SBM	+	+	698.3	196.7	4.03
			Probability		
Diet			0.533	0.996	0.849
CVC			0.001	<0.0001	<0.0001
Enzyme			0.472	0.373	0.061
Diet x CVC			0.961	0.523	0.721
Diet x enzyme			0.402	0.259	0.056
Enzyme x CVC			0.547	0.094	0.003
Diet x CVC x enzyme			0.758	0.625	0.485

[1]n for main effects: CS = 22, WCS = 24, CVC- = 24, CVC+ = 22, Enzyme- = 23, Enzyme+ = 23;
[2]CVC = coccidia vaccine challenge; VHCD = villi height-to-crypt-depth ratio

Figure 3.1 Product bulletin description for *Eimeria* contents in coccidiosis vaccine

PRODUCT BULLETIN

COCCIVAC®-B52

Coccidiosis Vaccine

(Eimeria acervulina, E. maxima, E. maxima MFP, *E. mivati, and E. tenella)*
(Live Oocysts)

For Animal Use Only.

Description

This product contains live oocysts of the following species of coccidia: *E. acervulina*, {*E. maxima*, *E. maxima* MFP}, *E. mivati*, and *E. tenella*.

Indications for Use

For vaccination of healthy chickens at 1 day of age by spray cabinet administration as an aid in the prevention of coccidiosis due to *E. mivati* and *E. tenella* and as an aid in the reduction of lesions related to *E. acervulina* and *E. maxima*.

Vaccination Programs

Many factors must be considered in determining the vaccination program for a particular farm or poultry operation. To be fully effective, the vaccine must be administered to healthy receptive birds held in proper environment under good management. In addition, the response may be modified by the age of the birds and their immune status. Seldom does 1 vaccination under field conditions produce complete protection for all individuals in a given flock. The amount of protection required will vary with the type of operation and the degree of exposure that a flock is likely to encounter.

Precautions

ONLY VACCINATE HEALTHY BIRDS

Consult your poultry pathologist for further recommendations based on conditions existing in your area at any given time. This product is not ordinarily recommended for use with prestarter or starter feeds containing coccidiostats. Birds must have access to their droppings as reinfection is required to induce full immunity.

Spray Cabinet Administration

FOR CHICKENS 1 DAY OF AGE

The vaccine should be prepared {or mixed} at the rate of 210 ml of distilled water per 1000 doses of vaccine. Each 100 chicks should receive 21 ml of vaccine solution {dye may be added as a marker}.

Full directions for use of the spray cabinet are available from the company.

Notice

All Merck Animal Health vaccines released for sale meet U.S. and local regulatory requirements in regard to safety, purity, potency and the capacity to immunize normal susceptible birds.

Caution

DO NOT FREEZE

The capacity of this vaccine to produce satisfactory results depends on many factors including, but not limited to conditions of storage and handling by the user, administration of the vaccine, health and responsiveness of individual animals and degree of field exposure. Therefore, directions for use should be followed carefully.

Contact our sales or technical services representatives to help design a custom vaccination program.

MERCK
Animal Health

US/CVB/0715/0007

CHAPTER 4 – SUMMARY AND CONCLUSION

It is apparent that many factors influence energy utilization in broiler chickens. Not only does diet matrix influence the digestion and absorption, the physicochemical properties of the individual ingredients may be different based on how a feed ingredient was processed, season it was grown in, or even the location it was grown. This research did not find any observations that adaptation length beyond four d may influence AMEn with the particular diets and feed ingredients used in these studies but there may be more factors found in the diet matrix that may be the cause for this observation.

Ingredients high in soluble NSP was observed to reduce the AMEn of the birds in one case (study 1: Exp. 1) and not in two other cases (study 1: Exp. 2; study 2). In study 2, the enzymes used reduced the increased digesta viscosity in the diet containing wheat but did not influence the AMEn values of the diets. This lack of effect on AMEn values could be attributed to the fact that the enzymes were added to diets that already met the requirements of the birds for energy.

The use of a 20x dose of a coccidia vaccine containing mixed *Eimeria* species greatly reduced the analyzed AMEn values of the diets and wheat ingredient. The damage to the villi of the ileum in the small intestine may have been a key factor for the decreased energy retention observed. Despite the drop in energy retention and reduced performance during the peak of coccidia infection (seven d post-CVC; day 21), the birds were able to recover and compensate for these reductions 14 d post challenge (day 28).

The research conducted successfully obtained values for AME and AMEn of diets and selected feed ingredients, however more research into the factors influencing the energy utilization of broiler chickens is needed. Analysis of the soluble NSP components of the diets and feed ingredients may provide a clearer understanding of the different observations occurring from the same feed ingredient. Additionally, adaptation length can be further explored using birds of a different age (e.g. younger birds with less developed digestive systems) and the many other feed ingredients used in poultry production.

APPENDIX

Appendix 1 Simple effects – Table 2.3[1]

Diet Type[2]	AL, d	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
Wheat-soybean meal	12	70.17	62.17	75.33	3 510.8	3 374.3
Wheat-soybean meal	8	70.17	62.50	75.33	3 516.2	3 381.5
Wheat-soybean meal	4	70.50	62.67	75.67	3 527.3	3 393.0
Corn-wheat-soybean meal	12	73.50	68.50	77.33	3 511.2	3 422.7
Corn-wheat-soybean meal	8	74.00	68.33	77.50	3 524.2	3 434.8
Corn-wheat-soybean meal	4	73.50	67.50	76.67	3 484.3	3 393.5
Wheat mid-wheat-soybean meal	12	62.17	60.50	66.67	3 030.8	2 904.3
Wheat mid-wheat-soybean meal	8	60.67	56.50	65.33	2 974.3	2 836.0
Wheat mid-wheat-soybean meal	4	61.50	59.50	66.17	3 003.5	2 874.5
	SEM[3]	0.384	0.836	0.383	17.149	18.721
				Probability		
Length of feeding x ingredient type		0.106	0.059	0.123	0.126	0.094

[1]Number of replicates were 18

[2]DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = nitrogen-corrected apparent metabolizable energy; AL = adaptation length

[3]SEM = standard error of the mean

Appendix 2 Simple effect – Table 2.5[1]

Diet Type[2]	AL, d	DM, %	N, %	En, %	AME, kcal/kg	AMEn, kcal/kg
Oats-soybean meal	12	67.33	63.83	70.00	3 311.5	3 178.3
Oats-soybean meal	8	62.00	59.83	65.67	3 112.8	2 964.7
Oats-soybean meal	4	63.83	65.33	67.00	3 169.3	3 042.2
Corn-oats-soybean meal	12	67.00	53.50	70.50	3 259.2	3 122.7
Corn-oats-soybean meal	8	67.17	56.33	70.67	3 222.5	3 094.5
Corn-oats-soybean meal	4	67.50	54.00	70.83	3 235.5	3 102.2
Wheat mid-oats-soybean meal	12	67.17	59.83	71.33	3 302.8	3 169.8
Wheat mid-wheat-soybean meal	8	64.00	51.67	68.33	3 152.3	2 992.3
Wheat mid-wheat-soybean meal	4	65.17	61.00	69.50	3 202.3	3 072.8
	SEM[3]	1.82	3.77	1.71	78.0	86.9
				Probability		
Length of feeding x ingredient type		0.650	0.544	0.742	0.874	0.858

[1]Number of replicates were 18

[2]DM = dry matter; N = nitrogen; En = energy; AME = apparent metabolizable energy; AMEn = nitrogen-corrected apparent metabolizable energy; AL = adaptation length

[3]SEM = standard error of the mean

REFERENCES

Adebiyi, A.O. and Olukosi, O.A. 2015. Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without a mixture of carbohydrases and protease for broilers and turkeys. Poult Sci **94**:1270-6.

Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 903-916 *in* L. L. S. Austin J. Lewis, ed. Swine Nutrition. CRC Press, Washington, DC.

Adeola, O. and Ileleji, K.E. 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.

Akhtar, M., Tariq, A.F., Awais, M.M., Iqbal, Z., Muhammad, F., Shahid, M. and Hiszczynska-Sawicka, E. 2012. Studies on wheat bran Arabinoxylan for its immunostimulatory and protective effects against avian coccidiosis. Carbohydr Polym **90**:333-339.

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

Amerah, A.M., Ravindran, V., Lentle, R.G. and Thomas, D.G. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult Sci **87**:2320-2328.

Annison, G. 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler-chickens. J Agric Food Chem **39**:1252-1256.

Antoniou, T.C. and Marquardt, R.R. 1983. The utilization of rye by growing chicks as influenced by autoclave treatment, water extraction, and water soaking. Poultry Science **62**:91-102.

AOAC. 2006. Official methods of analysis. Association of Official Analytical Chemist, Arlington, VA, USA.

Assis, R.C.L., Luns, F.D., Beletti, M.E., Assis, R.L., Nasser, N.M., Faria, E.S.M. and Cury, M.C. 2010. Histomorphometry and macroscopic intestinal lesions in broilers infected with Eimeria acervulina. Vet Parasitol **168**:185-189.

Banfield, M.J., Kwakkel, R.P. and Forbes, J.M. 2002. Effects of wheat structure and viscosity on coccidiosis in broiler chickens. Anim Feed Sci Technol **98**:37-48.

Bederska-Lojewska, D., Swiatkiewicz, S., Arczewska-Wlosek, A. and Schwarz, T. 2017. Rye non-starch polysaccharides: Their impact on poultry intestinal physiology, nutrients digestibility and performance indices - a review. Annals of Animal Science **17**:351-369.

Bedford, M.R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim Feed Sci Technol **53**:145-155.

Bedford, M.R., Scott, T.A., Silversides, F.G., Classen, H.L., Swift, M.L. and Pack, M. 1998. The effect of wheat cultivar, growing environment, and enzyme supplementation on digestibility of amino acids by broilers. Canadian Journal of Animal Science **78**:335-342.

Berrocoso, J.D., Yadav, S. and Jha, R. 2017. Nitrogen-corrected apparent metabolizable energy value of macadamia nut cake for broiler chickens determined by difference and regression methods. Anim Feed Sci Technol **234**:65-71.

Blake, D.P., Shirley, M.W. and Smith, A.L. 2006. Genetic identification of antigens protective against coccidia. Parasite Immunol **28**:305-314.

Bolarinwa, O.A. and Adeola, O. 2012. Energy value of wheat, barley, and wheat dried distillers grains with solubles for broiler chickens determined using the regression method. Poult Sci **91**:1928-1935.

Boros, D., Marquardt, R.R. and Guenter, W. 1998. Site of exoenzyme action in gastrointestinal tract of broiler chicks. Canadian Journal of Animal Science **78**:599-602.

Bourdillon, A., Carre, B., Conan, L., Duperray, J., Huyghebaert, G., Leclercq, B., Lessire, M., McNab, J. and Wiseman, J. 1990. European reference method for the in vivo determination of metabolisable energy with adult cockerels: reproducibility, effect of food intake and comparison with individual laboratory methods. Br Poult Sci **31**:557-65.

Broch, J., de Oliveira, N.T.E., Nunes, R.V., Henz, J.R., da Silva, I.M., Frank, R. and Schone, R.A. 2015. Chemical composition and energetic values of wheat and its sub-products for broiler chicken. Semin-Cienc Agrar **36**:3481-3488.

Burnett, G.S. 1966. Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. Br Poult Sci **7**:55-75.

Carre, B., Gomez, J. and Chagneau, A.M. 1995. Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic-acid and short-chain fatty-acids, to dietary metabolizable energy values in broiler-chickens and adult cockerels. Br Poult Sci **36**:611-629.

Choct, M. 2015. Feed non-starch polysaccharides for monogastric animals: classification and function. Animal Production Science **55**:1360-1366.

Choct, M., Dersjant-Li, Y., McLeish, J. and Peisker, M. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australasian Journal of Animal Sciences **23**:1386-1398.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G. 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.

Cowieson, A.J., Hruby, M. and Pierson, E.E.M. 2006. Evolving enzyme technology: impact on commercial poultry nutrition. Nutr Res Rev **19**:90-103.

Cowieson, A.J. and Ravindran, V. 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. Br Poult Sci **49**:37-44.

D'Agostini, P., Gomes, P.C., Albino, L.F.T., Rostagno, H.S. and Sa, L.M. 2004. Values of chemical and energy composition of some feedstuffs for broiler chicks. Braz J Poult Sci **33**:128-134.

Dixon, J.M. and Wilkinson, W.S. 1957. Surgical technique for the exteriorization of the ureters of the chicken. Am J Vet Res **18**:665-667.

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

Fetterer, R.H., Miska, K.B., Jenkins, M.C. and Wong, E.A. 2014. Expression of nutrient transporters in duodenum, jejunum, and ileum of Eimeria maxima-infected broiler chickens. Parasitol Res **113**:3891-3894.

Gallardo, C., Dadalt, J.C., Kiarie, E. and Trindade Neto, M.A. 2017. Effects of multi-carbohydrase and phytase on standardized ileal digestibility of amino acids and apparent metabolizable energy in canola meal fed to broiler chicks. Poult Sci **96**:3305-3313.

Garcia-Carreon, F.L. 1997. Classification of Proteases without tears. Biochemical Education **25**:161-167.

Generoso, R.A.R., Gomes, P.C., Rostagno, H.S., Albino, L.F.T., Barreto, S.L.D. and Brumano, G. 2008. Chemical and energy composition of some feeds for broiler chicks and two ages. Rev Bras Zootecn **37**:1251-1256.

Haritova, A.M. and Stanilova, S.A. 2012. Enhanced expression of IL-10 in contrast to IL-12B mRNA in poultry with experimental coccidiosis. Exp Parasitol **132**:378-382.

Hashemipour, H., Khaksar, V., Rubio, L.A., Veldkamp, T. and van Krimpen, M.M. 2016. Effect of feed supplementation with a thymol plus carvacrol mixture, in combination or not with an NSP-degrading enzyme, on productive and physiological parameters of broilers fed on wheat-based diets. Animal Feed Science and Technology **211**:117-131.

Hetland, H., Choct, M. and Svihus, B. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. Worlds Poult Sci J **60**:415-422.

Hetland, H., Svihus, B. and Krogdahl, A. 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Br Poult Sci **44**:275-282.

Hew, L.I., Ravindran, V., Mollah, Y. and Bryden, W.L. 1998. Influence of exogenous xylanase supplementation on apparent metabolisable energy and amino acid digestibility in wheat for broiler chickens. Anim Feed Sci Technol **75**:83-92.

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

Hughes, R.J. and Choct, M. 1999. Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. Aust J Agric Res **50**:689-701.

Igbasan, F.A. and Guenter, W. 1996. The evaluation and enhancement of the nutritive value of yellow-, green- and brown-seeded pea cultivars for unpelleted diets given to broiler chickens. Animal Feed Science and Technology **63**:9-24.

Im, H.L., Ravindran, V., Ravindran, G., Pittolo, P.H. and Bryden, W.L. 1999. The apparent metabolisable energy and amino acid digestibility of wheat, triticale and wheat middlings for broiler chickens as affected by exogenous xylanase supplementation. J Sci Food Agric **79**:1727-1732.

Janssen, W.M.M.A. and Carré, B. 1989. Influence of fibre on digestibility of poultry feeds. Butterworths, England.

Jorgensen, H., Zhao, X.Q., Knudsen, K.E. and Eggum, B.O. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br J Nutr **75**:379-95.

Jozefiak, D., Rutkowski, A., Jensen, B.B. and Engberg, R.M. 2006. The effect of beta-glucanase supplementation of barley- and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. Br Poult Sci **47**:57-64.

Kermanshahi, H., Shakouri, M.D. and Daneshmand, A. 2018. Effects of non-starch polysaccharides in semi-purified diets on performance, serum metabolites, gastrointestinal morphology, and microbial population of male broiler chickens. Livestock Science **214**:93-97.

Knudsen, K.E.B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poultry Science **93**:2380-2393.

Knudsen, K.E.B., Jensen, B.B. and Hansen, I. 1993. Digestion of polysaccharides and other major components in the small and large-intestine of pigs fed on diets consisting of oat fractions rich in beta-d-glucan. Br J Nutr **70**:537-556.

Kong, C. and Adeola, O. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas J Anim Sci **27**:917-925.

Leinonen, I. and Williams, A.G. 2015. Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment. J Sci Food Agric **95**:3041-6.

Liu, W., Liu, G.H., Liao, R.B., Chang, Y.L., Huang, X.Y., Wu, Y.B., Yang, H.M., Yan, H.J. and Cai, H.Y. 2017. Apparent metabolizable and net energy values of corn and soybean meal for broiler breeding cocks. Poult Sci **96**:135-143.

Marquardt, R.R., Boros, D., Guenter, W. and Crow, G. 1994. The nutritive-value of barley, rye, wheat and corn for young chicks as affected by use of a trichoderma-reesei enzyme preparation. Animal Feed Science and Technology **45**:363-378.

Masey O'Neill, H.V., Smith, J.A. and Bedford, M.R. 2014. Multicarbohydrase enzymes for non-ruminants. Asian-Australas J Anim Sci **27**:290-301.

Mateos, G.G., Jiménez-Moreno, E., Serrano, M.P. and Lázaro, R.P. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics1. The Journal of Applied Poultry Research **21**:156-174.

Mateos, G.G., Lopez, E., Latorre, M.A., Vicente, B. and Lazaro, R.P. 2007. The effect of inclusion of oat hulls in piglet diets based on raw or cooked rice and maize. Anim Feed Sci Technol **135**:100-112.

Mathlouthi, N., Mallet, S., Saulnier, L., Quemener, B. and Larbier, M. 2002. Effects of xylanase and beta-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. Animal Research **51**:395-406.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D. and Annison, E.F. 1983. Sudies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. Br Poult Sci **24**:81-89.

Munir, K. and Maqsood, S. 2012. A review on role of exogenous enzyme supplementation in poultry production.

Munyaka, P.M., Nandha, N.K., Kiarie, E., Nyachoti, C.M. and Khafipour, E. 2016. Impact of combined beta-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poultry Science **95**:528-540.

Myers, W.D., Ludden, P.A., Nayigihugu, V. and Hess, B.W. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci **82**:179-183.

NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. The National Academies Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine. 11th ed. National Academy Press, Washington, D.C.

Olukosi, O.A., Adedokun, S.A. and Agboola, J.O. 2017. Species-dependent response to the influence of adaptation length during assay for metabolisable energy of cereal grains employing the difference method. Anim Feed Sci Technol **231**:111-118.

Olukosi, O.A. and Adeola, O. 2009. Estimation of the metabolizable energy content of meat and bone meal for swine. J Anim Sci **87**:2590-2599.

Olukosi, O.A. and Adeola, O. 2010. Metabolizable energy content of meat and bone meal in corn-soybean meal or corn, wheat and soybean meal diets for broilers. J Poult Sci **47**:244-249.

Peek, H.W. and Landman, W.J.M. 2011. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Vet Q **31**:143-161.

Peuhkuri, K., Vapaatalo, H. and Korpela, R. 2010. Even low-grade inflammation impacts on small intestinal function. World Journal of Gastroenterology **16**:1057-1062.

Quiroz-Castaneda, R.E. and Dantan-Gonzalez, E. 2015. Control of avian coccidiosis: Future and present natural alternatives. Biomed Res Int:11.

Ravindran, V. 2013. Feed enzymes: The science, practice, and metabolic realities. The Journal of Applied Poultry Research **22**:628-636.

Ravindran, V., Selle, P.H. and Bryden, W.L. 1999. Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. Poult Sci **78**:1588-95.

Rosenfelder, P., Eklund, M. and Mosenthin, R. 2013. Nutritive value of wheat and wheat by-products in pig nutrition: A review. Anim Feed Sci Technol **185**:107-125.

Saki, A.A., Ranjbari, T., Tabatabaei, M.M., Ahmadi, A., Aflaki, F., Rabani, M., Abbasinezhad, M. and Mahmoudi, H. 2009. Composition of Metabolic Energy Value and Amino Acid Digestibility of Wheat, Wheat Screening and Barley Between Ileum and Faces of Broiler Chicken. Journal of Poultry Science **46**:188-192.

Schneiders, J.L., Nunes, R.V., Schone, R.A., Frank, R., Savoldi, T.L., Tsutsumi, C.Y., Scherer, C. and Castilha, L.D. 2017. Energy coefficients of plant foods for broiler chickens at different ages. Semin-Cienc Agrar **38**:2119-2128.

Selle, P.H., Ravindran, V., Caldwell, A. and Bryden, W.L. 2000. Phytate and phytase: consequences for protein utilisation. Nutr Res Rev **13**:255-78.

Shirley, M.W., Smith, A.L. and Tomley, F.M. 2005. The biology of avian Eimeria with an emphasis on their control by vaccination. Pages 285-330 *in* J. R. Baker, R.

Muller, D. Rollinson, eds. Advances in Parasitology, Vol 60. Elsevier Academic Press Inc, San Diego.
Sibbald, I.R. 1976. A bioassay for true metabolizable energy in feedingstuffs. Poult Sci **55**:303-8.
Sibbald, I.R. and Price, K. 1975. Variation in the metabolizable energy values of diets and dietary components fed to adult roosters. Poult Sci **54**:448-56.
Steenfeldt, S., Hammershøj, M., Müllertz, A. and Fris Jensen, J. 1998. Enzyme supplementation of wheat-based diets for broilers: 2. Effect on apparent metabolisable energy content and nutrient digestibility. Anim Feed Sci Technol **75**:45-64.
Theander, O., Westerlund, E., Åman, P. and Graham, H. 1989. Plant cell walls and monogastric diets. Anim Feed Sci Technol **23**:205-225.
Umar, S., Arif, M., Shah, M.A.A., Munir, M.T., Yaqoob, M., Ahmed, S., Khan, M.I., Younus, M. and Shahzad, M. 2015. Application of avian cytokines as immuno-modulating agents. Worlds Poult Sci J **71**:643-653.
USDA. 2019. Red meat and poultry production. *in* R. Knight, ed. ERS, www.ers.usda.gov.
Wigley, P. and Kaiser, P. 2003. Avian cytokines in health and disease. Brazilian Journal of Poultry Science **5**:1-14.
Williams, R.B. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int J Parasit **29**:1209-1229.
Wiseman, J., Nicol, N.T. and Norton, G. 2000. Relationship between apparent metabolisable (AME) values and in vivo/in vitro starch digestibility of wheat for broilers. Worlds Poult Sci J **56**:305-318.
Woyengo, T.A., Kiarie, E. and Nyachoti, C.M. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poultry Science **89**:1182-1189.
Woyengo, T.A. and Nyachoti, C.M. 2011. Review: Supplementation of phytase and carbohydrases to diets for poultry. Canadian Journal of Animal Science **91**:177-192.
Yaghobfar, A. and Kalantar, M. 2017. Effect of non-starch polysaccharide (nsp) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (sglt1) and mucin producer (muc2) genes of broiler chickens. Brazilian Journal of Poultry Science **19**:629-638.
Yuan, L., Wang, S.Q., Wang, Z.X., Zhu, H. and Huang, K. 2015. Effects of exogenous protease supplementation on endogenous trypsin activity and gene expression in broilers. Genetics and molecular research : GMR **14**:13633-41.
Zyla, K., Gogol, D., Koreleski, J., Swiatkiewicz, S. and Ledoux, D.R. 1999. Simultaneous application of phytase and xylanase to broiler feeds based on wheat: in vitro measurements of phosphorus and pentose release from wheats and wheat-based feeds. J Sci Food Agric **79**:1832-1840.

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