NUTRITIONAL EVALUATION OF PALM KERNEL MEAL FOR POULTRY

BY

JENNIFER BLAIR

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois Urbana-Champaign, 2023

Urbana, Illinois

Master's Committee:

Professor Carl M. Parsons, Adviser
Professor Jason L. Emmert
Professor Ryan N. Dilger

ABSTRACT

Four experiments were conducted to evaluate the nutritional value of palm kernel meal (PKM) from Colombia, Costa Rica, Indonesia, Mexico, and Thailand. Extensive chemical analyses were initially conducted to determine nutritional composition of 10 PKM. The first and second experiments were conducted to determine nitrogen-corrected true metabolizable energy (TME_n) in conventional roosters and standardized amino acid (AA) digestibility in cecectomized roosters for 12 PKM, respectively. The TME_n differed among PKM ($P<0.05$) and ranged from 1,644 to 2,439 kcal/kg (DM basis) for the 12 PKM. Standardized digestibility of AA varied among samples ($P<0.05$), e.g., Lys varied from 35 to 60%, Met varied from 65 to 86%, Cys varied from and 29 to 69%, and Thr varied from 53 to 77%. Chemical composition results (%), mean (range), were as follows: CP, 13.7 (11.2-16.6); fat, 7.5 (5.86-10.47); NDF, 64.4 (60.8-67.3); Ca, 0.4 (0.2-0.5); P, 0.7 (0.6-0.8); phytic acid, 1.3 (1.1-1.6). In Experiment 3, phosphorus (P) bioavailability of 2 PKM samples from Mexico (M-PKM) and Costa Rica (CR-PKM) relative to potassium phosphate (KH_2PO_4) based on tibia bone ash was determined. From 8 to 18 d-of-age, P-deficient corn-soybean meal diets supplemented with 0.05 and 0.1% P from KH_2PO_4 or 15 and 30% M-PKM and CR-PKM were fed. All diets were fed to 6 replicate pens of 5 chicks. Data were analyzed using a one-way ANOVA and multiple regression analyses. Multiple regression of bone ash (mg/tibia) on supplemental P intake yielded a P bioavailability of 21 and 40% relative to KH_2PO_4 for M-PKM and CR-PKM, respectively. In Experiment 4, an ad libitum-fed broiler chicken assay was conducted to determine apparent pre-cecal ileal P digestibility for M-PKM and CR-PKM at 2 dietary Ca levels in 2 × 2 factorial treatment arrangement. Diets were fed from 18 to 22 d of age. Semi-purified diets containing 45% M-PKM or CR-PKM as the sole source of P were fed at a Ca:total P ratio of 1.4 and 3.6, respectively. The latter ratio was achieved by adding limestone to increase dietary Ca from 0.3% to 0.75%.

Titanium dioxide (TiO_2) was used as an indigestible marker and P was kept constant across all diets at 0.21%. Data were analyzed as a 2-way ANOVA with PKM inclusion and Ca:total P ratio as main effects. When Ca level increased from 0.30% to 0.75% for diets containing M-PKM, ileal P digestibility decreased from 37.7% to 23.7%, respectively, while for diets containing CR-PKM, it decreased from 48.1% to 30.0%, respectively.

This thesis is dedicated to Roscoe, the best dog and companion I could have asked for. You were there for me constantly throughout this journey. I know you have been watching over me as I crossed the finish line.

"Success is not final, failure is not fatal - it is the courage to continue that counts."

- Winston Churchill

ACKNOWLEDGEMENTS

I would like to give my sincerest thanks to both of my advisors Dr. Carl Parsons and Dr. Jason Emmert. It has been wonderful to work with the best researchers and professors in the poultry industry. Both of your support, knowledge, wisdom, and guidance throughout my research, writing, and graduate school education gave me the strength to get through these last two and a half years.

I would also like to sincerely thank Dr. Ryan Dilger for being on my defense committee and being a great mentor. Your kindness has been wonderful.

Thank you so much to my lab group and helping me with my trials. Thank you to Rebekah Drysdale, Minoy Cristobal, and Andrew Hack for dealing with my stress and anxiety when it came to both of my trials. My deepest gratitude goes to our research manager, Pam Utterback. Thank you for answering all of my questions, helping me through mistakes, your guidance, and patience when it came to helping me with my trials. You are a wonderful work mom. Thank you for always keeping this lab running smoothly.

I do not know how I would have been able to get through these past two and a half years without my parents. Your unconditional love and support throughout my master's has kept me motivated, even when I doubt myself. It's hard to put into words how much you guys mean to me. Your unwavering support to me throughout my entire life has not gone unnoticed. Your constant understanding and support throughout my graduate school career gives me the reminder that I am never alone in this journey. Mom, you have instilled in me to be such a hard worker, and that even though life gets tough, it is only temporary. Dad, your constant jokes always put a smile on my face, even though I pretend they do not. I hope to have half the knowledge you have

one day. I am forever grateful to have such supporting and loving parents. I love you both so much.

Lastly, a huge shout out to my amazing friends. Thank you so much to Rebekah Drysdale, Alissa Moritz, Juli Jesperson, Kaitlyn Sommer, Cameron White, and Loreta Sutkutė for the constant laughs, unwavering support, and advice. You all have made my master's experience wonderful with amazing memories, despite the stress that comes with it. You guys are amazing friends. I am so thankful to have met you all and I don't know what I would do without you.

TABLE OF CONTENTS

CHAPTER 1:
LITERATURE REVIEW
INTRODUCTION

Feed is the most expensive attribute in poultry production, accounting for up to 70% of the total cost of broiler production (Spring, 2013). Since the 1960s, there have been advancements in crop and animal genetics and an increase in the quality of meat through nutritional improvements in monogastric and ruminant animals (Brameld and Parr, 2016). However, due to increasing costs of feed ingredients and the competition with human consumption and biofuels, producers and integrators are seeking ways to decrease the cost of their feed while maintaining a nutritionally balanced diet for poultry. For this reason, some countries are relying on alternative feedstuffs to partially replace corn and soybean meal (SBM).

Palm kernel meal (PKM) is a by-product of two marketable products, palm oil and palm kernel oil, from the African oil palm (*Elaeis guineensis Jacq.*) (Balandrán-Quintana et al., 2019). Palm kernel meal is typically grown in tropical climates such as Central America, South America, and East Asia (Ahmad et al., 2014). With the increased global demand of palm oil and the utilization of feed enzymes, PKM as a byproduct is becoming more acceptable for the poultry industry to include in their diets. It has been reported that PKM can replace corn up to a level of 25% in broiler diets with enzyme supplementation without impacting production performance (Natsir et al., 2018) and can be included up to 40% of the total diet in laying hens without affecting egg production (Perez et al., 2000).

Alternative feedstuffs that are nutritionally of high quality and have no negative effect on performance or any cost contingencies can help reduce the dependence on corn and SBM in poultry diets, while increasing the availability of these feed ingredients for human consumption or biofuel production. With the increased production of PKM, this alternative feedstuff can help

poultry producers located in tropical countries have easier access to feed ingredients and that can be more cost-effective in the future. Moreover, due to limited data on the feeding of PKM to poultry, more research on this alternative feed ingredient is required to decide what the best use of this ingredient in poultry feed formulation.

ALTERNATIVE FEEDSTUFFS – PALM KERNEL MEAL

History

The oil palm is native to west and central Africa and had been used for many purposes in addition to cooking, such as oil being burned for light, medicinally for soothing ointment, and kernel shells for fire (Henderson and Osborne, 2000). Furthermore, oil palm leaves were used for roofing, fencing, and brooms, fiber was used for rope and baskets, and the sap drained from immature flowers was used to make palm wine and vinegar. According to Henderson and Osborne (2000), it was not until the 18th century that palm oil revolutionized the soap and candle industry with the discovery of glycerin during the Industrial Revolution. However, due to the demand of palm oil being higher than the supply, starting in 1935, the company Unilever invested research into the propagation of oil palm (Henderson and Osborne, 2000). Unilever's research helped produce oil palm that is more resistant to disease and has increased fruit yield.

Currently, palm kernel is one the largest produced oilseeds globally, with an estimated production of 20 million metric tons in 2022 (USDA, 2023). Indonesia and Malaysia, the leading suppliers of palm oil, dominate the global vegetable oil trade by accounting for about 60% of global exports and are projected to account for 82% of global production by 2031 (OECD and FAO, 2022). The ideal conditions to grow oil palm trees are warm and wet conditions, and they grow optimally in soil that is rich in clay (Pirker et al., 2016). Due to the oil palm having to grow

in these specific conditions, there has been controversy associated with the palm oil industry causing deforestation of the tropical rainforest in South-East Asia (Vijay et al., 2016) and is raising questions and concerns about long-term sustainability. This has led to several Central American countries to start oil palm production with global sustainability certifications (OCED and FAO, 2022).

Palm kernel meal has been historically used in ruminant and rabbit feeds due to its high fiber content, which monogastric animals (i.e., swine and poultry) have difficulty digesting (Pickard, 2005). Due to the increased production of palm oil, there is an economic incentive to use the inexpensive by-product PKM in broiler diets (Sundu et al., 2006). Palm kernel meal has typically been excluded from broiler diets due to the high fiber content and low amino acid (AA) content, particularly lysine and methionine (Sundu et al., 2008), with the latter making it often unsuitable to use in starter diets (Sundu et al., 2006). It has been noted that the low digestibility of PKM could be due to the higher dietary fiber content and the heat treatments during processing (Sundu et al., 2008 and O'Mara et al., 1999).

Solvent and Mechanical Extraction

Palm kernel oil is typically extracted with two methods, solvent and mechanical extraction, creating two by-products, PKM and palm kernel cake (PKC), respectively. Although these two products are extracted differently, many papers use the terms PKM and PKC interchangeably, even though there is a slight difference in nutrient values between the two products. The nutritional composition (i.e. crude protein, crude fiber, ether extract and ash) of PKM and PKC vary depending on the method of extraction, soil type, and geographical source, the amount of endocarp remaining post processing, and oil extraction efficiency (Abdeltawab and Khattab, 2018). According to Ruben et al. (2020), crude protein, crude fiber, phosphorus (P)

and dry matter contents are higher in solvent extracted PKM, whereas fat and ash contents are higher in mechanical extracted PKC.

There are three methods by which solvent PKM can be solvent extracted: pre-press, full press, and direct solvent extraction. All three methods begin when the palm kernel is cleaned of impurities using rotary drum cleaners and screens and magnetic separation is then used to remove any potential metals using a rotary drum magnet or stationary magnet (Goyum Group, 2021). The cleaned palm kernel contains 43-48% oil depending on the size and origin of the seed. The first method, pre-press with solvent extraction, consists of the palm kernels being sent to short screw presses where up to 28-30% of the oil is extracted. The second method, full press with solvent extraction, consists of the palm kernels being sent through short screw presses to extract up to 28-30% of the oil and then the PKC being sent through a second screw press to reduce the PKC to 6-7% oil. The PKC has a total of 15-18% oil before being sent through the second screw press. This method is the most commonly used in the palm kernel extraction industry (Goyum Group, 2021). The third method is direct solvent extraction, where the palm kernels are sent to a hammer mill and passed through 6-8 mm perforated sheets. The hammered kernels then pass through cracking rollers to reduce the size to 3-4 mm to create flakes with approximately 43-45% oil. After one of these three methods is completed, the PKC or flakes are sent to the solvent extraction plant where hexane is added to these products. The hexane will percolate through the PKC and flakes to extract additional oil, leaving up to 1% oil, in the final meal. After the solvent extracts the oil, the product then becomes PKM. The PKM is then desolventized with heat and cooled. After desolventization, the hexane is condensed and then reused for future solvent extractions (Goyum Group, 2021).

NUTRIENT REQUIREMENTS FOR POULTRY

More than 90% of the cost of poultry feeds is due to supplying metabolizable energy, digestible amino acids, and available or digestible P to meet the bird's requirements. Thus, the value of PKM will mainly be composed of these three nutritional components. A discussion of these is provided in the following sections.

Metabolizable energy

Energy in poultry is utilized for the growth of tissues, to carry out physically motivated activities, and for the maintenance and regulation of body temperature and metabolism (Leeson and Summers, 2001). While energy is not considered a nutrient, it has been called the "fire of life" by Kleiber (1961), and is physiologically important for poultry diets. Energy is also often the first criterion used when formulating diets due to poultry's physiological requirements for metabolism, maintenance, tissue growth, and heat production, as previously stated (Wu et al., 2020).

Energy has a multitude of definitions and interrelationships. Gross energy (GE) is the first energy value found and is typically determined by bomb calorimetry. The GE provides a starting point for the evaluation of further energy values and is not otherwise valuable for nutritional studies (Leeson and Summers, 2001). In poultry energy assays, apparent metabolizable energy (AME) is defined as the GE of the feed consumed minus the fecal and urine energy (NRC, 1994) and has been the most commonly used energy value in the poultry industry since the 1950s (Hill and Anderson, 1958). Digestible energy (DE) is not often determined due to poultry excreting fecal and urine energy together; thus, it is an impractical energy assay to use. After accounting for endogenous energy losses, which are primarily made

up of intestinal cells, hormones, and enzymes, true metabolizable energy (TME) can be determined.

Guillaume and Summers (1970) conducted one of the first studies in attempt to describe the relationship between AME and TME in roosters. This relationship was evaluated during a study that was conducted to measure the maintenance energy requirement of adult roosters. Guillaume and Summers (1970) used 12 White Leghorn roosters, divided into two groups, where each group was fed a predominantly yellow corn or wheat diet, respectively. These diets were not formulated to meet nutrient requirements. The birds were fed *ad libitum* during the first period of this study, which was seven weeks, and the birds' food consumption and live weight were recorded with the maintenance energy requirement estimated. The second period was four weeks, when the birds were fed enough of the diets to just meet their maintenance energy requirement. The third period was two weeks, but the two group's diets were switched. Metabolizable energy was measured by the total collection method and the maintenance requirement was calculated by dividing weekly gain and feed intake by average live weight and then regressing the feed intake values on the weight gain values. The maintenance energy requirement was estimated as 117 kcal of metabolizable energy per kg of body weight pen per day. The authors concluded that metabolic and endogenous energy should be taken into account when food intake is low and close to maintenance.

Although AME is the most commonly used energy assay, the TME bioassay, also known as the precision-fed rooster assay (Sibbald, 1976), was invented to account for endogenous energy excretion. However, according to Parsons et al. (1982), in this bioassay, fasted birds have a greater loss of nitrogen than fed birds due to the greater breakdown of body proteins to meet energy requirements. Therefore, TME needs to be corrected to a nitrogen equilibrium or zero

nitrogen retention (TME_n) to have a more accurate measurement of metabolizable energy (Parsons et al., 1982). The precision-fed rooster assay consists of fasting roosters for 24 hours, and then intubating 25 to 30 g of a feed ingredient into the crop. Usually, the feed is given as a single ingredient; however, the ingredient can be mixed with other ingredients, such as corn, to help facilitate the feeding process. The roosters are then once again fasted for 48 hours while the total excreta are collected.

Amino acid digestibility

According to the NRC (1994), the dietary protein requirement of poultry is actually a requirement for specific AA. Accordingly, this has created a shift to focus on formulating to AA requirements. However, AA in feedstuffs are not all totally or equally digested and absorbed due to the body being in a constant state of synthesis and degradation of proteins (Garcia et al., 2007; NRC, 1994). According to Leeson and Summers (2001), AA digestibility is a primary function of endogenous enzyme secretion as digesta travels throughout the gastrointestinal tract. Amino acids are nutritionally divided into two categories: essential and nonessential. Essential AA are those that cannot be synthesized and nonessential are those that can be synthesized in the body (NRC, 1994).

As stated before, the most common methods for determining AA digestibility are the precision-fed cecectomized rooster assay and the standardized ileal AA digestibility (SIAAD) chick assay. While the precision-fed cecectomized rooster assay yields consistent results, it is not a normal feeding pattern. The SIAAD chick assay has a more normal feeding pattern, ad libitum, and can be used on different aged birds. The precision-fed rooster cecectomized assay is the same as the precision-fed rooster conventional assay procedure used for TME; however, this assay is done with roosters whose ceca have been surgically removed. The ceca have been

known to influence AA excretion due to bacterial modification of AA that occurs there (Parsons, 1984). The latter can affect both digestibility values for diet AA and also endogenous AA losses. For example, Kessler et al. (1981) showed that for roosters that were fasted for 24 hours, cecectomized roosters had higher endogenous AA excretion compared with fasted conventional roosters.

The SIAAD chick assay is one in which birds are fed ad libitum with diets containing the test ingredient and an indigestible marker (i.e., chromic oxide, titanium dioxide, or acid insoluble ash) (Parsons, 2020). Then the birds are humanely euthanized, and the ileum section of the gastrointestinal tract is removed. The ileum is located between the Meckel's diverticulum and the ileo-cecal junction. Collecting digesta from this section helps prevent any error from microbial fermentation that occurs in the ceca (Lemme et al., 2004). Digesta from the ileum is either flushed with distilled water or gently squeezed, pooled from each replicate, freeze-dried, and analyzed for AA content.

Currently, there is a large amount of literature on whether the rooster precision-fed assay and the chick ileal methodologies for determining AA digestibility are accurate (Garcia et al., 2007; Kim et al., 2011; Ravindran et al., 1999; Parsons, 2020). These assays have different methodologies, meaning that resulting AA digestibility values may differ between the SIAAD chick assay and the precision-fed cecectomized rooster assay (Kim et al., 2011). According to Ravindran et al. (1999), excreta digestibility is an inaccurate form of measurement due to microbial protein excretion that is unavoidable. Furthermore, a criticism has been that excreta analysis actually represents AA metabolizability instead of AA digestibility due to feces and urine being combined in avians (Ravindran et al., 1999). There have been concerns that comparisons between the assays have been limited in scope due to the lack of feedstuffs

evaluated between the two assays. Garcia et al. (2007) conducted an experiment where the authors compared the SIAAD chick assay and precision-fed cecectomized rooster assay for different ingredients such as SBM, cottonseed meal, poultry by-product meal, fish meal, corn, and wheat. The results of these experiments show that the precision-fed rooster assay often yielded higher AA digestibility values compared with the SIAAD chick assay. This is in agreement with Parsons (2020) that the rooster assay sometimes yields higher AA digestibility values; however, the author stated that the differences are not consistent and the methods can be generally interchangeable with each other.

Phosphorus digestibility and relative bioavailability

Phosphorus and calcium (Ca) are two of the most important structural constituents of bone. Phosphorus is also usually the third most expensive nutrient in poultry feed formulations, following protein and energy (Potchanakorn and Potter, 1987). Approximately 85% of total P in animals is stored in the bone, while the other 15% is distributed between tissues and extracellular fluid (Penido and Alon, 2012). Phosphorus is not only a major component of bone; it also plays an important role in muscle coordination, in energy, carbohydrate, AA, fat, and nervous tissue metabolism, in normal blood chemistry, and in the transport of fatty acids and other lipids (Leeson and Summers, 2001).

There are two main types of methods to measure P bioavailability in poultry feedstuffs: qualitative and quantitative. The qualitative measures include blood, bone, and growth studies (Shastak and Rodehutscord, 2013). There are also quantitative measures such as retention tests and pre-cecal digestibility. One of the most common qualitative methods used to determine relative bioavailability of P is based on bone ash weight, bone breaking strength, or percentage bone ash of birds fed the test ingredient and comparing it with the same parameters of birds fed a

standard P source. According to a study done by Nelson and Walker (1964), the zone of proliferation in the bone of developing chicks is extremely sensitive and is influenced by P deficiencies. The standard reference P sources typically used to determine relative bioavailability are potassium phosphates, sodium phosphates, and mono-, di-, and tri-calcium phosphates (Leske and Coon, 2002). In order to accurately understand the P bioavailability of the ingredient that is being tested, the dietary P level needs to be below the requirement; otherwise, the excess P excreted will be higher regardless of the bioavailability of the P source that is being tested (Rodehutscord, 2009). When using the tibia ash method, the birds are euthanized, then the right or left tibias are collected. The legs are typically autoclaved, then cleaned of muscle, skin, and feathers. Once the tibias are cleaned, the tibias are weighed and dried at 100°C and then dry-ashed at 600°C for ~24 hours. After the tibias are dry-ashed, they are weighed once again to determine the ash content for each bone.

The preferred assay for quantitative measurements is the ileal pre-cecal digestible P determination. This method is preferred over the P retention tests due to having a linear response over a wider range of dietary P (Rodehutscord et al., 2012). Ileal P digestibility follows a method similar to the ileal AA digestibility determination, where the digesta contents are collected from between the Meckel's diverticulum and the cecal junction. This is to avoid cecal and colonic microbial activity and urinary P excretion. In order to conduct this method, birds are usually fed an experimental diet with the desired test ingredient and an indigestible marker (i.e., chromic dioxide or titanium dioxide) for 3 to 5 days. The birds are then euthanized, and the ileal contents are either squeezed or flushed with distilled water into respective containers for each replicate. The samples are then freeze-dried, ground, and analyzed for their indigestible marker and P concentration.

In order to determine total tract P retention, the difference is taken between the P consumed and the P in excreta. The P retention test can be done at the same time as the ileal P digestibility method. The excreta are collected from each replicate with an indigestible marker as an index, freeze-dried, ground, and analyzed for the concentration of P and indigestible marker.

Phytic acid

There has been interest in improving P utilization due to excess P excretion causing environmental concerns, increasing prices of inorganic phosphate supplements, and depletion of inorganic phosphate reserves (Leeson and Summers, 2001; Mutucumarana et al., 2014). The majority of plant P is bound as phytic acid. Phytic acid serves as principal storage form of P, being 50 to 80% of the total P in cereals and plant seeds (Pallauf and Rimbach, 1997). Due to the nature of the molecular structure of phytic acid, it is considered to have antinutritive effects (Pallauf and Rimbach, 1997). Phytic acid is usually bound to minerals such as Ca, Cu, Zn, Mn, and Fe, with these complexes known as phytate. Phytate will chelate to these minerals and make them unavailable for the birds to digest (Leeson and Summers, 2001).

Phytic acid is in the form of IP6, myo-inositol hexakis phosphate, and exists as an anionic form in plants (Angel et al., 2002). Poultry cannot digest most phytic acid and young birds are less able to digest phytate compared with older birds. This is due to poultry lacking the effective endogenous enzymes for hydrolyzing the ester bonds found in phytates (Cowieson et al., 2003). Incorporating phytases into diets greatly increases P digestibility and may also improve bone strength, mineral retention, feed conversion ratio, and AA digestibility coefficients (Rutherfurd et al., 2002).

Phosphorus and calcium metabolism

As stated prior, P and Ca are the two most important macro minerals and constituents of bone. These minerals are required for adequate performance, production, and growth in poultry. These macro minerals are extremely important for leg strength due to increased broiler growth rate and weight gain over recent decades. According to the National Chicken Council (2023), the average weight of a chicken in the 1920's was 2.5 pounds, compared with today's average weight of 6 pounds at 47 days of age. Additionally, Ca serves as an integral part of skeletal growth, and helps with blood clotting, enzyme activation, nerve transmissions, and protein synthesis (David et al., 2021). The nutritional requirements and interrelationships of P and Ca and vitamin D_3 show the importance of adequate nutritional supply of all of these nutrients to prevent deficiencies and interference of their respective homeostasis.

There are multiple factors that can affect P and Ca digestion, metabolism, and absorption. These factors include the dietary Ca to P ratio, phytic acid content of feedstuffs, vitamin D_3 levels, sodium, and even stocking density (Adedokun and Adeola, 2013; Proszkowiec-Weglarz and Angel, 2013). Calcium is the most abundant mineral in the body and is found as three different forms: hydroxyapatite ($Ca_5 (PO_4)_3 (OH)$), ionized Ca, and Ca bound to anions (Adedokun and Adeola, 2013; Leeson and Summers, 2001). These different forms make up bone in a ratio of 2:1, reflecting Ca bound to anions and P in the extracellular matrix, respectively. Calcium and P homeostasis is modulated through the endocrine system through a series of hormones: parathyroid hormone (PTH), 1,25 dihydroxycholecalciferol, Fibroblast Growth Factor 23(FGF23), and Klotho (Li et al., 2017). These hormones highly regulate Ca and P metabolism through intestinal, renal, and skeletal mechanisms; depending on the quantity of these minerals, the rates of intestinal absorption, bone accretion and resorption, and intestinal endogenous losses

will vary (Li et al., 2017). Calcium is typically absorbed from the intestine via transcellular and paracellular routes; however, Ca and P are predominantly absorbed via the transcellular pathway in the duodenum and upper jejunum with the stimulation of vitamin D_3 (Adedokun and Adeola, 2013; Proszkowiec-Weglarz and Angel, 2013). It has been recently found that there are two axes that control Ca and P metabolism. According to Li et al. (2016), P metabolism differed from Ca metabolism. These authors found that kidneys are more in control of the metabolism of P, whereas Ca metabolism is controlled more through the intestines. The PTH/1,25 dihydroxycholecalciferol axis controls Ca balance, while the FGF23/Klotho axis is thought to control P balance.

Dietary supplementation of enzymes

Palm kernel meal has been known to have high acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude fiber. The main components of fiber in PKM are insoluble non-starch polysaccharides (NSP) and β-mannans (Abdollahi et al., 2016). Beta-mannans are linear polysaccharides that are composed with repeating β-1-4 mannose, α-1-6 galactose, and glucose units that are attached to a β-mannan backbone (Jackson et al., 2004). According to Jackson et al. (2004), β-mannans are exceedingly antinutritional in monogastric animals. Products of the oil palm, such as PKM, have mannans located in the endosperm tissue that are crystalline, hard, and highly insoluble (Daud and Jarvis, 1992). According to Knudsen (1997), about 81% of the total carbohydrates in PKM are in the form of NSPs.

A study was conducted to investigate the dietary inclusion of PKM and two dietary enzymes (i.e., β-mannanase and NSP-degrading) on growth performance, energy utilization, and nutrient digestibility in broilers. This study, conducted by Abdollahi et al. (2016), used four treatments with increasing dietary inclusion levels of PKM at 0, 8, 16, 24%, respectively, with

and without enzyme supplementation. These diets were corn, SBM, and PKM based and formulated to meet Ross 308 nutrient recommendations. This study found that increasing the PKM inclusion rate had a significant negative impact on weight gain when no enzyme was supplemented. However, it was found that supplemental enzymes increased weight gain and feed conversion ratio, regardless of PKM inclusion level. There was no significant difference in feed intake and feed efficiency with diets containing a PKM inclusion rate at 16% and higher with the supplemented enzymes. It was noted that the main effect of increasing PKM inclusion rate is that total tract retention of nitrogen, fat, and starch was significantly reduced; however, the inclusion of enzymes had increased GE retention. This study concluded that having an inclusion rate of PKM up to 16% would have no detrimental effects on growth performance, and that enzyme supplementation for diets with high inclusion rates of PKM may not be sufficient enough to hydrolyze β-1,4-glucosidic linkages and reduce antinutritional effects in broilers.

CONCLUSION

With the increasing prices of feed ingredients, it is important to seek suitable alternatives to partially replace ingredients of high cost, such as corn and SBM. Due to increasing production of palm oil, the byproduct, PKM, is increasing, thereby incentivizing its use in animal feeds. Palm kernel meal has not typically been used in monogastric diets due to its high fiber and NSP content and low AA and crude protein levels; however, the use of carbohydrase enzymes and phytases may help to increase the nutritional quality of this ingredient. More research needs to be conducted to better understand the best use of this ingredient in poultry diets.

The objective of this thesis was to evaluate the nutritional value of PKM for poultry. Extensive chemical analysis was initially conducted to determine the nutritional composition of

PKM from several countries. Four experiments were then conducted to evaluate PKM when fed to chickens. The first and second experiment was conducted to determine the TME_n concentration and standardized digestibility of AA in roosters for 12 global PKM samples. The other two experiments were conducted to determine apparent ileal P digestibility, the effect of dietary Ca level on P digestibility, and relative P bioavailability of two PKM samples from Mexico and Costa Rica in commercial broiler chicks.

LITERATURE CITED

Abdeltawab, A. M., and M. S. Khattab. 2018. Utilization of palm kernel cake as a ruminant feed for animala: a review. Asian J. Biol. Sci. 11:157-164.

Abdollahi, M., B. Hosking, D. Ning, and V. Ravindran. 2016. Influence of palm kernel meal inclusion and exogenous enzyme supplementation on growth performance, energy utilization, and nutrient digestibility in young broilers. Anim. Biosci. 29:539-548.

Adedokun, S. A., and O. Adeola. 2013. Calcium and phosphorus digestibility: metabolic limits. J. Appl. Poult. Res. 22:600-608.

Ahmad, S., A. Alimon, A. Kasim, M. H. Mohd Noor, and A. A. Samsudin. 2014. Improving nutritional values of palm kernel cake (PKC) as poultry feeds: a review. Malays. J. of Anim. Sci. 17:1-18.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-460.

Balandrán-Quintana, R. R., A. M. Mendoza-Wilson, G. R.-C. Montfort, and J. Á. Huerta-Ocampo. 2019. Chapter 4 - Plant-based proteins. Pages 97-130 in Proteins: Sustainable Source, Processing and Applications. C. M. Galanakis, ed. Academic Press, Chania, Greece

Brameld, J. M., and T. Parr. 2016. Improving efficiency in meat production. Proc. Nutr. Soc. 75:242-246.

Cowieson, A. J., T. Acamovic, and M.R. Bedford. 2003. The effect of phytase and phytic acid on endogenous losses from broiler chickens. Br. Poult. Sci. 44:23-24.

Daud, M. J., and M. C. Jarvis. 1992. Mannan of oil palm kernel. Phytochemistry 31:463-464.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2021. Comparison of the apparent ileal calcium digestibility of limestone in broilers and layers. Br. Poult. Sci. 62: 852-857.

Garcia, A. R., A. B. Batal, and N. M. Dale. 2007. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci. 86:94-101.

Goyum Group. 2021. Palm kernel solvent extraction plant. Accessed Mar. 2023. https://www.oilexpeller.com/palm-kernel-solvent-extraction-plant/

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

Henderson, J., and D. J. Osborne. 2000. The oil palm in all our lives: how this came about. Endeavour. 24:63-68.

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

Jackson, M. E., K. Geronian, A. Knox, J. McNab, and E. McCartney. 2004. A dose-response study with the feed enzyme beta-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. Poult. Sci. 83:1992-1996.

Kessler, J. W., T. H. Nguyen, and O. P. Thomas. 1981. The amino acid excretion values in intact and cecectomized negative control roosters used for determining metabolic plus endogenous urinary losses. Poult. Sci. 60:1576-1577.

Kim, E. J., P. L. Utterback, T. J. Applegate, and C. M. Parsons. 2011. Comparison of amino acid digestibility of feedstuffs determined with the precision-fed cecectomized rooster assay and the standardized ileal amino acid digestibility assay. Poult. Sci. 90:2511-2519.

Kleiber, M. 1961. The fire of life. An introduction to animal energetics. In the fire of life: an introduction to animal energetics. RE Krieger Pub Co.

Knudsen, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Tech. 67:319-338.

Leeson, S., and J. D. Summers. 2001. Scott's nutrition of the chicken. 4th ed. Univ. Books, Guelph, Ontario, Canada.

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

Leske, K., and C. Coon. 2002. The development of feedstuff retainable phosphorus values for broilers. Poult. Sci. 81:1681-1693.

Li, X., D. Zhang, T. Y. Yang, and W. L. Bryden. 2016. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. Agriculture. 6:1-15.

Li, X., D. Zhang, and W. L. Bryden. 2017. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? Anim. Prod. Sci. 57:2304-2310.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. J. Anim. Sci. 92:5520-5529.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

TNC Council. 2023. The National Chicken Council. Accessed Mar. 2023. https://www.chickencheck.in/

Natsir, H, I. Djunaidi, O. Sjofjan, A. Suwanto, E. Puspitasari, and L. J. Virginia. 2018. The effect of corn substitution with palm kernel meal treated by enzyme on production performance and carcass quality of broilers. Bulletin Peternakan. 42:103-108.

Nelson, T. S., and A. C. Walker. 1964. The biological evaluation of phosphorus compounds: a summary. Poult. Sci. 43:94–98.

OECD, and FAO. 2022. OECD-FAO Agricultural Outlook 2022-2031. OECD Publishing. Paris.

O'Mara, F. P., F. J. Muligan, E. J. Cronin, M. Rath, and P. J. Caffrey. 1999. The nutritive value of palm kernel meal measured in vivo and using rumen fluid and enzymatic techniques. Liv. Prod. Sci. 60: 305-316.

Pallauf J., and G. Rimbach. 1997. Nutritional significance of phytic acid and phytase. Arch. Tierernahr. 50:301-319.

Parsons. C. M. 1984. Influence of caecectomy and source of dietary fibre or starch on excretion of endogenous amino acids by laying hens. Br. J. Nutr. 51:541-548.

Parsons, C. M. 2020. Unresolved issues for amino acid digestibility in poultry nutrition. J. Appl. Poult. Res. 29:1-10.

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

Penido, M. G., and U. S. Alon. 2012. Phosphate homeostasis and its role in bone health. Pediatr. Nephrol. 27:2039-2048.

Perez, J. F., A. G. Gernat, and J. G. Murillo. 2000. Research notes: The effect of different levels of palm kernel meal in layer diets. Poult. Sci. 79:77-79.

Pickard, M. D. 2005. By-products utilization. In: Bailey's industrial oil products. Edible Oil and Fat Products: Products and applications. Shahidi, F. (Ed). 6th ed. Vol. 4. Wiley-Interscience.

Pirker, J., A. Mosnier, F. Kraxner, P. Havlík, and M. Obersteiner. 2016. What are the limits to oil palm expansion? Global Environ. Change. 40:73-81.

Potchanakorn, M., and L. M. Potter. 1987. Biological values of phosphorus in various sources for young turkeys. Poult. Sci. 66:505-51.

Proszkowiec-Weglarz, M, and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility. J. Appl. Poult. Res. 22:609-627.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Br. Poult. Sci. 40:266-274.

Rodehutscord, M. 2009. Approaches and challenges for evaluating phosphorus sources for poultry. 17th Eur. Symp. Poult. Nutr. 17:2–6.

Rodehutscord, M., A. Dieckmann, M. Witzig, and Y. Shastak. 2012. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. Poult. Sci. 91:965-971.

Ruben, N. T., K. J. Raphaël, M. K. Hervé, L. T. Brice, E. N. LangstonWilfried, and T. Alexis. 2020. Comparative study of artisanal and industrial palm kernel meal as a potential substitutes of soybean meal in broiler chickens diet. Am. J. Food Sci. and Technol. 8:154-160.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598-606.

Shastak, Y., and M. Rodehutscord. 2013. Determination and estimation of phosphorus availability in growing poultry and their historical development. World's Poult. Sci. J. 69:569–586.

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

Spring, P. 2013. The challenge of cost effective poultry and animal nutrition: Optimizing existing and applying novel concepts. Lohmann Inf. 48:38-46.

Sundu, B., A. Kumar, and J. Dingle. 2006. Palm kernel meal in broiler diets: effect on chicken performance and health. World's Poult. Sci. J. 62:316-325.

Sundu, B., A. Kumar, A., and J. Dingle. 2008. Amino acid digestibilities of palm kernel meal in poultry. J. Indones. Trop. Anim. Agric, 33:139-144.

United States Department of Agriculture. 2023. U.S. soybean oil exports forecast at record low. https://usda.library.cornell.edu/

Vijay, V., S. L. Pimm, C. N. Jenkins, and S. J. Smith. 2016. The Impacts of oil palm on recent deforestation and biodiversity loss. PLOS ONE. 11.

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

CHAPTER 2:

DETERMINATION OF NUTRITIONAL COMPOSITION, TME_n, AND STANDARDIZED AMINO ACID DIGESTIBILITY OF PALM KERNEL MEAL FROM SEVERAL COUNTRIES

ABSTRACT

Extensive nutritional analyses were conducted for palm kernel meal (PKM) sourced from 5 countries. Two precision-fed rooster trials were then conducted to evaluate the nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid (AA) digestibility of 10 PKM samples (PKM 1-10). The TME_n was determined using conventional Single Comb White Leghorn roosters, and the standardized AA digestibility was determined using cecectomized roosters. Roosters were fasted for 26 h prior to crop intubation with 25 g of each PKM. Excreta were then collected for 48 h post-intubation, then freeze dried for analysis. Statistical analyses were conducted using a one-way ANOVA for a completely randomized design. The least significant difference test was conducted to determine if differences between or among individual treatments were significant at $P < 0.05$. The PKM samples 1-10 were found to have an average of 14% CP (DM basis), 8% fat, and 64% NDF, 0.4% Ca, 0.7% P, and 1.3% phytic acid. The average TME_n for all 10 PKM samples were 2,082 kcal/kg (DM basis). On average, the Lys, Met, Cys, and Thr digestibility values for all 10 PKM samples were 47%, 73%, 47%, and 64%, respectively. All PKM samples contained highly variable and often low levels of TME_n and digestible amino acids. The nutrient composition also varied substantially among the different PKM samples.

INTRODUCTION

Palm oil is one of the highest produced oilseeds globally and is expected to continue to grow with the increasing population and economy. This oilseed is essential in many

underdeveloped and developing countries as a food and fuel source (Chew et al., 2021); however, with increasing production of palm oil for human use, there is heightened interest to use the by-product, palm kernel meal (PKM), in animal feeds. While PKM has been typically been used in rabbit and ruminant feed due to the high fiber content of this ingredient, there has been an economic incentive to use PKM in poultry diets (Sundu et al., 2006). However, there is limited research conducted on PKM included within poultry diets. More research needs to be conducted on this alternative feed ingredient to better understand the nutritional composition and values, particularly its metabolizable energy and AA composition and availability.

While apparent metabolizable energy (AME) is the most commonly used method to determine energy values for feed ingredients within the poultry industry, true metabolizable energy (TME) is a faster, alternative method. Apparent metabolizable energy is the gross energy of the feed ingredient with the gross energy of feces and urine subtracted (Leeson and Summers, 2001; NRC, 1994). The TME is calculated with fecal and urinary energy and metabolic and endogenous energy losses subtracted from ingredient gross energy (Leeson and Summers, 2001). The TME is calculated through the precision-fed rooster assay developed by Sibbald (1976) and has since been further modified to correct to zero nitrogen retention, known as TME_n (Parsons et al., 1982). The assay can also be used to determine AA digestibility (Engster et al., 1985). Single Comb White Leghorn roosters are usually used in the TME_n assays, while cecectomized Single Comb White Leghorn roosters are used in the AA digestibility assays (Parsons et al., 1985). This assay would be effective for PKM nutritional evaluation due to it being fast and less expensive, and not requiring a large amount of sample or large number of animals.

While there has been little research conducted on PKM, there has been some research done with adding carbohydrase enzymes (e.g., mannanase) to broiler diets to increase

digestibility values of the nutrients. Supplementation of enzymes to diets containing PKM was shown to improve protein, fat, and fiber digestibility and increase broiler weight gain (Iyayi and Davies, 2005; Abdollahi et al., 2016). Therefore, using PKM in diets, despite the high fiber content, may still have beneficial uses in poultry diets as an alternative feed ingredient. The objective of this study was to determine the nutritional composition, TME_n, and AA digestibility of 10 PKM samples from 5 countries (Colombia, Costa Rica, Mexico, Indonesia, and Thailand) using the precision-fed rooster assay.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Ingredients and analysis

Ten palm kernel meals (PKM) were obtained from a total of 5 countries by Elanco (2500 Innovation Way, Greenfield, IN 46140, USA), including Colombia, Costa Rica, Mexico, Indonesia, and Thailand. Analyses were conducted by the University of Missouri-Columbia Experiment Station Chemical Laboratories to determine crude protein (CP) by measuring N content via combustion (Method 990.03; AOAC International, 2007), crude fat via ether extract (Method 920.39 (A); AOAC International, 2007), neutral detergent fiber (Method 2002.04; AOAC International, 2007), ash (Method 942.05; AOAC International, 2007), Ca, P, and Na via inductively coupled plasma-mass spectrometry (Method 958.01; AOAC International, 2007), and AA concentrations (Method 982.30 E [a, b, and c]; AOAC International, 2007). Phytic acid content of samples was determined at Eurofins Nutrition Analysis Center, Des Moines, IA. Dry matter content of samples were determined at the University of Illinois (Method 930.15; AOAC

International 2007). Gross energy analyses were performed by N*P Analytical Laboratories, St. Louis, MO.

Diets and Experimental Design

The 10 PKM samples from Colombia, Costa Rica, Mexico, Indonesia, and Thailand were evaluated for TME_n and standardized AA digestibility. Experiments were conducted using the precision-fed rooster assay consisting of Single Comb White Leghorn roosters. While conventional Leghorn roosters were used to evaluate TME_n, cecectomized Leghorn roosters were used to evaluate AA digestibility. There were 4 replicates of 1 individually-caged rooster for each sample. The roosters were fasted for 26 h prior to being crop-intubated with 25 g of the assigned PKM sample with a tube to ensure that there was no other feed in their gastrointestinal tract. After the roosters were precision-fed the PKM sample, they were placed in an individual wire cage with a tray underneath to quantitatively collect all the excreta for a total of 48 h post-intubation. The excreta samples were then freeze-dried, weighed, and ground prior to being analyzed. The excreta from the conventional roosters were analyzed for GE and N, as described previously, and then the TME_n was calculated (Parsons et al., 1982). Excreta from the cecectomized roosters were analyzed for AA, as described previously, and then standardized AA digestibility values were calculated (Engster et al., 1985). The basal endogenous correction for standardization was determined using AA excreted by roosters that were fasted for 48 h.

Statistical Analysis

Data were initially analyzed using SAS software (SAS Institute., 2010) by using an ANOVA procedure for a completely randomized design. Significant differences between or among treatment means were determined using Fisher's least significant difference test with

differences in values considered significant at $P < 0.05$. The individual roosters were used as the experimental unit for all statistical analysis.

RESULTS AND DISCUSSION

Nutrient Composition

The analyzed nutrient composition of PKM samples 1-10 is found in Table 2.1. The CP of the PKM samples ranged from 11.2% to 16.6% with PKM 1 being the lowest and PKM 7 being the highest, on a DM basis. These CP values are lower compared with Onwudike (1986) and Perez et al. (2000), where CP values of 19.2% and 17.20%, respectively, were reported. The fat content of the 10 PKM samples was highly variable and ranged from 5.9% to 10.5%. The PKM 2 had the lowest value, while PKM 10 had the highest value. The average of these fat values was approximately the same as that reported by Kim et al. (2001) (8.1%, DM basis). The crude fiber values ranged from 18.4% (PKM 9) to 31.6% (PKM 1). The average of the crude fiber values (24.2%) was higher compared with the 17.4% crude fiber value reported by Kim et al. (2001). The neutral detergent fiber (NDF) was consistent among PKM samples 1-10, ranging from 60.8% to 67.3%, on a DM basis (Table 2.1). These values are higher than the value of 53.1% reported by Huang et al. (2017). The acid detergent fiber values did not vary greatly among samples and ranged from 40.5% to 49.3%, with PKM 7 being the lowest and PKM 1 being the highest. These values are similar compared with the Onifade and Babatunde (1998) value of 42.7%. All these fiber levels were expected to be high, and the high fiber content is a primary reason why PKM has historically been used in ruminant and rabbit feeds.

Calcium (Ca), chloride (Cl), sodium (Na), and potassium (K) (Table 2.1) overall were substantially variable among the 10 PKM samples with averages of 0.36%, 0.15%, 0.002%, and

0.61%, respectively. The Na values were much lower compared with the Rostagno (2011) value of 0.03%, while the K value of 0.61% is close to the value of 0.68% (Rostagno, 2011). There is limited research on PKM, making it difficult to find comparable values for Ca and Cl. The P content of all samples were highly variable ranging from 0.55% (PKM 1) to 0.84% (PKM 7); however, the average of the 10 samples was lower than the 0.8% P content reported by Nwokolo and Bragg (1977). The phytic acid content of the 10 PKM samples ranged from 1.07% to 1.61%, with PKM 3 being the lowest and PKM 7 being the highest. These phytic acid values are not greatly different from the value of 1.25% reported by Almaguer et al. (2014).

The gross energy of PKM 1-10 were consistent and ranged from 4,350 to 4,670 kcal/kg DM (Table 2.2). These values are less than the value of 4,735 kcal/kg (DM basis) as reported by Agunbiade et al. (1999), but similar to the value reported by Huang et al. (2017; 4,625 kcal/kg DM basis). There was substantial variability ($P < 0.05$) for TME_n (Table 2.2) among PKM samples; values ranged from 1,644 kcal/kg to 2,511 kcal/kg DM, with PKM 2 being the lowest and PKM 10 being the highest. The average TME_n for the 10 PKM samples (2,082 kcal/kg DM) was lower than the value of 2,603 kcal/kg (DM basis) reported by Muangkeow and Chinajariyawong (2011). The variation in TME_n among samples within this study and difference compared with the earlier study is likely due partially to differences in fat content. Indeed, the 2 PKM with the lowest TME_n values in the current study had the lowest fat levels (less than 6% fat). Although an in-depth, detailed comparison of PKM among the different countries is probably not valid since there were only 2 samples from each country, there were some interesting differences among countries. The PKM samples from Mexico, 5 and 6, had higher TME_n ($P < 0.05$) than PKM samples from Colombia, 1 and 2, and PKM samples from Costa Rica, 3 and 4. Part of the difference was probably due to the fiber content as the crude fiber,

NDF, and ADF in the Mexico samples were lower than the Colombia and Costa Rica samples. In other instances, however, TME_n was inconsistent for samples within the same country. For example, the 2 samples that had the highest TME_n were PKM 8 and 10 from Indonesia and Thailand, respectively. However, the TME_n of the other sample from these countries was much lower ($P < 0.05$).

Total AA concentrations, standardized AA digestibility values, and standardized AA concentrations for PKM samples 1-10 are found Tables 2.3, 2.4, and 2.5, respectively. The total AA concentrations among the 10 PKM samples were generally low and often variable. However, these values are not greatly different from values reported by Abdollahi et al. (2015) (i.e., 0.37% for Lys compared to PKM 1 Lys at 0.37%) The standardized AA digestibility values ranged from 14.6% to 88.2% among the individual AA. Among the 10 PKM samples, the standardized AA digestibility for Lys, Met, Cys, and Thr ranged from 34.5% to 59.7%, 65.5% to 80.3%, 29.3% to 68.8%, and 53.5% to 76.9%, respectively, having significant variation ($P < 0.05$). There is limited research in the literature to which AA digestibility of PKM in poultry can be compared among studies. Standardized AA concentrations for the 10 PKM samples (Table 2.5) for some individual AA were highly variable (i.e., Pro ranging from 0.05% to 0.44%), while other individual AA were consistent among the samples (i.e., Trp ranging from 0.06% to 0.08%). Overall, the standardized digestible AA concentrations were low for the PKM samples tested.

When comparing AA digestibility among samples from different countries, there was no consistent pattern. In general, even the 2 samples from the same country often differed greatly. The values in PKM 5 and 6 from Mexico were more consistent than most other countries. PKM 9 and 10 from Thailand did have 2 of the lowest Lys digestibility values, suggesting possible heat damage. Lysine and Cys were two of the AA showing the greatest variation among samples.

Part of the reason for the large variation in Lys among samples may be due to heat damage, since some of the samples were very dark in color (i.e., PKM 9 and 10 from Thailand). The reason for the particularly large variation in Cys digestibility among samples is unknown, although digestibility of this AA can also be affected somewhat by over processing and heat damage (Papadopoulos, 1989).

In summary, the TME_n, AA digestibility, and nutrient composition values were highly variable among the 10 PKM samples. The high mean NDF content, 64.4%, indicates that this feed ingredient would be poorly digested by poultry. Consequently, the average TME_n of the PKM samples (2,082 kcal/kg DM) is much lower than the TME_n of other standard ingredients such as corn (3,899 kcal/kg DM) and soybean meal (2,761 kcal/kg DM) as reported by the NRC (1994). The variability in nutrient composition among PKM could be due to the country that the sample originated from, the processing method or both.

TABLES

Table 2.1 Analyzed composition of palm kernel meal[1]

	Palm kernel meal sample number[2]										
Item, %	1	2	3	4	5	6	7	8	9	10	Mean
DM	93.2	93.4	92.7	95.3	92.6	90.8	96.6	88.7	90.3	93.8	92.7
CP	11.20	14.31	11.24	12.01	14.48	13.63	16.57	13.26	15.34	15.21	13.73
Crude fat	8.57	5.86	9.03	6.88	6.62	6.86	5.89	7.90	6.70	10.47	7.48
Crude fiber	31.63	27.80	28.43	27.36	24.01	22.44	22.46	18.76	18.44	20.43	24.18
NDF	67.19	65.78	67.26	65.81	64.14	62.88	65.04	60.78	63.24	61.61	64.37
ADF	49.33	48.98	47.44	45.71	42.82	45.35	40.53	42.23	41.61	40.64	44.46
Ca	0.18	0.18	0.19	0.31	0.40	0.33	0.53	0.50	0.41	0.51	0.36
Cl	0.16	0.17	0.11	0.13	0.14	0.14	0.16	0.14	0.15	0.15	0.15
P	0.55	0.63	0.56	0.66	0.74	0.67	0.84	0.64	0.77	0.75	0.68
Na	0.002	0.001	0.0009	0.002	0.002	0.003	0.002	0.003	0.002	0.0004	0.002
K	0.51	0.63	0.50	0.64	0.63	0.57	0.72	0.59	0.64	0.63	0.61
Phytic acid	1.15	1.16	1.07	1.32	1.44	1.4	1.61	1.31	1.44	1.46	1.34

[1]Values are expressed on a DM basis, excluding DM which is expressed on an as-fed basis.

[2]Samples 1-2 are from Colombia, 3-4 from Costa Rica, 5-6 from Mexico, 7-8 from Indonesia, and 9-10 from Thailand.

Table 2.2 Gross energy and true metabolizable energy (TME_n) of palm kernel meal[1]

	Palm kernel meal sample number[2]										
Item	1	2	3	4	5	6	7	8	9	10	Mean
Gross energy (kcal/kg)	4,670	4,540	4,610	4,520	4,470	4,490	4,510	4,450	4,350	4,570	4,518
TME_n[3] (kcal/kg)	1,896^{cd}	1,644^{d}	1,956^{cd}	1,832^{cd}	2,293^{ab}	2,406^{a}	1,759^{d}	2,439^{a}	2,081^{bc}	2,511^{a}	2,082
Pooled SEM for TME_n											111

[a-d]Values within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Values are expressed on a DM basis.

[2]Samples 1-2 are from Colombia, 3-4 from Costa Rica, 5-6 from Mexico, 7-8 from Indonesia, and 9-10 from Thailand.

[3]TME_n values are means of 4 individually-caged conventional roosters.

Table 2.3 Total amino acid concentrations in palm kernel meal (%, DM basis)

	Palm kernel meal sample number[1]									
Amino acid	1	2	3	4	5	6	7	8	9	10
Asp	0.83	1.07	0.89	0.97	1.18	1.04	1.30	1.09	1.20	1.17
Thr	0.33	0.40	0.36	0.38	0.46	0.41	0.50	0.43	0.47	0.45
Ser	0.39	0.48	0.43	0.46	0.55	0.49	0.59	0.53	0.56	0.53
Glu	1.83	2.52	1.98	2.25	2.66	2.26	2.96	2.40	2.71	2.60
Pro	0.35	0.50	0.40	0.50	0.60	0.54	0.67	0.56	0.65	0.65
Gly	0.47	0.64	0.51	0.57	0.68	0.59	0.75	0.60	0.68	0.65
Ala	0.42	0.58	0.45	0.50	0.60	0.52	0.68	0.54	0.61	0.59
Cys	0.15	0.20	0.15	0.16	0.21	0.19	0.20	0.21	0.22	0.21
Val	0.54	0.75	0.58	0.66	0.79	0.67	0.88	0.71	0.79	0.78
Met	0.19	0.25	0.20	0.23	0.29	0.25	0.31	0.26	0.27	0.27
Ile	0.39	0.52	0.41	0.46	0.55	0.47	0.61	0.49	0.55	0.54
Leu	0.66	0.90	0.71	0.79	0.95	0.82	1.07	0.86	0.96	0.94
Tyr	0.23	0.31	0.26	0.27	0.34	0.30	0.37	0.31	0.32	0.33
Phe	0.43	0.59	0.47	0.51	0.64	0.55	0.71	0.58	0.65	0.62
Lys	0.37	0.42	0.37	0.46	0.44	0.45	0.44	0.43	0.42	0.48
His	0.18	0.24	0.20	0.23	0.26	0.23	0.29	0.22	0.24	0.26
Arg	1.15	1.57	1.28	1.52	1.70	1.57	1.83	1.66	1.71	1.76
Trp	0.08	0.09	0.08	0.09	0.10	0.10	0.10	0.10	0.10	0.10

[1]Samples 1-2 are from Colombia, 3-4 from Costa Rica, 5-6 from Mexico, 7-8 from Indonesia, and 9-10 from Thailand.

Table 2.4 Standardized digestibility values of amino acids in palm kernel meal (%)

	Palm kernel meal sample number[1]											
Amino acid	1	2	3	4	5	6	7	8	9	10	Mean	Pooled SEM
Asp	51.5^{c}	63.8^{ab}	69.9^{a}	61.9^{abc}	66.3^{ab}	64.6^{ab}	58.3^{abc}	68.5^{a}	59.7^{abc}	56.2^{bc}	62.07	4.23
Thr	53.5^{c}	63.8^{abc}	76.9^{a}	61.1^{abc}	71.9^{ab}	69.5^{abc}	63.8^{abc}	68.3^{abc}	62.3^{abc}	55.1^{bc}	64.63	5.86
Ser	61.1^{b}	67.1^{ab}	81.3^{a}	68.4^{ab}	75.8^{ab}	72.9^{ab}	68.9^{ab}	74.9^{ab}	66.9^{ab}	61.5^{b}	69.88	5.23
Glu	68.2^{c}	72.9^{bc}	82.9^{a}	76.1^{abc}	76.0^{abc}	76.4^{abc}	67.5^{c}	77.5^{ab}	69.4^{bc}	70.2^{bc}	73.72	3.10
Pro	14.6^{b}	40.5^{ab}	66.5^{a}	45.0^{a}	56.5^{a}	52.4^{a}	45.8^{a}	53.4^{a}	49.2^{a}	67.7^{a}	49.16	10.49
Ala	55.4^{b}	63.9^{ab}	75.6^{a}	62.5^{ab}	70.8^{ab}	64.2^{ab}	62.4^{ab}	68.7^{ab}	62.0^{ab}	54.9^{b}	64.04	6.15
Cys	29.3^{c}	43.1^{bc}	68.8^{a}	43.9^{abc}	53.7^{abc}	55.7^{ab}	35.7^{bc}	57.9^{ab}	45.4^{abc}	40.9^{bc}	47.47	8.74
Val	56.3^{c}	65.4^{abc}	75.5^{a}	64.9^{abc}	72.7^{ab}	67.0^{abc}	64.9^{abc}	69.1^{abc}	64.6^{abc}	61.3^{bc}	66.17	4.71
Met	67.0^{bc}	70.8^{abc}	80.3^{a}	71.1^{abc}	77.8^{ab}	74.4^{abc}	71.6^{abc}	76.1^{abc}	70.7^{abc}	65.5^{c}	72.50	3.83
Ile	59.6^{b}	67.9^{ab}	77.0^{a}	65.9^{ab}	73.5^{ab}	68.1^{ab}	67.9^{ab}	71.5^{ab}	66.7^{ab}	61.1^{b}	67.85	4.93
Leu	62.9^{b}	69.4^{ab}	80.2^{a}	69.9^{ab}	76.4^{ab}	72.1^{ab}	69.2^{ab}	74.7^{ab}	68.9^{ab}	64.4^{b}	70.73	4.72
Tyr	45.5^{c}	60.9^{abc}	75.4^{a}	59.2^{abc}	69.2^{ab}	67.0^{ab}	64.7^{ab}	69.3^{ab}	62.1^{abc}	54.9^{bc}	62.80	6.02
Phe	63.4^{c}	71.0^{abc}	80.5^{a}	70.2^{abc}	77.9^{ab}	73.2^{abc}	71.6^{abc}	76.1^{ab}	71.3^{abc}	67.2^{bc}	72.25	4.21
Lys	44.9^{ab}	49.9^{ab}	59.7^{a}	51.0^{ab}	52.6^{ab}	51.1^{ab}	36.3^{ab}	51.4^{ab}	38.6^{ab}	34.5^{b}	47.01	8.16
His	56.2^{b}	65.8^{ab}	74.9^{a}	63.3^{ab}	68.3^{ab}	67.9^{ab}	62.7^{ab}	65.9^{ab}	61.7^{ab}	59.0^{b}	64.57	4.59
Arg	78.2^{c}	84.2^{ab}	88.2^{a}	83.8^{abc}	84.9^{ab}	84.5^{ab}	81.5^{bc}	85.1^{ab}	80.2^{bc}	80.6^{bc}	83.12	2.08
Trp	69.3^{abc}	69.2^{abc}	74.2^{ab}	65.7^{bc}	74.3^{ab}	79.9^{a}	68.9^{abc}	74.2^{ab}	70.9^{abc}	60.9^{c}	70.74	4.25

[a-c] Values within a row with no common superscript are significantly different ($P < 0.05$).

[1]Samples 1-2 are from Colombia, 3-4 from Costa Rica, 5-6 from Mexico, 7-8 from Indonesia, and 9-10 from Thailand.

Values are means of 4 individually-caged cecectomized roosters.

Table 2.5 Standardized digestible amino acid concentrations in palm kernel meal (%)[1]

	Palm kernel meal sample number[2]										
Amino acid	1	2	3	4	5	6	7	8	9	10	Mean
Asp	0.43	0.68	0.62	0.60	0.78	0.67	0.76	0.75	0.72	0.66	0.67
Thr	0.18	0.26	0.28	0.23	0.33	0.28	0.32	0.29	0.29	0.25	0.27
Ser	0.24	0.32	0.35	0.31	0.42	0.36	0.41	0.40	0.37	0.33	0.35
Glu	1.25	1.84	1.64	1.71	2.02	1.73	2.00	1.86	1.88	1.83	1.78
Pro	0.05	0.20	0.27	0.23	0.34	0.28	0.31	0.30	0.32	0.44	0.27
Ala	0.23	0.37	0.34	0.31	0.42	0.33	0.42	0.37	0.38	0.32	0.35
Cys	0.04	0.09	0.10	0.07	0.11	0.11	0.07	0.12	0.10	0.09	0.09
Val	0.30	0.49	0.44	0.43	0.57	0.45	0.57	0.49	0.51	0.48	0.47
Met	0.13	0.18	0.16	0.16	0.23	0.19	0.22	0.20	0.19	0.18	0.18
Ile	0.23	0.35	0.32	0.30	0.40	0.32	0.41	0.35	0.37	0.33	0.34
Leu	0.42	0.62	0.57	0.55	0.73	0.59	0.74	0.64	0.66	0.61	0.61
Tyr	0.10	0.19	0.20	0.16	0.24	0.20	0.24	0.21	0.20	0.18	0.19
Phe	0.27	0.42	0.38	0.36	0.50	0.40	0.51	0.44	0.46	0.42	0.42
Lys	0.17	0.21	0.22	0.23	0.23	0.23	0.16	0.22	0.16	0.17	0.20
His	0.10	0.16	0.15	0.15	0.18	0.16	0.18	0.14	0.15	0.15	0.15
Arg	0.90	1.32	1.13	1.27	1.44	1.33	1.49	1.41	1.37	1.42	1.31
Trp	0.06	0.06	0.06	0.06	0.07	0.08	0.07	0.07	0.07	0.06	0.07

[1]Values calculated as: (amino acid concentration × standardized digestibility) / 100.

[2]Samples 1-2 are from Colombia, 3-4 from Costa Rica, 5-6 from Mexico, 7-8 from Indonesia, and 9-10 from Thailand.

LITERATURE CITED

Abdollahi, M., B. Hosking, and V. Ravindran. 2015. Nutrient analysis, metabolisable energy and ileal amino acid digestibility of palm kernel meal for broilers. Anim. Feed Sci. Technol. 206:119-125.

Abdollahi, M., B. Hosking, D. Ning, and V. Ravindran. 2016. Influence of palm kernel meal inclusion and exogenous enzyme supplementation on growth performance, energy utilization, and nutrient digestibility in young broilers. Anim. Biosci. 29:539-548.

Agunbiade, J. A., J. Wiseman, and D. J. A. Cole. 1999. Energy and nutrient use of palm kernels, palm kernel meal and palm kernel oil in diets for growing pigs. Anim. Feed Sci. Technol. 80:165-181.

Almaguer, B. L., R. C. Sulabo, Y. Liu, and H. H. Stein. 2014. Standardized total tract digestibility of phosphorus in copra meal, palm kernel expellers, palm kernel meal, and soybean meal fed to growing pigs. J. Anim. Sci. 92:2473–2480.

AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.

Chew, C. L., C. Y. Ng, W. O. Hong, T. Y. Wu, Y. Y. Lee, L. E. Low, P. S. Kong, and E. S. Chan. 2021. Improving sustainability of palm oil production by increasing oil extraction rate: a review. Food and Bioprocess Technol. 14:574-586.

Engster H. M., N. A. Cave, H. Likuski, J. M. McNab, C. M. Parsons, and F. E. Pfaff. 1985. A collaborative study to evaluate a precision-fed rooster assay for true amino acid availability in feed ingredients. Poult. Sci. 64:487-498.

Huang C., S. Zhang, H. Stein, J. Zhao, D. Li, and C. Lai. 2017. Effect of inclusion level and adaptation duration on digestible energy and nutrient digestibility in palm kernel meal fed to growing-finishing pigs. Anim. Biosci. 31:395-402.

Iyayi, E. A., and B. I. Davies. 2005. Effect of enzyme supplementation of palm kernel meal and brewer's dried grain on the performance of broilers. Int. J. Poult. Sci. 4:76-80.

Kim, B. G., J. H. Lee, H. J. Jung, Y. K. Han, K. M. Park, and I. K. Han. 2001. Effect of partial replacement of soybean meal with palm kernel meal and copra meal on growth performance, nutrient digestibility and carcass characteristics of finishing pigs. Asian Australas. J. Anim. Sci. 14:821-830.

Leeson, S., and J. D. Summers. 2001. Scott's nutrition of the chicken. 4th ed. Univ. Books. Guelph, Ontario, Canada.

Muangkeow, N., and C. Chinajariyawong. 2011. Determination of true amino acid digestibility and metabolizable energy in fermented palm kernel meal with aspergillus wentii TISTR 3075 for chickens. Walailak J. Sci. Technol. 6:231–241.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Nwokolo, E. N., and D. B. Bragg. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci. 57:475-477.

Onifade A. A., and G. M. Babatunde. 1998. Comparison of the utilisation of palm kernel meal, brewers' dried grains and maize offal by broiler chicks. Br. Poult. Sci. 39:245-250.

Onwudike, O. C. 1986. Palm kernel meal as a feed for poultry. Composition of palm kernel meal and availability of its amino acids to chicks. Anim. Feed Sci. Technol. 16:179-186.

Papadopoulos, M. C. 1989. Effect of processing on high-protein feedstuffs: A review. Biol. Wastes. 29:123-138.

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

Parsons C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agric. Sci. 104:469-472.

Perez, J. F., A. G. Gernat, and J. G. Murillo. 2000. The effect of different levels of palm kernel meal in layer diets. Poult. Sci. 79:77-79.

Rostagno, H. S. 2011. Brazilian tables for poultry and swine. Composition of feedstuffs and nutritional requirements. 3rd ed. Univ. Federal de Viçosa. Vicosa, Brazil.

SAS Institute. 2010. SAS ® User's Guide: Statistics. Version 9.2 Edition. SAS Institute, Inc., Cary, NC.

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

Sundu, B., A. Kumar, and J. Dingle. 2006. Palm kernel meal in broiler diets: effect on chicken performance and health. World's Poult. Sci. J. 62:316-325.

CHAPTER 3:

DETERMINATION OF TME_n, STANDARDIZED AMINO ACID DIGESTIBILITY, PHOSPHORUS DIGESTIBILITY AND PHOSPHORUS BIOAVAILIBILITY OF PALM KERNEL MEAL FROM TWO COUNTRIES

ABSTRACT

Four experiments were conducted to determine nitrogen-corrected true metabolizable energy (TME_n), amino acid (AA) digestibility, bioavailability of P using a tibia ash bone bioassay, and ileal digestibility of P of 2 palm kernel meal (PKM) samples, Mexico PKM (M-PKM) and Costa Rica PKM (CR-PKM), in commercial Ross 308 broiler chicks. In Experiments 1 and 2, TME_n and AA digestibility of M-PKM and CR-PKM were determined using the precision-fed rooster assay in conventional and cecectomized roosters, respectively. The TME_n of M-PKM and CR-PKM were 2,058 and 1,870 kcal/kg, respectively (DM basis). The AA digestibility values for M-PKM and CR-PKM did not differ ($P > 0.05$) between the 2 samples for most AA. The digestibility values for Lys, Met, Cys, and Thr for M-PKM were 60%, 86%, 56%, and 67%, respectively, and for CR-PKM were 54%, 74%, 34%, and 61%, respectively. In Experiment 3, the bioavailability of P in the 2 PKM relative to KH_2PO_4 was determined using a tibia ash bone bioassay by feeding P-deficient diets supplemented with increasing levels of KH_2PO_4 or PKM from 8 to 18 d of age. Multiple regression of bone ash in mg/tibia on supplemental P intake yielded a slope-ratio P bioavailability value of 22% for M-PKM and 41% for CR-PKM. In Experiment 4, the ileal P digestibility of M-PKM and CR-PKM was determined by feeding dextrose-cornstarch diets containing PKM as the only source of P from 18 to 21 d of age. Diets contained a Ca:total P ratio of either 1.4 or 3.6. Diets were arranged with 2 × 2 factorial arrangement of dietary treatments including 2 PKM (M-PKM and CR-PKM) and 2 Ca inclusion rates (0.30% and 0.75%). Apparent ileal P digestibility at 21 d of age for M-PKM at 0.30% and 0.75% Ca was 38% and 24%, respectively, whereas CR-PKM was 48% and 30%,

respectively. Thus, ileal P digestibility was lower in M-PKM ($P < 0.05$) than CR-PKM, and P digestibility was negatively impacted by the increased dietary Ca level.

INTRODUCTION

Palm oil is one the highest produced oilseeds globally, with an estimated production of 79.46 million metric tons as of July 2023 (USDA, 2023), and is expected increase in the future due to increased demands for human use. With this increased production, quantities of the by-product PKM have grown significantly as well and there is an increased economic incentive to use the by-product in diets of monogastric animals (i.e., poultry and swine) in addition to the current use in ruminant and rabbit diets. Therefore, more research is needed to better understand the best use of PKM in monogastric animals, particularly with regard to P content and bioavailability.

Phosphorus is the third most expensive ingredient in poultry diets behind protein and energy (Potchanakorn and Potter, 1987) and is one of the most important structural components of bone. There is considerable interest in improving P utilization due to environmental concerns, caused by excess P excretion, and increasing prices of inorganic phosphate supplements (Mutucumarana et al., 2014). Up to 70% of P is stored as phytic acid in cereal grains and oilseeds (Leeson and Summers, 2001) causing reduced digestibility in poultry. Due to P not being optimally utilized from plant feedstuffs in poultry diets, enzymes, such as phytases, are increasingly being used to improve digestion and reduce P excretion (Abbasi et al., 2019). According to Abdollahi et al. (2015), 80% of the P in PKM is in the form of phytate. There has been very little research conducted on PKM to determine its digestible and bioavailable P content, or both; therefore, more research is needed.

There are multiple methodologies to determine to P bioavailability and digestibility in poultry feed ingredients, such as bone ash assays for relative bioavailability and ileal pre-cecal digestibility assays (Shastak and Rodehutscord, 2013). The determination of relative P bioavailability in a feed ingredient is done by comparing the specific feed ingredient to a highly digestible standard, such as KH_2PO_4, due to the P assumed to be 100% bioavailable (Gilles et al., 1954). Chicks are typically used in these assays because the zones of proliferation in their bones are sensitive to nutritional deficiencies, such as P (Nelson and Walker, 1964). The P bioavailability assay usually uses the slope-ratio method with a multiple linear regression analysis to determine P bioavailability of feedstuffs (Hurwitz, 1964). The ileal pre-cecal digestible P determination is the one of the preferred methods due to having a linear response across a wide range of dietary P levels (Rodehutscord et al., 2012). Even though this is the preferred assay for determining P digestibility, the dietary Ca to P ratio or Ca level can influence the digestion and absorption of P due to its antagonistic effects (Hurwitz and Bar, 1971). While there is a large amount of literature for true digestible P values for swine, historically, there have been limited attempts to determine digestible P feedstuffs for poultry (Mutucumarana et al., 2014).

The primary objectives of this study were to determine P bioavailability relative to potassium phosphate (KH_2PO_4) and apparent ileal P digestibility of 2 PKM samples from 2 countries, Mexico and Costa Rica, using different assays and dietary Ca levels. The nitrogen-corrected true metabolizable energy (TME_n) and amino acid (AA) digestibility were also determined to provide additional data to those presented in Chapter 2.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Ingredients and Analysis

Two PKM samples, 1 from Mexico (M-PKM) and 1 from Costa Rica (CR-PKM), were obtained from Elanco (2500 Innovation Way, Greenfield, IN 46140, USA). Dry matter content of samples was determined at the University of Illinois (Method 930.15; AOAC International, 2007) and gross energy analyses were performed by N*P Analytical Laboratories, St. Louis, MO. Phytic acid content of samples was determined at Eurofins Nutrition Analysis Center, Des Moines, IA. Analyses were conducted by the University of Missouri-Columbia Experiment Station Chemical Laboratories to determine Ca and total P via inductively coupled plasma-mass spectrometry (Method 958.01; AOAC International, 2007), AA concentrations (Method 982.30 E [a, b, and c]; (AOAC International, 2007), and titanium concentrations in experimental diets and ileal digesta using UV spectroscopy (Myers et al., 2004).

Diets and Experimental Design

Experiment 1 was conducted to determine the TME_n of M-PKM and CR-PKM. The precision-fed rooster assay was used to determine the TME_n using Single Comb White Leghorn roosters (Parsons et al., 1982). The roosters were fasted for 26 h prior to crop intubating with 25 g of each PKM sample. The roosters were then placed in individual cages with a tray underneath to quantitatively collect the excreta for 48 h. Four replicates of 1 individually-caged rooster were used for each PKM samples. The endogenous energy correction was determined in roosters that were fasted for 48 h. The excreta were then freeze-dried, ground, and analyzed for gross energy

and N as previously described. The TME_n was then calculated as described as Parsons et al. (1982).

Experiment 2 was conducted to determine the AA digestibility of M-PKM and CR-PKM following the same methodology as Experiment 1, except that cecectomized Single Comb White Leghorn roosters were used. Roosters were fasted for 26 h, then were precision-fed 25 g of each PKM sample. Roosters were placed in individual cages post crop-intubation with a tray underneath each cage to collect excreta for 48 h. Six replicates of 1 individually-caged rooster were used for M-PKM and 5 replicates for CR-PKM. The excreta were then freeze-dried, ground, and analyzed for AA as described previously. Basal endogenous AA losses were determined using cecectomized roosters that were fasted for 48 h, and then standardized AA digestibility values were calculated (Engster et al., 1985).

Experiment 3 was conducted to determine the relative bioavailability of P of 2 PKM samples, M-PKM and CR-PKM, relative to potassium phosphate (KH_2PO_4) using a chick bone ash assay. The chicks were housed in Petersime starter batteries with raised wire floors in a temperature-controlled room and they had ad libitum access to water and feed. This experiment was conducted using commercial Ross 308 male broiler chicks. During this 18-d trial, all chicks were fed a nutritionally complete corn-SBM pretest diet from 1 to 7 d of age. On d 8 of age, the chicks were weighed, wing-banded, then allotted to their respective dietary treatments. From 8 to 18 d of age, the chicks were fed 1 of 7 diets. A P-deficient corn-soybean meal-cornstarch-dextrose diet (0.18% non-phytate P) was formulated for diet 1 (Table 3.1). Diets 2 and 3 contained 0.05% and 0.10% supplemental P from KH_2PO_4, respectively, diets 4 and 6 contained an added 15% or 30% M-PKM, respectively, and diets 5 and 7 contained an added 15% or 30% CR-PKM, respectively (Table 3.1). The M-PKM, CR-PKM, and KH_2PO_4 were added to the diets

in place of cornstarch and dextrose. There were 6 replicate pens of chicks for each dietary treatment, with 5 chicks per pen assigned to each diet. At d 18 of age, chicks were weighed, then euthanized using CO_2 gas, and feed intake was recorded. The right tibia was then collected, autoclaved, cleaned, oven-dried at 100° C for 24 h, then ashed at 600° C for 24 h in a muffle furnace.

Experiment 4 was conducted to determine ileal P digestibility of 2 PKM samples, M-PKM and CR-PKM. Chicks were housed in Petersime starter batteries with raised wire floors in a temperature-controlled room, and they had ad libitum access to water and feed. This experiment was conducted on commercial Ross 308 male broiler chicks. During this 22-d trial, all chicks were fed a nutritionally complete corn-SBM pretest diet from 1 to 17 d of age. At d 18 of age the chicks were weighed, wing-banded, then allotted to their respective dietary treatments. From 18 to 22 d of age, the chicks were fed 1 of the 4 diets. Cornstarch-dextrose based diets were used in this experiment, with M-PKM and CR-PKM being the sole source of P (Table 3.2). Diets were arranged in a 2 × 2 factorial arrangement where PKM sample (M-PKM vs. CR-PKM) and Ca inclusion rate (0.30% vs. 0.75%) were considered as the fixed effects. The first two diets were formulated to contain 0.30% Ca (Ca:total P ratio of 1.4), and diets 3 and 4 were formulated to contain 0.75% Ca (Ca:total P ratio of 3.6). A Ca:total P ratio of 1.4 was used for diets 1 and 2, based on the 1.3 to 1.4 Ca to total P ratio that is recommended by the World's Poultry Science Association Working Group (WPSA, 2013). The 0.75% Ca level was used in diets 3 and 4 due to it being similar to the Ca level found in broiler finisher diets (NRC, 1994). Limestone was added in replacement of cornstarch to supplement the additional Ca, and 0.50% TiO_2 was used in all diets as an indigestible marker. There were 8 replicate pens of chicks for each dietary treatment, with 5 chicks per pen. At d 22 of age, chicks were weighed then euthanized using CO_2 gas, and

the feed intake was recorded. Ileal digesta were then collected from the Meckle's diverticulum to the ileal-cecal junction using a combination of gentle squeezing and flushing with water.

Statistical Analysis

The SAS software (SAS Institute INC., 2010) was used to analyze data obtained from all experiments. Data from all 4 experiments were analyzed using an ANOVA procedure for a completely randomized design, and differences between or among treatments were determined using Fisher's least significant difference test. Mean values among treatments were considered to be significantly different at $P < 0.05$. In Experiments 1 and 2, the experimental unit was the individually-caged roosters. In Experiments 3 and 4, the experimental unit was each pen of 5 chicks. Data from Experiment 3 were also analyzed using multiple linear regression (SAS GLM procedure) by regressing either tibia ash in mg/tibia or percent on supplemental P intake (g/chick) from KH_2PO_4 or PKM. The slope ratio method was then used to calculate the bioavailability of P in M-PKM and CR-PKM relative to the P in KH_2PO_4. Phosphorus bioavailability for KH_2PO_4 was assumed to be 100%.

RESULTS AND DISCUSSION

Experiment 1

The analyzed nutritional composition and TME_n for M-PKM and CR-PKM, on a DM basis, are found in Table 3.3. The M-PKM had a higher level of Ca, 0.24%, compared with CR-PKM, 0.19%. These levels were similar to Panigrahi and Powell's (1991) 2 PKM samples with Ca levels of 0.26% and 0.21%; however, the M-PKM and CR-PKM Ca levels were higher than reported by Kim et al. (2001) at 0.12%. The P levels were similar between M-PKM and CR-PKM; however, M-PKM had a higher phytic acid content of 1.46% compared with CR-PKM

with a phytic acid content of 1.06%. The M-PKM value is similar to a study by Nwokolo and Bragg (1977) where their PKM had a phytic acid level of 1.42%. The M-PKM also had a numerically higher TME_n than CR-PKM (2,058 and 1,870 kcal/kg DM, respectively). While there were some differences between these 2 PKM samples, their analyzed values were within the range and similar to the average of the PKM samples 1-10 reported in Chapter 2.

Experiment 2

Total AA concentrations, standardized AA digestibility values, and standardized AA concentrations for M-PKM and CR-PKM are found in Tables 3.4, 3.5, and 3.6, respectively. All total AA concentrations were slightly higher for M-PKM in comparison with CR-PKM. These total AA concentrations were similar with concentrations reported by Abdollahi et al. (2015; Lys at 0.37%, Met at 0.30%, Cys at 0.19%, and Thr at 0.44%). The M-PKM also had higher numerical digestibility of some AA compared with CR-PKM (Table 3.5), which ranged from 34.1% to 86.9% among individual AA and PKM samples; however, only Cys, Val, Met, Ile, Phe, and Arg were significantly different ($P < 0.05$) between PKM samples. Comparing the AA digestibility values reported by Sundu et al. (2008) with the average AA digestibilities obtained in Experiment 2 herein, the values were generally similar, with Lys, Met, and Thr digestibility being 57.1, 80.1, and 65.0% in the Sundu et al. (2008) study. Due to the concentration of total AA and higher digestibilities, standardized AA concentrations (Table 3.6) for M-PKM were higher compared with CR-PKM.

Experiment 3

The growth performance and tibia ash values for this experiment are shown in Table 3.7. Weight gain and feed intake increased ($P < 0.05$) with graded inclusion of KH_2PO_4, M-PKM,

and CR-PKM compared with the P-deficient basal (diet 1). Responses were inconsistent, probably due primarily to changes in ME among the diets. The bone ash (mg/tibia and %) presented a positive linear relationship ($P < 0.05$) for all 3 supplemental P sources in comparison with the P-deficient basal, with R^2 values from the multiple linear regression analysis being 0.91 and 0.86, respectively (see footnotes in Table 3.7).

The relative bioavailability values of P for each PKM (Table 3.8) equations shown in the footnotes of Table 3.7 were calculated using the slope-ratio method by multiple linear regression (Table 3.8). Relative P bioavailability values on mg/tibia basis for M- and CR-PKM were 21.5 and 40.5%, respectively. When expressed on a percent tibia ash basis, the values were 30.3% (M-PKM) and 55.6% (CR-PKM). The bioavailability values for M-PKM were lower ($P < 0.05$) than the respective values for CR-PKM. The bioavailable content was determined using the total P and bioavailable values (see footnotes in Table 3.8). The bioavailable content values calculated using mg/tibia data for M- and CR-PKM were 0.10 and 0.19%, respectively. When based on percent tibia ash data, the values were 0.14% and 0.26% for M-PKM and CR-PKM, respectively. The higher bioavailability values for CR-PKM could be at least partially due to M-PKM having a higher phytic acid content (Table 3.1). There is limited research on P bioavailability for PKM with which to compare results of the current study. However, Abdollahi et al. (2015) reported that 80% of the P in PKM is bound to phytate, which suggests the digestibility of P in PKM may be approximately 20%. The latter values agree well with the bioavailability value obtained for M-PKM based on total bone ash (mg/tibia) in the current study.

Experiment 4

Apparent ileal P digestibility values are shown Table 3.9. Due to the short duration of this experiment, weight gain, feed intake, and feed efficiency were not taken into consideration. Increased dietary Ca content reduced ($P < 0.05$) apparent ileal P digestibility for both M-PKM and CR-PKM. When Ca level increased from 0.30% to 0.75%, for diets containing M-PKM, ileal P digestibility decreased from 37.7% to 23.7%, respectively, while CR-PKM decreased from 48.1% to 30.0%, respectively. These results are in agreement with Hurwitz and Bar (1971) that there is an antagonistic relationship between increased levels of dietary Ca with P, because increased levels of Ca reduce intestinal digestion and absorption of P, at least partially. Ileal P digestibility was lower for M-PKM than CR-PKM, and as previously stated, CR-PKM (1.06% phytic acid) having a higher digestibility could be due to the fact that M-PKM has a higher phytic acid content (1.46%) in comparison.

In summary, M-PKM had an overall higher TME_n, total AA concentrations, standardized AA digestibility values, and digestible AA concentrations compared with CR-PKM. The CR-PKM had higher relative P bioavailability and ileal P digestibility than M-PKM. Increasing the dietary Ca level and dietary Ca:total P ratio decreased P digestibility for both PKM samples. The ileal P digestibility values determined in diets containing 0.75% Ca, a more practical level, were generally in good agreement with the relative bioavailability values based on bone ash (mg/tibia), suggesting that P digestibility in feed ingredients should be determined in diets containing more practical dietary Ca levels rather than diets containing deficient Ca levels. When averaging relative P bioavailability values based on bone ash as mg/tibia and ileal P digestibility determined in diets containing 0.75% Ca, the overall results indicate that the bioavailability or digestibility of P in PKM is approximately 30%. This value agrees with the generally accepted

conclusion that the availability of P in plant-based ingredients is approximately 33% (Leeson and Summers, 2001).

TABLES

Table 3.1 Ingredient composition of experimental diets in Experiment 3 for determination of relative phosphorus bioavailability for Mexico and Costa Rica palm kernel meals in broiler chickens.

	Dietary treatment						
Ingredient, %	1	2	3	4	5	6	7
Dextrose	10.00	10.00	10.00	5.00	5.00	-	-
Cornstarch	20.00	19.77	19.54	10.00	10.00	-	-
Corn	18.66	18.66	18.66	18.66	18.66	18.66	18.66
KH_2PO_4	-	0.23	0.46	-	-	-	-
Soybean meal	42.00	42.00	42.00	42.00	42.00	42.00	42.00
M-PKM[1]	-	-	-	15.00	-	30.00	-
CR-PKM[2]	-	-	-	-	15.00	-	30.00
Soybean oil	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Limestone	1.59	1.59	1.59	1.59	1.59	1.59	1.59
Dicalcium phosphate	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.36	0.36	0.36	0.36	0.36	0.36	0.36
L-Thr	0.07	0.07	0.07	0.07	0.07	0.07	0.07
L-Lys HCl	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Cl (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutrients:							
Ca	0.80	0.80	0.80	0.83	0.83	0.87	0.87
Nonphytate P	0.18	0.23	0.28	0.20	0.20	0.22	0.22

[1]M-PKM = Mexico palm kernel meal.

[2]CR-PKM = Costa Rica palm kernel meal.

[3]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL-αtocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

[4]Provided as milligrams per kilogram of diet: manganese, 75 mg from $MnSO_4 \cdot H_2O$; iron, 75 mg from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 mg from ethylene diamine dihydroiodide; selenium, 0.1 mg from Na_2SeO_3.

Table 3.2 Ingredient composition of experimental diets in Experiment 4 for determination of apparent ileal phosphorus digestibility for Mexico and Costa Rica palm kernel meal in broiler chickens

	Dietary treatment			
Ingredient, %	1	2	3	4
Dextrose	15.00	15.00	15.00	15.00
Cornstarch	30.17	30.10	28.97	28.90
M-PKM[1]	45.00	-	45.00	-
CR-PKM[2]	-	45.00	-	45.00
Soybean oil	8.00	8.00	8.00	8.00
Limestone	0.48	0.55	1.68	1.75
Salt	0.40	0.40	0.40	0.40
Vitamin mix[3]	0.20	0.20	0.20	0.20
Mineral mix[4]	0.15	0.15	0.15	0.15
Choline Cl (60%)	0.10	0.10	0.10	0.10
Titanium dioxide	0.50	0.50	0.50	0.50
Calculated nutrients:				
Ca	0.30	0.30	0.75	0.75
Total P	0.21	0.21	0.21	0.21

[1]M-PKM = Mexico palm kernel meal.
[2]CR-PKM = Costa Rica palm kernel meal.
[3]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-αtocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.
[4]Provided as milligrams per kilogram of diet: manganese, 75 mg from $MnSO_4 \cdot H_2O$; iron, 75 mg from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 mg from ethylene diamine dihydroiodide; selenium, 0.1 mg from Na_2SeO_3.

Table 3.3 Analyzed composition and TME_n of palm kernel meal from Experiment 1[1]

Item	M-PKM[2]	CR-PKM[3]
DM (%)	92.2	94.3
CP (%)	13.3	13.6
Ca (%)	0.24	0.19
P (%)	0.47	0.46
Phytic acid (%)	1.46	1.06
Gross energy (kcal/kg)	4,380	4,560
TME_n[4] (kcal/kg)	2,058	1,870
SEM of TME_n	60	30

[1]Values are expressed on a DM basis, excluding DM which is expressed on an as-fed basis.
[2]M-PKM = Mexico palm kernel meal.
[3]CR-PKM = Costa Rica palm kernel meal.
[4]TME_n values were not significantly different ($P > 0.05$). TME_n values are means of 6 individually-caged cecectomized roosters for M-PKM and 5 cecectomized roosters for CR-PKM.

Table 3.4 Total amino acid concentrations in palm kernel meal (%, DM basis)

	Palm kernel meal	
Amino acid	M-PKM[1]	CR-PKM[2]
Asp	1.13	0.94
Thr	0.41	0.34
Ser	0.49	0.42
Glu	2.57	2.21
Pro	0.46	0.39
Gly	0.64	0.55
Ala	0.56	0.48
Cys	0.22	0.16
Val	0.73	0.63
Met	0.27	0.22
Ile	0.55	0.48
Leu	0.89	0.78
Tyr	0.27	0.23
Phe	0.57	0.49
Lys	0.51	0.35
His	0.26	0.21
Arg	1.76	1.26
Trp	0.09	0.07

[1]M-PKM = Mexico palm kernel meal.
[2]CR-PKM = Costa Rica palm kernel meal.

Table 3.5 Standardized digestibility values of amino acids in palm kernel meal (%)[1]

	Palm kernel meal			
Amino acid	M-PKM[2]	SEM	CR-PKM[3]	SEM
Asp	65.0	2.97	55.6	3.92
Thr	67.2	4.81	60.8	3.90
Ser	75.8	3.73	71.2	3.60
Glu	78.4	2.57	70.7	2.24
Pro	65.9	5.04	55.8	4.25
Ala	73.3	2.72	65.8	2.52
Cys	55.8[a]	4.64	34.1[b]	5.92
Val	79.4[a]	2.60	71.3[b]	2.24
Met	86.3[a]	2.72	73.8[b]	2.24
Ile	76.4[a]	2.22	68.5[b]	2.20
Leu	77.9	3.00	71.8	1.99
Tyr	80.4	6.06	64.4	3.26
Phe	79.3[a]	2.39	71.9[b]	2.07
Lys	59.7	8.97	54.4	0.93
His	57.1	4.54	46.8	3.24
Arg	86.9[a]	1.36	80.2[b]	1.55
Trp	73.2	7.44	59.9	3.56

[a-b]Standardized digestibility values within a row lacking a common superscript differ ($P < 0.05$).
[1]Values are means of 6 individually-caged cecectomized roosters for M-PKM and 5 cecectomized roosters for CR-PKM.
[2]M-PKM = Mexico palm kernel meal.
[3]CR-PKM = Costa Rica palm kernel meal.

Table 3.6 Standardized amino acid concentrations in palm kernel meal (%)[1]

	Palm kernel meal	
Amino acid	M-PKM[2]	CR-PKM[3]
Asp	0.73	0.52
Thr	0.28	0.21
Ser	0.37	0.30
Glu	2.01	1.56
Pro	0.30	0.22
Ala	0.41	0.32
Cys	0.12	0.05
Val	0.58	0.45
Met	0.23	0.16
Ile	0.42	0.33
Leu	0.69	0.56
Tyr	0.22	0.15
Phe	0.45	0.35
Lys	0.30	0.19
His	0.15	0.10
Arg	1.53	1.01
Trp	0.07	0.04

[1]Values calculated by (amino acid concentration × standardized digestibility) / 100.
[2]M-PKM = Mexico palm kernel meal.
[3]CR-PKM = Costa Rica palm kernel meal.

Table 3.7 Growth performance and tibia ash for broiler chicks in Experiment 3[1]

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Tibia ash[2] (mg/tibia)	Tibia ash[3] (%)
1. P deficient cornstarch - dextrose	299.3^{e}	379.4^{d}	788.5^{bcd}	224.2^{d}	30.1^{e}
2. As 1 + 0.05% P[4]	359.5^{b}	422.1^{c}	851.5^{a}	299.8^{b}	35.6^{c}
3. As 1 + 0.1% P[4]	388.1^{a}	457.7^{ab}	847.3^{ab}	366.0^{a}	39.3^{a}
4. As 1 + 15% M-PKM[5]	323.1^{de}	369.3^{d}	817.3^{abc}	228.0^{d}	29.3^{e}
5. As 1 + 15% CR-PKM[6]	362.2^{ab}	435.4^{bc}	833.0^{ab}	262.2^{c}	33.1^{d}
6. As 1 + 30% M-PKM	328.4^{cd}	444.7^{abc}	739.0^{d}	271.5^{c}	34.8^{c}
7. As 1 + 30% CR-PKM	352.4^{bc}	459.7^{a}	767.2^{cd}	305.2^{b}	37.7^{b}
Pooled SEM	9.51	8.10	20.6	6.45	0.32

[a-e] Means within a column with no common superscript differ ($P < 0.05$).

[1] Values are means of five pens of five chicks; average initial BW was 92.6 g. Diets were fed from 8 to 18 days of age.

[2] Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), M-PKM (X_2), or CR-PKM (X_3) yielded the equation: $Y = 221.5 + 327.2 \pm 17.56\ X_1 \pm 70.4 \pm 12.81\ X_2 + 132.5 \pm 12.57X_3$ ($R^2 = 0.91$) The (±) values are standard errors of the regression coefficients.

[3] Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), M-PKM (X_2), or CR-PKM (X_3) yielded the equation: $Y = 29.5 + 22.7 \pm 1.56X_1 + 6.9 \pm 1.14\ X_2 + 12.7 \pm 1.12\ X_3$ ($R^2 = 0.86$) The (±) values are standard errors of the regression coefficients.

[4] From KH_2PO_4.

[5] M-PKM = Mexico palm kernel meal.

[6] CR-PKM = Costa Rica palm kernel meal.

Table 3.8 Relative phosphorus bioavailability in broiler chicks in Experiment 3.[1]

PKM Type	Total P (%)	Bioavailability values[1] (%)		Bioavailable content[2] (%)	
		Tibia ash (mg/tibia)	Tibia ash (%)	Tibia ash (mg/tibia)	Tibia ash (%)
M-PKM[3]	0.47	21.5[b]	30.3[b]	0.10	0.14
CR-PKM[4]	0.46	40.5[a]	55.6[a]	0.19	0.26

[a-b] Means within a column with no common superscript differ (P<0.05).
[1]Calculated by the slope-ratio method using the regression equation in footnotes 2 and 3 in Table 3.7. Bioavailability values are relative to the P in KH_2PO_4 which was set at 100%.
[2]Bioavailable content = (Total P x bioavailability value)/100. Values are presented on as-fed basis.
[3] M-PKM = Mexico palm kernel meal.
[4] CR-PKM = Costa Rica palm kernel meal.

Table 3.9 Apparent ileal P digestibility for broiler chicks in Experiment 4[1]

PKM type	Diet Ca level[2] (%)	Ileal P digestibility[3] (%)
M-PKM[4]	0.30	37.7[b]
CR-PKM[5]	0.30	48.1[a]
M-PKM	0.75	23.7[c]
CR-PKM	0.75	30.0[bc]
Pooled SEM	-	2.80

[a-c]Means within a column with no common superscript differ ($P < 0.05$).

[1]Values are means of five pens of five chicks at 18 days of age for ileal P digestibility.

[2]Diet Ca levels are calculated values.

[3]Significant main effect of diet Ca level ($P < 0.05$) and no significant interaction between PKM type and diet Ca level.

[4]M-PKM = Mexico palm kernel meal.

[5]CR-PKM = Costa Rica palm kernel meal.

LITERATURE CITED

Abbasi, F., T. Fakhur-un-Nisa, J. Liu, X. Luo, and I. H. R. Abbasi. 2019. Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. Environ. Sci. Pollut. Res. 26:9469–9479.

Abdollahi, M., B. Hosking, V. Ravindran. 2015. Nutrient analysis, metabolisable energy and ileal amino acid digestibility of palm kernel meal for broilers. Anim. Feed Sci. and Technol. 206:119-125.

AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.

Engster H. M., N. A. Cave, H. Likuski, J. M. McNab, C. M. Parsons, and F. E. Pfaff. 1985. A collaborative study to evaluate a precision-fed rooster assay for true amino acid availability in feed ingredients. Poult. Sci. 64:487-498.

Gillis, M. B., L. C. Norris and G. F. Heuser, 1954. Studies on the biological value of inorganic phosphates. J. Nutr. 52:115-126.

Hurwitz, S. 1964. Estimation of net phosphorus utilization by the slope method. J. of Nutr. 84:83-91.

Hurwitz, S., and A. Bar. 1971. Calcium and phosphorus interrelationships in the intestine of the fowl. J. Nutr. 101:677–685.

Kim, B. G.,J. H. Lee, H. J. Jung, Y. K. Han, K. M. Park, and I. K. Han. 2001. Effect of partial replacement of soybean meal with palm kernel meal and copra meal on growth

performance, nutrient digestibility and carcass characteristics of finishing pigs. Asian Australas. J. Anim. Sci. 14:821-830.

Leeson, S., and J. D. Summers. 2001. Scott's nutrition of the chicken. 4th ed. Univ. Books. Guelph, Ontario, Canada.

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

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. J. Anim. Sci. 92:5520-5529.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Nelson, T. S., and A. C. Walker. 1964. The biological evaluation of phosphorus compounds: a summary. Poult. Sci. 43:94–98.

Nwokolo, E. N., and D. B. Bragg. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci. 57:475-477.

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

Panigrahi, S., and C. J. Powell. 1991. Effects of high rates of inclusion of palm kernel meal in broiler chick diets. Anim. Feed Sci. and Technol. 34:37-47.

Potchanakorn, M., and L. M. Potter. 1987. Biological values of phosphorus in various sources for young turkeys. Poult. Sci. 66:505-51.

Rodehutscord, M., A. Dieckmann, M. Witzig, and Y. Shastak. 2012. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. Poult. Sci. 91:965-971.

SAS Institute. 2010. SAS ® User's Guide: Statistics. Version 9.2 Edition. SAS Institute, Inc., Cary, NC.

Shastak, Y., and M. Rodehutscord. 2013. Determination and estimation of phosphorus availability in growing poultry and their historical development. World's Poult. Sci. J. 69:569–586.

Sundu, B, A. Kumar, and J. Dingle. 2008. Amino acid digestibilities of palm kernel meal in poultry. J. Indones. Trop. Anim. Agric. 33:139-144.

United States Department of Agriculture. 2023. U.S. soybean production decline curtails exports while crush remains strong https://usda.library.cornell.edu/

WPSA. 2013. Working group report No 2: nutrition of the European Federation of Branches of WPSA. Determination of phosphorus availability in poultry. World's Poult. Sci. J. 69:687-698.