

THE EFFECT OF DIFFERENT MINERAL SOURCES ON GROWTH PERFORMANCE OF
TURKEYS

By

Salah Mahdi Hadi AL-Sherify

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science - Master of Science

2013

ABSTRACT

THE EFFECT OF DIFFERENT MINERAL SOURCES ON GROWTH PERFORMANCE OF TURKEYS

By

Salah Mahdi Hadi AL-Sherify

Producers are finding unique ways to turn turkey litter into a benefit instead of a cost. One approach is to use starved air low temperature gasification to create energy while providing an ash that could be used as a mineral source in turkey diets. The hypothesis of this study is gasification will result in minimal calcium and phosphorus availability due to non-specific binding to other minerals. The aim of the studies is to evaluate calcium and phosphorus bioavailability from turkey litter ash and the effect on the growth performance of turkeys. Two separate experiments, each with seven diets, were conducted. The concentrations of the diets ranged from 9.2 to 12.9 g/kg (calcium diets) and 7.4 to 12.4 g/kg (phosphorus diets). Each experiment had six replicates per treatment with eight birds per pen from 7 to 28 days of age. In the first experiment, no significant differences were found in production parameters or bone measurements. However, significant differences were found in ileal calcium digestibility between the lowest and highest concentrations of limestone and litter ash. The results suggest that using approximately 11 g/kg calcium from turkey litter ash in the diet is comparable to a standard limestone diet. Phosphorus source in the second experiment had an effect on the body weight gain, feed intake, and bone parameters. The ileal phosphorus digestibility was significantly different between the litter ash and monosodium phosphate diets. The data indicated that using 8.4 g/kg phosphorus from the litter ash in the diet resulted in performance similar to the monosodium phosphate diet. Therefore, litter ash can be substituted into turkey starter diets for the first 28 days of production with no significant impact on production parameters.

I would like to devote this work to my country Iraq. I would like to dedicate this work to my wife and my daughters. I would like also to dedicate this work to my parents, brothers and sisters, and uncles, their support and encouragements are appreciated.

ACKNOWLEDGEMENTS

First of all, I would like to express my great thanks to Allah Almighty and start with the two sayings from the Quran “be grateful to Allah if it is [indeed] Him that you worship” (2:172) and “Allah will raise those who have believed among you and those who were given knowledge, by degrees” (58:11). Then, I would like to thank my country Iraq represented by the Ministry of Higher Education and Scientific Research for providing me the scholarship. My appreciation and thanks to my great advisor Dr. Darrin Karcher for his guidance and support during my study. Without his help and encouragements this degree would not be possible. I would like to thank my committee members Dr. Gretchen Hill, Dr. Steven Bursian, and Dr. Sardar Sardary for their help and encouragements. I would like to thank the poultry farm manager Angelo Napolitano for the kindness help during my project in the farm. I would like to thank Jane Link, David Main, and Cara Robison for helping me during my research. I want to thank the graduate students Robert Van Wyhe and Prafulla Regmi for their help and I am really appreciated. Also, I would like to thank the undergraduate students for their help. I would like to thank the department chair Dr. Janice Swanson and all the faculty and staff at the Department of Animal Science for the great community and friendship I found during the few years of my study. I would like to thank the Iraqi Cultural Office in Washington DC represented by Dr. Abdulhadi Al Khalili for the help and support during my staying in the U.S. I would like to thank AL-Qasim Green University/ College of Agriculture for giving me the permission to study at Michigan State University and obtain this degree. I would like to appreciate the help of my family and friends and I am really missed them a lot, especially my family. Finally, special thanks to my wife for the support and help she provided during my study and I would really appreciate all of that.

TABLE OF CONTENTS

LIST OF TABLES	vii
KEY TO SYMBOLS AND ABBREVIATIONS	ix
Chapter One: Literature Review	1
Introduction	1
Poultry Litter Gasification	3
Calcium	4
Calcium Requirements of Turkeys	5
Calcium Availability	6
Calcium Absorption and Retention	7
Phosphorus	9
Phosphorus Requirements for Turkeys	10
Phosphorus Availability	11
Phosphorus Absorption and Retention	11
Calcium and Phosphorus Deficiency	12
Summary	13
APPENDIX	15
REFERENCES	24
Chapter Two: Calcium	33
Introduction	33
Materials and Methods	35
<i>Birds and Husbandry</i>	35
<i>Diet formulation</i>	36
<i>Sample Collection</i>	36
Sample Analyses	37
<i>Microwave Digestion Preparation</i>	37
<i>Calcium determination</i>	38
<i>Phosphorus determination</i>	38
<i>Ether extraction assay</i>	39
<i>Dry matter</i>	40
<i>Energy determination</i>	40
<i>Nitrogen determination</i>	41
<i>Chromium determination</i>	42
<i>Bone</i>	43
Production Measurements	43
Statistical analysis	44
Results	44
<i>Parameters measured</i>	44
<i>Bone</i>	45
<i>Nutrient Digestibility</i>	45

Discussion	46
<i>Body weight and feed intake</i>	46
<i>Bone</i>	47
<i>Nutrient Digestibility</i>	48
Summary	50
APPENDIX	51
REFERENCES	61
 Chapter Three: Phosphorus	 65
Introduction	65
Materials and Methods	67
<i>Birds and Husbandry</i>	67
<i>Diet formulation</i>	67
<i>Sample Collection</i>	68
Sample Analyses	68
<i>Microwave Digestion Preparation</i>	68
<i>Calcium determination</i>	69
<i>Phosphorus determination</i>	70
<i>Ether extraction assay</i>	70
<i>Dry matter</i>	71
<i>Energy determination</i>	71
<i>Nitrogen determination</i>	72
<i>Chromium determination</i>	74
<i>Bone</i>	75
Production Measurements	75
Statistical analysis	75
Results	76
<i>Parameters measured</i>	76
<i>Bone</i>	77
<i>Nutrient digestibility</i>	77
Discussion	78
<i>Body Weight and Feed Intake</i>	78
<i>Bone</i>	79
<i>Nutrient digestibility</i>	80
Summary	82
APPENDIX	83
REFERENCES	93
 Chapter Four: Overall Conclusion	 97

LIST OF TABLES

Table 1. Compilation of studies reporting the nutrient composition of excreta from various poultry species and other animals	16
Table 2. Chemical properties of turkey manure ash ¹	17
Table 3. Calcium requirements for Japanese quail, broilers, ducks, and geese	18
Table 4. Calcium sources used in poultry diets	19
Table 5. Calcium requirements for turkeys suggested by the National Research Council (1994), Hybrid and Nicholas turkey management guides	20
Table 6. Effects of different calcium sources on apparent and true calcium availability in broiler chicks (0 to 2 weeks) ¹	21
Table 7. National Research Council (1994) requirements for nonphytate phosphorus (NPP) for turkeys, Japanese quail, broilers, ducks, and geese	22
Table 8. Phosphorus sources used in poultry diets	23
Table 9. Diet formulation and nutrient composition of experimental diets (as-fed basis) for calcium	52
Table 10. Lighting program used for the 28-day calcium trial ¹	53
Table 11. Body weight gain (g/bird) for turkey poults fed different dietary calcium sources from 7 to 28 days of age	54
Table 12. Body weight gain (g/pen) for turkey poults fed different dietary calcium sources from 7 to 28 days of age	55
Table 13. Feed intake (g/pen) for turkey poults fed different dietary calcium sources from 7 to 28 days of age	56
Table 14. Feed conversion ratio for turkey poults fed different dietary calcium sources from 7 to 28 days of age	57

Table 15. Ileal digestibility mean and standard error for turkey poult fed different dietary calcium sources from 7 to 28 days of age.....	58
Table 16. Excreta digestibility mean and standard error for turkey poult fed different dietary calcium sources from 7 to 28 days of age.....	59
Table 17. Bone measurements, ash, and dry weight for turkey poult fed different dietary calcium sources from 7 to 28 days of age.....	60
Table 18. Diet formulation and nutrient composition of experimental diets (as-fed basis) for phosphorus	84
Table 19. Lighting program used for the 28 day phosphorus trial ¹	85
Table 20. Body weight gain (g/bird) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	86
Table 21. Body weight gain (g/pen) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	87
Table 22. Feed intake (g/pen) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	88
Table 23. Feed conversion ratio for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	89
Table 24. Ileal digestibility mean and standard error for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	90
Table 25. Excreta digestibility mean and standard error for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	91
Table 26. Bone measurements, ash, and dry weight for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age	92

KEY TO SYMBOLS AND ABBREVIATIONS

ATP	adenosine triphosphate
ADP	adenosine diphosphate
BWG	body weight gain
C	Celsius
Ca	calcium
cal	calorie
Cu	copper
CP	crude protein
cm	centimeter
dd H ₂ O	double deionized water
dL	deciliter
d	day
DM	dry matter
EAAP	experimental animal allotment program
F	Fahrenheit
FCR	feed conversion ratio
FI	feed intake
GE	gross energy
g	gram
gal	gallon
HAC	high ash calcium

HAP	high ash phosphorus
hr	hour
K	potassium
Kcal	kilocalorie
kg	kilogram
LAC	low ash calcium
LAP	low ash phosphorus
LLC	low limestone calcium
LMP	low monosodium phosphate
MAC	medium ash calcium
MAP	medium ash phosphorus
ME	metabolizable energy
MLC	medium limestone calcium
MMP	medium monosodium phosphate
Mg	magnesium
mL	milliliter
mm	millimeter
mg	milligram
μL	microliter
NC	negative control
NPP	nonphytate phosphorus
N	nitrogen
NaCl	salt

NRC	National Research Council
nm	nanometer
PC	positive control
P	phosphorus
ppm	part per million
PSI	pounds per square inch
S	sulfur
SEM	standard error of the mean
TN	total nitrogen
vs.	Versus
wk	week
Zn	zinc

Chapter One

Literature Review

Introduction

Manure is considered a waste product of farm animals (poultry, cattle, sheep, pigs, etc.). Due to the large number of animals in livestock facilities, the production of manure has increased over time, which has resulted in concerns for animal producers and for the environment as well (Zhang, 2010). However, new ways of dealing with animal waste are constantly being explored. Poultry producers must deal with environmental problems encompassing odors, noise, feathers, dust, water run-off and insects (Bell and Weaver, 2002). Additionally, animal waste may contain pathogenic organisms and heavy metals that could contribute to agricultural nonpoint source pollution (Reddy et al., 1981).

The traditional method of land application utilizing manure as a fertilizer is no longer an option due to phosphorus loads within the soil (Eghball and Barbarick, 2007). The timing of manure application to land is also challenging during the winter months when the ground is frozen or covered with snow (Bell and Weaver, 2002). The limitation of land application during the winter months was overcome by storing the manure until the spring. Nevertheless, storing manure for long periods of time does not solve the problem for the producers, but increases their expenses (Sharply et al., 2004). Therefore, alternative methods of reducing and utilizing poultry manure are continually being explored.

Poultry do not utilize 100% of the dietary nutrients consumed throughout their lives. Nutritionists have continually worked to evaluate feed ingredients for digestibility to maximize the inclusion levels to promote effective nutrient utilization. Typically, poultry are phase-fed allowing nutritionists to feed poultry nutrients to meet the bird's nutrient requirements, thus

minimizing the nutrients wasted during the period of production (Rao and Clandinin, 1970; Mateos and Sell, 1980; McNab and D'Mello 1994; Rochell et al., 2012). Any nutrients not utilized by the bird or produced from intestinal bacteria are excreted. Sistani et al. (2001) reported that poultry manure was considered to be a very rich source of calcium, phosphorus, and nitrogen compare to other animals. For instance, the nitrogen and phosphorus concentrations in broiler litter were reported to be considerably higher than in horse manure (Hansen, 2006). Camberato et al. (1997) and Walker et al. (1997) reported that millions of tons of nutrients such as magnesium were produced in the United States from animal facilities due to the high amounts of manure that these facilities generated. Table 1 (Hansen, 2006; Oluyemi et al., 1979) is a compilation of studies reporting the nutrient composition of excreta from various poultry species.

The nutrient profile of poultry litter lends itself as a great source of nutrients. As a result, the use of poultry litter as a fertilizer could have a negative effect on the soil depending on the nutrient profile of the soil and the amount of the nutrients contained in the manure. For example, high concentrations of nutrients such as phosphorus may exceed the amounts needed by plants, which then results in accumulation of phosphorus in soil potentially causing pollution (Eghball and Barbarick, 2007). The amino acid composition of the diet is important for proper growth and maintenance of the bird. Any exogenous amino acids not utilized and any endogenous proteins secreted into the digestive system that are not reabsorbed will be lost in the excreta. Nitrogen excretion can be reduced by feeding amino acids at concentrations that match their requirements. Otherwise, high amounts of nitrogen in the litter can lead to ammonia emission (Powers and Angel, 2008).

Ammonia emission can be reduced by treating the litter with alum, ferrous sulfate, and phosphoric acid (Moore et al., 1996, 1999; Choi and Moore, 2008). Kim and Patterson (2003)

found that using Zn and Cu would reduce the emission of ammonia because these two elements were used to inhibit microbial uricase that is involved in the production of ammonia. Manure nitrogen is affected by the temperature as reported by Parker and Perkins (1959) in that they found that drying manure at high temperature leads to reduced nitrogen concentration in the manure.

Poultry Litter Gasification

Poultry litter and excreta can be processed and used in many different ways to reduce environmental concerns. More recently, alternative methods have been developed to generate energy using animal manure (Moller et al, 2007; Burns and Raman, 2010). The reduction in dollars spent annually on storage, transport, and treatment of animal manure can save producers millions (Appleford et al., 2005). The gasification process converts a solid (poultry litter and excreta) into products such as gases, energy, and fuel (U.S. Department of Energy, 2004). Manure gasification is accomplished by converting manure into a liquid oil and char using pyrolysis without oxygen and then into gas and energy; high temperature was required with all manure gasification processes (Antal et al., 1984; Raman et al., 1980; Appleford et al., 2005; Abboud and Rahbar, 2010). Raman et al. (1980) reported that gas and energy production from gasified manure increased linearly with temperature. Prapasongsa et al. (2010) found that thermal gasification of animal manure increased energy output and reduced greenhouse gases. An advantage to gasification is the conversion of poultry litter into methane, thus reducing the overall impact on the environment. Manure gasification is reported to be different between poultry species. For example, turkey manure results in higher levels of gas production compared to chicken manure (Hills, 1982). Further, Thygesen et al. (2011) indicated that phosphorus will

be a limited resource in the future, leading to increase recycling of phosphorus from animal waste.

Therefore, converting poultry litter and excreta to energy with a byproduct of ash would aid the producer by reducing energy costs and potentially creating an opportunity to utilize the ash as a mineral source in poultry diets. Strock et al. (2006) provide the chemical properties of turkey manure ash (Table 2).

Calcium

Calcium is an important mineral for animal growth and development. Calcium plays an important role in animals such as neurotransmission, muscle contraction, clotting of blood, skeletal structure, bone formation, egg shell formation, and kidney function (Joint, 2001). Calcium is stored in many different parts of the body. The skeleton contains approximately 99% of the body calcium and the other 1% is distributed between teeth and other tissues such as the plasma and cellular compartments. Circulating calcium is derived from absorption of calcium from the gut and by resorption of calcium from the bones (Joint, 2001; Pond et al, 1995). Blood carries calcium in three forms: 60% as the free ion, 35% bound with protein, and 5 to 7% associated with organic or inorganic acids (Pond et al, 1995). Calcium requirements vary between the different poultry species. There are several factors influencing nutrient requirements in poultry including genetics, age, sex, environment, health, production aims, nutrient and vitamin levels and ratios (Applegate and Angel, 2008; Mussehl and Ackerson, 1935). For example, laying hens require approximately 1.35% more calcium than turkeys due to the calcium needed for eggshell formation (NRC, 1994). The National Research Council (1994) recommendations for the nutrient requirements for poultry were based on data published more than 30 years ago. However, genetic modifications have increased poultry performance and that

would imply that the mineral requirements should be evaluated continually. Table 3 is a summary of the calcium requirements for different poultry species (Nutrient Requirements of Poultry, 1994). Table 4 presents information about different calcium sources that are used to supplement poultry diets.

Calcium Requirements of Turkeys

The refinement of calcium requirements for turkeys was studied over 70 years ago by Mussehl and Ackerson in 1935 (Sanders et al. 1992). The calcium requirement for all animals including birds, changes over time with the requirement being higher earlier in life and then declining as the animal ages. The calcium requirement for starter poult was estimated to be no more than 1% (Lindblad et al., 1954; Slinger et al., 1961; Neagle et al., 1968). However, Sanders et al. (1992) estimated the calcium requirement was 1.25% in very young turkeys based on performance, bone ash measurements, incidence of tibia dyschondroplasia, plasma calcium, and calcium retention. Lilburn et al. (1997) used bone measurements (bone weight, fat extraction, tibia and femur length and width) to evaluate the calcium requirement and reported that 0.6% calcium was deficient, but no significant differences in performance were observed with 0.8, 1.0, and 1.2% calcium. The improvement in genetics since the 1994 NRC Nutrient Requirements of Poultry has necessitated increased nutrients, including dietary calcium, to support the increased growth rates and skeletal mass (Atia et al. 2000; Roberson, 2004). As a result, genetic company management guides are used to determine the nutrient recommendations that vary from the 1994 NRC Nutrient Requirements of Poultry (1994) (Table 5).

Calcium Availability

Calcium availability for poultry is evaluated by using bone ash, egg shell thickness, and apparent calcium retention (Reid and Weber, 1976). Ajakaiye et al. (2003) reported that apparent and true calcium availability can be calculated by using the following two formulas:

$$\frac{\text{Ca intake} - \text{excreta Ca}}{\text{Ca intake}} \times 100$$

$$\frac{\text{Ca intake} - (\text{excreta Ca of test chicks} - \text{excreta Ca of Ca deficient chicks})}{\text{Ca intake}} \times 100$$

The first formula is used to calculate apparent calcium availability, which is the difference between calcium intake and fecal calcium. Biological availability or true calcium availability is calculated using the second formula that considers the calcium contribution from a diet deficient in calcium (Ajakaiye et al. 2003). Biological availability was defined by Peeler (1972) as “a measure of the element or ion under consideration to support some physiological process”.

Factors including source of calcium, nonphytate phosphorus and phytase can influence calcium availability to the bird. Initially, Bethke et al. (1930) found no difference in calcium availability from different calcium sources such as limestone, calcium sulfate, and calcium carbonate when evaluating bone formation in chicks fed equivalent amounts of calcium. Furthermore, Waldroup et al. (1965) indicated equal calcium availability for the same calcium sources used by Bethke and his group (1930), but using bone ash and growth for determining calcium availability. However, Ajakaiye et al, (2003) determined the apparent and true calcium availability in different calcium sources (Table 6). Calcium bioavailability of some other calcium

sources such as mono-, di-, and tricalcium phosphate were 90% compared to the standard (100% calcium bioavailability) for calcium carbonate (Baker, 1991; Soares, 1995).

The relationship between phosphorus and calcium is important both within the bird and in the diet being fed. The amount of phosphorus in the diet affects the calcium requirement. Johnson and Karunajeewa (1985) found that the body weight of live birds decreased when they were fed a diet containing 0.81% available phosphorus compared to diet with 0.45% available phosphorus when calcium concentrations were identical for both diets. Calcium has to be supplied in the diet within the requirements, otherwise feeding birds calcium at concentrations greater or less than the requirement leads to negative effects. For example, calcium deficiency leads to rickets; however, providing more calcium in the diet reduces body weight. Johnson and Karunajeewa (1985) found that using 44% more calcium than what is recommended for 7-week-old birds led to a reduction in body weight by 5%. Qian et al. (1996) reported that calcium availability for turkey poults was improved with phytase supplementation. However, calcium availability for laying hens was decreased with higher supplementation of calcium and nonphytate phosphorus in the diet (Lim et al., 2003). An important aspect of calcium availability is determining absorption during metabolism.

Calcium Absorption and Retention

Absorption is the physiological activity that occurs during metabolism, resulting in the passage of nutrients from the intestinal lumen into the blood (Allen, 1982; Bronner, 1987; Kebreab et al, 2009). In poultry, calcium is absorbed in the duodenum and jejunum of the small intestine (Klunzinger, 2002; Hurwitz et al. 1978; Kebreab et al, 2009). There are two different mechanisms responsible for calcium absorption in the small intestine.

One mechanism is passive diffusion (paracellular) and it operates when calcium concentrations in the gastrointestinal tract are high (Bronner, 1987, 1990; Ledwaba, 2002). The second mechanism is the active absorption (transcellular) and it operates when calcium concentrations are low in the blood (Bronner, 1987, 1990; Ledwaba, 2002). Therefore, when the calcium concentration is high in the gut, passive diffusion will be operate to move it to the blood when it has a low level of calcium by using calcium channels. There are calcium receptors found in animals and the number of receptors is different between species and calcium status. The number of receptors in small animals was low compared to large animals as was indicated by a low number of receptors for $1,25\text{-(OH)}_2\text{-D}_3$ in small animals compared to larger animals (Halloran and Deluca, 1981). Also, Cross and Peterlik (1984) indicated that the number of receptors can be increased by release of hormones such as insulin.

Numerous factors affect calcium absorption. One factor is calcium intake, which influences calcium absorption in that absorption increases with low calcium intake and decreases with high calcium intake. The other factor affecting calcium absorption was phosphorus deficiency in that the absorption of calcium and Calcium Binding Protein (CaBP) from intestinal mucosa depended on the calcium and phosphorus concentrations in the diet, especially when the concentration of calcium was normal or higher than normal with a phosphorus deficiency (Morrissey and Wasserman, 1971). The parathyroid gland is responsible for calcium regulation in the animal's body. Therefore, when the calcium concentration is deficient in the blood, the parathyroid gland will release parathyroid hormone (PTH) to induce absorption of calcium from the small intestine through the calcindin protein carrier and to induce resorption of calcium from the bone by action of the hormone. Therefore, the absorption and resorption of calcium from the gut and bones occur after kidney activation of vitamin D_3 resulting in dihydroxycholecalciferol

1, 25-(OH)₂-D₃ (Lloyd et al., 1978; Groff and Gropper, 2000; Underwood and Suttle, 1999; Klunzinger, 2002). Calcium absorption is also affected by phytase and phytic acid. Intestinal absorption of calcium is limited by phytic acid; however, phytase makes calcium more available for absorption by release of it from phytic acid (Qian et al. (1997). Activation of phytate hydrolysis can improve calcium absorption by using cholecalciferol (Shafey et al., 1990; Mohammed et al., 1991). However, Ruschkowski and Hart (1992) reported that deficient vitamin D₃ with sufficient calcium in the diet leads to a decrease in calcium absorption and at the same time cause bone loss.

Calcium is excreted from poultry through elimination of uric acid whereas pigs excrete calcium with the feces and urine (Klunzinger, 2002). The difference between calcium excretion in the manure and calcium intake is calcium retention. Similar to calcium absorption, phytase and dietary calcium concentrations highly influence calcium retention. As reported by Juanpere et al. (2005), using phytase in poultry diets improves calcium retention. Rush et al. (2005) indicated that increasing calcium concentration in the diet leads to a decrease in apparent calcium retention.

Phosphorus

Phosphorus is one of the most important nutrients for animals. It has a positive effect on bone strength, skeletal development, egg production, growth, and metabolism of cellular constituents such as phospholipids (Kebreab et al., 2009). Phosphorus can be affected by other nutrients such calcium. Kebreab and Vitti (2005) reported that calcium and phosphorus are interrelated and the deficiency of one can affect the other. The majority of phosphorus is found in bones and the teeth, accounting for 80%, and the other 20% is associated with other body tissues (Klunzinger, 2002). Groff and Gropper (2000) reported that there are two forms of

phosphorus in blood serum, organic phosphorus that is associated with blood lipids and inorganic phosphorus. Genetic modifications have improved poultry performance, which has led to increase nutrient requirements such as phosphorus (Applegate and Angle, 2008). This change with genetics is compounded by nutrient requirements affected by factors including age, sex, health, production aim, nutrient concentrations, and environment (Applegate and Angel, 2008; Mussehl and Ackerson, 1935).

Phosphorus concentrations have to be within the requirements; otherwise, having animals with excessive or deficient dietary phosphorous will cause health problems. Waldroup (1999) reported that supplying high amounts of phosphorus in the diet led to increase phosphorus excretion. However, using low phosphorus levels in turkey diets will cause birds to have rickets (Waibel et al., 1984). Phosphorus is also affected by phytase supplementation in the diet. As indicated by Sebastian et al. (1996), using phytase in diet with low phosphorus levels had a positive effect on broiler performance as indicated by improved growth, increased bone strength, and retention of nutrients such as calcium and phosphorus. In addition, using phytase and non-phytase phosphorus together in poultry diets had a greater effect on feed intake and egg performance (Peter, 1992). Table 7 shows nonphytate phosphorus (NPP) requirements for different poultry species (Applegate and Angel, 2008; Nutrient Requirements of Poultry, 1994). Phosphorus sources used in poultry diets are many and most of them are represented in Table 8.

Phosphorus Requirements for Turkeys

The phosphorus requirement for turkeys has not been studied since the 1960s (Sullivan, 1962; Day and Dilworth, 1962). However, during these years genetics developed more than four to five decades ago have led to have higher nutrients requirements for poultry species (Roberson, 2004; Applegate and Angel, 2008). For example, in 1954 Almquist found that the nonphytae

phosphorus requirement for turkey poult was about 0.60%. More than 30 years later, the percentage of nonphytate phosphorus was increased to 0.72% as reported by Sanders et al. (1992). Therefore, Atia et al. (2000) found that turkey phosphorus requirements were higher than the NRC (1994) recommendation. However, even though NRC (1994) guideline is based on data published more than 40 years ago, it is still similar to what the modern turkey requires (Roberson et al., 2000; Roberson and Fulton, 2000; Thompson et al. 2002).

Phosphorus Availability

Phosphorus availability is affected by several factors such as animal health, environment, age, sex, nutrient concentrations and the type of phosphorus used in the diet (Applegate and Angel, 2008). Phosphorus availability is also affected by different types of soybeans. For example, use of soybean hulls in the diet increased the availability of phosphorus as reported by Griffith and Young (1966). The phosphorus availability from different sources can be evaluated by measuring body weight and toe ash for young turkeys (Potchanakorn and Potter, 1987). However, Potter (1988) reported that tibia and toe ash was a better measurement for phosphorus availability than body weight. Waldroup et al. (1965) reported that poultry phosphorus availability from mono- and dicalcium phosphate was lower than bone meal and meat phosphorus availability. Moreover, phosphorus availability for broilers had been increased due to the phytase supplements in the diets (Camden et al., 2001; Rutherford et al., 2004; Cowieson and Adeola, 2005),

Phosphorus Absorption and Retention

Phosphorus absorption occurs in the duodenum and jejunum of the small intestine via two mechanisms; the first mechanism is passive transport and the second mechanism is active transport (Klunzinger, 2002; Hurwitz et al. 1978; Kebreab et al, 2009). Phosphorus absorption

can be affected by nutrient concentrations in poultry diets. For example, intestinal absorption of phosphorus was reduced with high and low concentrations of calcium and nonphytate phosphorus respectively (Rousseau et al., 2012). In addition, phosphorus absorption improved with the intake of phosphate (Hurwitz et al, 1978). Another study by Yan et al. (2007) reported that sodium phosphate in the poultry intestine increased the absorption of phosphorus.

Phosphorus retention is another aspect taken under consideration during nutritional studies. Phosphorus retention is affected by the concentration of nutrients supplied in the diet. For instance, Hurwitz et al. (1978) found that gradually increasing phosphorus intake by way of the diet did not affect the retention of phosphorus. Similarly, phosphorus retention was not affected by the increase of calcium concentrations in the diets of young Pekin ducks as reported by Rush et al. (2005). In addition, the concentrations of phytase and nonphytate phosphorus also affect phosphorus retention. Qian et al. (1996) indicated that adding nonphytate phosphorus to turkey diets reduced phosphorus retention; however, phosphorus retention was improved with phytase supplementation depending on the Ca:tP ratio, especially with nonphytate phosphorus. Moreover, phosphorus retention was also improved by improving the availability of phosphorus in the diets of broiler chicks (Perney et al, 1993).

Calcium and Phosphorus Deficiency

Deficiency is defined as lack of a nutrient supplied in the diet. Formulating animal diets with insufficient nutrients has a negative impact on the animal. Calcium and phosphorus deficiencies cause health problems not only associated with growth but also with bones such as rickets, rachitis, dyschondroplasia and osteomalacia, and perosis (Lilburn et al., 1997; Whitehead 1997; Hester and Ferket, 1998). One of the very important diseases that affect most poultry species is rickets, which is caused by the insufficiency of the most important nutrients in poultry

diets including calcium, phosphorus, and vitamin D (Klunzinger, 2002). There are several signs indicative of rickets starting with growth reduction, bone weakness, and finally death (Lloyd et al., 1978; Pond et al. 1995). There is a relationship between calcium and phosphorus concentrations in the diet for animals having rickets. As a result, the ratio between calcium and phosphorus has to follow recommended requirements for poultry species; otherwise, having high calcium with insufficient phosphorus in the diet leads to rickets (Sanders et al., 1992). In addition, perosis is another disease in poultry species caused by deficiency of phosphorus and vitamin E as reported by Slinger et al. (1954). In order to prevent this kind of disease, nutrient deficiency has to be prohibited or the diet must be supplied with other nutrients such as phytase for preventing phosphorus deficiency (Sohail and Roland, 1999).

Summary

Calcium and phosphorus are the most important inorganic elements for poultry nutrition and sufficient concentrations need to be present in the diet. Otherwise, deficiencies will occur leading to performance and health issues. Numerous calcium and phosphorus sources have been used in poultry diets such as calcium carbonate, calcium monophosphate, dicalcium phosphate, and limestone (Tables 7 and 8). A study done by North Carolina State University Extension (Swine News, 2006) used four different types of manure ash (three of them were from swine manure and the fourth was from turkey litter) in swine diets. The results of this study showed no difference in the growth performance and digestibility between ash sources and positive control treatments; however, turkey litter ash has the highest phosphorus digestibility compared to other treatments. Therefore, using gasified litter ash as a mineral source is not novel. However, the improvement in technology and ash composition is unique to this study. Therefore, the hypothesis of this study is gasification will result in minimal calcium and phosphorus availability

due to non-specific binding to other minerals. The objective is to evaluate calcium and phosphorus bioavailability from turkey litter ash and the effect on the growth performance of turkeys.

APPENDIX

Table 1. Compilation of studies reporting the nutrient composition of excreta from various poultry species and other animals

Manure type	Composition					Nutrients content		
	%					Lb/ ton ¹		
	Moisture	Ash	CP ¹	GE (Kcal/kg) ¹		TN	(P ₂ O ₅)	(K ₂ O)
Poultry	48.3	17.1	15.1	2.8				
Broiler ³	N/A	N/A	N/A	N/A		59	63	40
Turkey ²	N/A	N/A	N/A	N/A		27	25	12
Layer ²	N/A	N/A	N/A	N/A		35	42	28
Horse ³	N/A	N/A	N/A	N/A		9	6	11
						Lb/1000 gal		
Swine ³	36.5	13.2	10.9	3.1		40	37	23
Cow	15.7	2.9	3.4	3.97				
Dairy ³						28	19	25
Sheep	30.5	6.1	6.3	3.84		N/A	N/A	N/A

¹ CP= crude protein, GE= gross energy, TN= total nitrogen, P₂O₅ = phosphorus, K₂O = potassium.

² Zublena et al., 1990.

³ Bandel, 1990.

Table 2. Chemical properties of turkey manure ash¹

Chemical property	Turkey manure ash
Acid-digestible elements	g kg ⁻¹
Aluminum	4
Calcium	159.2
Iron	3.8
Magnesium	28.6
Manganese	1.9
Phosphorus	76.5
Potassium	111.7
Sodium	19.9
Sulfur	17.2
Zinc	1.4
	mg kg ⁻¹
Arsenic	5.2
Barium	163.4
Boron	107.7
Beryllium	0.6
Cadmium	1.5
Chromium	34.1
Cobalt	10.2
Copper	418.3
Lead	17.1
Lithium	7.2
Molybdenum	24
Nickel	35.3
Rubidium	212.1
Selenium	N/A
Silicon	193.4
Strontium	217.9
Titanium	35.1
Vanadium	27.2

¹ Strock et al. (2006)

Table 3. Calcium requirements for Japanese quail, broilers, ducks, and geese

	Weeks of age			
	0 to 4	4 to 8	9 to 17	Breeding
Japanese quail				
Ca %	1.00	0.85	0.53	2.5
Broilers	0 to 3	3 to 6	6 to 8	-
Ca %	1.00	0.90	0.80	-
Ducks	0 to 2	2 to 7	Breeding	-
Ca %	0.65	0.60	2.75	-
Geese	0 to 4	After 4	Breeding	-
Ca %	0.65	0.60	2.25	-

Table 4. Calcium sources used in poultry diets

Calcium sources	Reference
Limestone	Bethke et al., 1930
Calcium sulfate	Bethke et al., 1930
Calcium carbonate	Bethke et al., 1930
Calcium lactate	Bethke et al., 1930
Calcium phosphate	Bethke et al., 1930
Calcium silicate	Bethke et al., 1930
Bone meal	Bethke et al., 1930
Rock phosphate	Bethke et al., 1930
Oyster shell	Bethke et al., 1930
Mono-calcium phosphate	Baker, 1991; Soares, 1995
Di-calcium phosphate	Baker, 1991; Soares, 1995
Tri-calcium phosphate	Baker, 1991; Soares, 1995

Table 5. Calcium requirements for turkeys suggested by the National Research Council (1994), Hybrid and Nicholas turkey management guides

	-----Calcium (%) -----		
Weeks of age	NRC	Hybrid	Nicholas ¹
0 to 4	1.20	1.40	1.45
4 to 8	1.00	1.40	1.35
8 to 12	0.85	1.30	1.25
12 to 16	0.75	1.15	1.10
16 to 20	0.65	1.00	1.00
20 to 24	0.55	1.00	1.00

¹ Nicholas requirements indicated that it is suggested for 4 to 6 weeks, 6 to 10 weeks, 10 to 16 weeks, 16 to 24 weeks, and as needed respectively.

Table 6. Effects of different calcium sources on apparent and true calcium availability in broiler chicks (0 to 2 weeks)¹

Calcium sources	Soap (g/kg of excreta fat)	Ash (g/kg of defatted bone)	Apparent calcium availability (g/kg)	True calcium availability (g/kg)
Calcium carbonate	52 ^b	397 ^{ab}	257	168
Bivalve shell	114 ^{ab}	416 ^{ab}	352	268
Periwinkle shell	105 ^{ab}	401 ^{ab}	431	344
Oyster shell	129 ^{ab}	389 ^b	267	185
Marble dust	143 ^a	404 ^{ab}	281	194
Snail shell	148 ^a	397 ^{ab}	302	214
S. E. M	29.9	9.3	80.5	86.4

^{a-b} Means within the same column with different letters are significantly different ($P < 0.05$).

¹ Ajakaiye et al (2003)

Table 7. National Research Council (1994) requirements for nonphytate phosphorus (NPP) for turkeys, Japanese quail, broilers, ducks, and geese

	Weeks of age					
	0 to 3	3 to 6	6 to 9	9 to 12	12 to 15	15 to 18
Turkey						
NPP %	0.60	0.50	0.42	0.38	0.32	0.28
Japanese quail						
NPP %	0.55	0.50	0.45	0.40	-	-
Broiler						
NPP %	0.45	0.35	0.30	-	-	-
Duck						
NPP %	0.40	0.30	-	-	-	-
Geese						
NPP %	0.30	0.30	0.30	-	-	-

Table 8. Phosphorus sources used in poultry diets

Phosphorus sources	References
Fish meal	Waldroup et al., 1965
Poultry by products meal	Waldroup et al., 1965
Meat and bone meal	Waldroup et al., 1965
Mono-sodium phosphate	Waldroup et al., 1965
Di-calcium phosphate	Waldroup et al., 1965
Mono-calcium phosphate	Van der Klis et al., 1994
Di-calcium phosphate	Coon et al., 2007
Defluorinated phosphate	Coon et al., 2007

REFERENCES

REFERENCES

- Abboud, S., and S. Rahbar. 2010. Potential for producing renewable natural gas from Canadian wastes. CISA, Environmental Sanitary Engineering Center, Venice, Italy.
- Ajakaiye, A., J.O. Atteh, and S. Leeson. 2003. Biological availability of calcium in broiler chicks from different calcium sources found in Nigeria. *Animal Feed Sci. and Technol.* 104:209-214.
- Appleford, J. M., Y. Zhang, L. Christianson, T. L. Funk, and K. C. S. Ocfemia. 2005. Thermochemical conversion of animal and human wastes: A Review. *American Society of Agriculture Engineering*, St. Joseph, Michigan. 292-300.
- Atia, F. A., P. E. Waibel, I. Hermes, C. W. Carlson, and M. M. Walser. 2000. Effect of dietary phosphorus, calcium, and phytase on performance of growing turkeys. *Poult. Sci.* 79:231-239.
- Antal Jr, M. J., W. H. Edwards, H. L. Friedman, and F. E. Rogers. 1984. Study of the steam gasification of organic wastes (No. PB-84-143148). Princeton Univ., NJ (USA). Dept. of Mechanical and Aerospace Engineering.
- Applegate, T. J. and R. Angel. 2008. Phosphorus requirements for poultry. Purdue Univ. and Univ. Maryland, College Park.
- Almquist, H.J. 1954. The phosphorus requirement of young chicks and poults: A review. *Poult. Sci.* 33:936-943.
- Bronner, F. 1990. Intestinal calcium transport: The cellular pathway. *Miner. Electrol. Metab.* 16:94-100.
- Bronner, F. 1987. Intestinal calcium absorption: Mechanisms and applications. *J. Nutr.* 117:1347-1352.
- Bell, D. D., and W. D. Weaver (eds.). 2002. Commercial chicken meat and egg production (5th ed.). Page 149 - 151 in waste managements. Kluwer Academic Pub. Norwell, Mass. MA, USA.
- Eghball, B., and K. A. Barbarick. 2007. Manure, compost and biosolids. *Encyclopedia of Soil Science*, Second Edition. Taylor and Francis: New York, 1049-1052.
- Burns, R.T., and D. R. Raman. 2010. Animal Waste: Utilization. In *Encyclopedia of Agricultural, Food, and Biological Engineering*, Second Edition. Taylor and Francis: New York, 65-66.
- Bethke, R. M., D. C. Kennard, and C. H. Kick. 1930. The availability of calcium in calcium salts and minerals for bone formation in the growing chick. *Poult. Sci.* 9:45-50.

- Baker, D. H. 1991. Bioavailability of mineral and vitamins. In *Swine Nutrition*, E. R. Miller, D. E. Ullrey, and A. J. Lewis (eds.). Butterworth-Heinemann, Boston.
- Camberato, J. J., E. D. Vance, and A. V. Someshwar. 1997. Composition and land application of paper manufacturing residuals. Washington, DC: American Chemical Society, In ACS Symposium Series. 668:185-203.
- Choi, I. H., and P. A. Moore Jr. 2008. Effects of liquid aluminum chloride additions to poultry litter on broiler performance, ammonia emissions, soluble phosphorus, total volatile fatty acids, and nitrogen contents of litter. *Poult. Sci.* 87:1955-1963.
- Cross, H. S., and M. Peterlik. 1984. Hormonal and ionic control of phosphate transport in the differentiating enterocyte. *Progress in clinical and biological research*, 168, 247.
- Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in 1126 Rutherford et al. improving the bioavailabilities of phosphorus and other nutrients in maize-soybean diets for broilers. *Anim. Sci.* 73:289-297.
- Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, proteases, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chickens. *Poult. Sci.* 84:1860-1867.
- Day, E.J., and B.C. Dilworth. 1962. Dietary phosphorus levels and calcium: phosphorus ratios needed by growing turkeys. *Poult. Sci.* 41:1324-1328.
- Groff, J.L. and S.S. Gropper. 2000. Macrominerals. In *advanced nutrition and human metabolism* (3rd Ed.) Wadsowrth, Belmont.
- Griffith, M., and R. J. Young. 1966. Influence of dietary calcium, vitamin D3 and fiber on the availability of phosphorus to turkey poults. *Poult. Sci.* 46:553-560.
- Hills, D. J. 1982. Chemical characteristics of and methane production from turkey manure. *Poult. Sci.* 61(4): 677-684.
- Hester, P.Y. and P.R. Ferket. 1998. Relationship between long bone distortion and tibial dyschondroplasia in male turkeys. *Poult. Sci.* 77(9):1300-1302.
- Hansen, D. J. 2006. Manure as a Nutrient Source. *The Mid-Atlantic Nutrient Management Handbook*, 207.
- Hurwitz, S., D. Dubrov, U. Eisner, G. Risenfeld, and A. Bar. 1978. Phosphate absorption and excretion in the young turkey, as influenced by calcium intake. *J. Nutr.* 108:1329-1335.
- Halloran, B. P., and H. F. Deluca. 1981. Appearance of the intestinal cytosolic receptor for 1,25-dihydroxy vitamin D3 during neonatal development of the rat. *Biol. Chem.* 256:7338-7342.

Johnson, R. J., and H. Karunajeewa. 1985. The effects of dietary minerals and electrolytes on the growth and physiology of the young chick. *J. Nutr.* 115(12), 1680.

Joint, F. A. O. 2001. Vitamin and mineral requirements in human nutrition. Bangkok, Thailand

Juanpere, J., A. M. Perez-Vendrell, E. Angulo, and J. Brufau. 2005. Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poult. Sci.* 84:571-580.

Kim, W. K., and P. H. Patterson. 2003. Effect of minerals on activity of microbial uricase to reduce ammonia volatilization in poultry manure. *Poult. Sci.* 82:223-231.

Kebreab, E., J. France, R. P. Kwakkel, S. Leeson, H. D. Kuhi, and J. Dijkstra. 2009. Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. *Poult. Sci.* 88 (3):680-689.

Kebreab, E., and D. M. S. S. Vitti. 2005. Mineral metabolism. Pages 469-486 in *Quantitative Aspects of Ruminant Digestion and Metabolism*. J. Dijkstra, J. M. Forbes, and J. France, ed. CAB Int., Wallingford, UK.

Klunzinger, M. W. 2002. Utilization of low phytic acid corn with phytase to reduce phosphorus excretion from growing turkeys and pigs. Thesis (M.S.) Michigan State University. Dept. of Animal Science.

Lindblad, G. S., S. J. Slinger, and I. Motsok. 1954. Effect of aureomycin on the calcium and phosphorus requirements of chicks and poults. *Poult. Sci.* 33:482-491.

Allen, L. H. 1982. Calcium bioavailability and absorption: a review. *The American journal of clinical nutrition*, 35(4):783-808.

Lilburn, M. S., G. W. Barbour, R. Nemasetoni, C. Coy, M. Werling, and A. G. Yersin, 1997. Protein quality and calcium availability from extruded and autoclaved turkey hatchery residue. *Poult. Sci.* 76:841-848.

Lim, H. S., H. Namkung, and I. K. Paik. 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. *Poult. Sci.* 82:92-99.

Ledwaba, M. F. 2002. Efficacy of 25-hydroxycholecalciferol on the prevention of tibial dyschondroplasia in Ross broiler chicks. Thesis (M.S.) Michigan State University. Dept. of Animal Science.

Lloyd, L. E., B. E. McDonald, and E. W. Crampton. 1978. Essential microelements. In *Fundamentals of Nutrition* (2nd Ed.). W. H. Freeman and Co., San Francisco.

- McNab, J. M., and J. P. F. D'Mello. 1994. Amino acid digestibility and availability studies with poultry. *Amino acids in farm animal nutrition*. 185-203.
- Mateos, G. G., and J. L. Sell. 1980. Influence of graded levels of fat on utilization of pure carbohydrate by the laying hen. *J. Nutr.* 110:1894-1903.
- Moore Jr, P. A., T. C. Daniel, and D. R. Edwards. 1999. Reducing phosphorus runoff and improving poultry production with alum. *Poult. Sci.* 78 (5):692-698.
- Moore Jr, P. A., T. C. Daniel, D. R. Edwards, and D. M. Miller. 1996. Evaluation of chemical amendments to reduce ammonia volatilization from poultry litter. *Poult. Sci.* 75:315-320.
- Mussehl, F. E., and C. W. Ackerson. 1935. Calcium and phosphorus requirements of growing turkeys. *Poult. Sci.* 14:147-151.
- Morrissey, R. L., and R. H. Wasserman. 1971. Calcium absorption and calcium-binding protein in chicks on differing calcium and phosphorus intakes. *Am. J. Physiol.* 220:1509-1515.
- Moller, H. B., H. S. Jensen, L. Tobiasen, and M. N. Hansen. 2007. Heavy metal and phosphorus content of fractions from manure treatment and incineration. *Environ. Technol.* 28:1403-1418.
- Mohammed, A., M. J. Gibney, and T. G. Taylor. 1991. The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytase-p by the chick. *Br. J. Nutr.* 66 (02):251-259.
- NRC, National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Neagle, L. H., L. G. Blaylock, and J. H. Goihl. 1968. Calcium, phosphorus, and vitamin D interactions in turkeys to 4 weeks of age. *Poult. Sci.* 47:174-180.
- Oluyemi, J. A., B. Longe, and R. Esubi. 1979. Replacing corn with sun-dried manure of laying pullet, mature pig, sheep, and cow. *Poult. Sci.* 58(4):852-857.
- Powers, W., and R. Angel. 2008. A review of the capacity for nutritional strategies to address environmental challenges in poultry production. *Poult. Sci.* 87:1929-1938.
- Parker, M. B., H. F. Perkins, and H. L. Fuller. 1959. Nitrogen, phosphorus and potassium content of poultry manure and some factors influencing its composition. *Poult. Sci.* 38(5):1154-1158.
- Prapasongsa, T., T. G. Poulsen, J. A. Hansen, and P. Christensen. 2010. Energy production, nutrient recovery and greenhouse gas emission potentials from integrated pig manure management systems. *Waste Management and Research*, 28(5):411-422.
- Pond, W. G., D. C. Church, and K. R. Pond. 1995. Basic animal nutrition and feeding (4th ed.). Page 169-174 in *Inorganic Mineral Elements*. John Wiley and Sons, New York.

Peeler, H. T., 1972. Biological availability of nutrients in feeds: availability of major mineral ions. *J. Anim. Sci.* 35:695-699.

Peter, W. 1992. Investigations on the use of phytase in the feeding of laying hens. In *worlds poultry congress*, 19:672.

Potter, L. M. 1988. Bioavailability of phosphorus from various phosphates based on body weight and toe ash measurements. *Poult. Sci.* 67:96-102.

Potchanakorn, M., and L. M. Potter. 1987. Biological values of phosphorus from various sources for young turkeys. *Poult. Sci.* 66:505-513.

Perney, K. M., A. H. Cantor, M. L. Straw, and K. L. Herkelman. 1993. The Effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. *Poult. Sci.* 72:2106-2114.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. *Poult. Sci.* 76:37-46.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. *Poult. Sci.* 75:69-81.

Rush, J. K., C. R. Angel, K. M. Banks, K. L. Thompson, and T. J. Applegate. 2005. Effect of dietary calcium and vitamin d3 on calcium and phosphorus retention in white pekin ducklings. *Poult. Sci.* 84:561-570.

Reddy, K.R., R. Khaleed, M.R. Overcash. 1981. Behaviour and transport of microbial pathogens and indicator organisms in soils treated with organic waste. *J. Environ. Qual.* 10(3):255-266.

Rao, P. V., and D. R. Clandinin. 1970. Effect of method of determination on the metabolizable energy value of rapeseed meal. *Poult. Sci.* 49:1069-1074

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

Raman, K. P., W. P. Walawender, and L. T. Fan. 1980. Gasification of feedlot manure in a fluidized bed reactor. The effect of temperature. *Industrial and Engineering Chemistry Process Design and Development*, 19(4):623-629.

Roberson, K. D. 2004. Estimation of the dietary calcium and nonphytase phosphorus needs of growing commercial tom turkeys weighing four to twelve kilograms. *International J. Poult. Sci.* 3(3):175-178.

Roberson, K. D., M. W. Klunzinger, M. F. Ledwaba, A. P. Rahn, M. W. Orth, R. M. Fulton, and B. P. Marks. 2000. Effects of dietary calcium and phosphorus regimen on growth performance, bone strength and carcass quality and yield of Large White tom turkeys. *Poult. Sci.*, 79 (Sup. 1): 68.

Roberson, K.D. and R. M. Fulton. 2000. Estimation of the calcium and phosphorus requirements of 4.5 to 12 kg commercial tom turkeys. *Poult. Sci.* 79 (Supl. 1):98.

Ruschkowski, S. R., and L. E. Hart. 1992. Ionic and endocrine characteristics of reproductive failure in calcium deficient and vitamin D deficient laying hens. *Poult. Sci.* 71:1722-1732.

Reid, B. L. and C. W. Weber, 1976. Calcium availability and trace mineral composition of feed grade calcium supplements. *Poult. Sci.* 55:600-605.

Rutherford, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on the ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a lowphosphorus diet for broilers. *Poult. Sci.* 83:61-68.

Rousseau, X., M. P. Letourneau-Montminy, N. Meme, M. Magnin, Y. Nys, and A. Narcy. 2012. Phosphorus utilization in finishing broiler chickens: Effects of dietary calcium and microbial phytase. *Poult. Sci.* 91:2829-2837.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn soybean diets. *Poult. Sci.* 75:729-736.

Shafey, T. M., M. W. McDonald, and R. A. Pym 1990. Effects of dietary calcium, available phosphorus and vitamin D on growth rate, food utilization, plasma and bone constituents and calcium and phosphorus retention of commercial broiler strains. *Br. Poult. Sci.* 31:587-602.

Sharply A., P. Kleinman, and J. Weld. 2004. Assessment of best management practices to minimise the runoff of manure-borne phosphorus in the United States. *New Zealand J. Agric.* 47:461-477.

Strock, J., C. Rosen, and P. Pagliari. 2006. Turkey Manure Ash Progress Report. Univ. Minnesota.

Sistani, K. R., D. E. Rowe, D. M. Miles, and J. D. May. 2001. Effects of drying method and rearing temperature on broiler manure nutrient content. *Commun. Soil Sci. Plant Anal.* 32 (13 and 14):2307-2316.

Slinger, S. J., W. F. Pepper, I. Motzok, and I. R. Sibbald. 1961. Studies on the calcium requirements of turkeys. 1. Influence of antibiotics during the starting period. 2. Interrelationship with reserpine during the growing period. *Poult. Sci.* 40:1281-1291.

Slinger, S. J., W. F. Peppee, and I. Motzok. 1954. Interrelationship between Vitamin E and Phosphorus In Preventing Perosis in Turkeys. *J. Nutrition*. 52(3):395-403.

Swine news. 2006. Combustion Ash can serve as a Mineral Supplement in Swine Diets. NCSU Extension Swine Husbandry. Vol. 29, Nos. 03.

Sohail, S. S. and D. A. Roland Sr. 1999. Influence of Supplemental Phytase on Performance of Broilers Four to Six Weeks of Age. *Poult. Sci.* 78(4):550-555.

Sanders, A.M., H.M. E. JR, and G.N. Rowland. 1992. Calcium and phosphorus requirements of the very young turkey as determined by response surface analysis. *Br. J. Nutr.*, 67:421-435.

Sullivan, T.W., 1962. Studies on the calcium and phosphorus requirements of turkeys, 8 to 20 weeks of age. *Poult. Sci.* 41:253-259.

Soares, J. H., 1995. Calcium bioavailability. In bioavailability of nutrients for animals, C. B. Ammerman, D. H. Baker, and A. J. Lewis (eds.). Academic Press. New York.

Thompson, K.L., T.J. Applegate, R. Angel, K. Ondracek, and P. Jaynes. 2002. Effect of phosphorus level, 25-hydroxycholecalciferol (HyD), and phytase supplementation on performance of male turkeys from 0 to 18 weeks of age. *Poult. Sci.* 81(Supl. 1):13.

Thygesen, A.M., O. Wernberg, E. Skou, and S.G. Sommer. 2011. Effect of incineration temperature on phosphorus availability in bio-ash from manure. *Environ. Technol.* 32 (6):633-638.

Underwood, E. J., and N. F. Suttle. 1999. Calcium. phosphorus. in the mineral nutrition of livestock (3rd Ed.). CAB International Publishing, New York.

Walker, J. M., R. M. Southworth, and A. B. Rubin. 1997. US Environmental Protection Agency regulations and other stakeholder activities affecting the agricultural use of by-products and wastes. American Chemical Society, In ACS Symposium Series, 668:28-49.

Waldroup, P. W. 1999. Nutritional approaches to reducing phosphorus excretion by poultry. *Poult. Sci.* 78:683-691.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms. 1965. The utilization of phosphorus from animal protein sources for chicks. *Poult. Sci.* 44:1302-1306.

Waibel, P. E., N. A. Nahorniak, H. E. Dziuk, M. M. Walser, and W. G. Olson. 1984. Bioavailability of phosphorus in commercial phosphate supplements for turkeys. *Poult. Sci.* 63:730-737.

Whitehead, C.C. 1997. Dischondroplasia in poultry. *Proceedings of the nutrition society* 56:957-966.

Yan, F., R. Angel, and C. M. Ashwell. 2007. Characterization of the chicken small intestine type IIb sodium phosphate cotransporter. *Poult. Sci.* 86:67-76.

Zhang, Y. 2010. Thermochemical Conversion (TCC): Swine Manure to Crude Oil. In *Encyclopedia of Agricultural, Food, and Biological Engineering*, Second Edition. Taylor and Francis: New York, 1717-1721.

Chapter Two

Calcium

Introduction

Calcium is one of the most important inorganic elements for the growth and performance of different poultry species. One of the key roles for calcium is the development of the animal's skeletal structure (Mussehl and Ackerson, 1935; Siebrits, 1993). Calcium is vital for egg production due to the large amount of calcium carbonate required for egg shell formation (Elaroussi et al., 1994; Highfill, 1998; Klasing, 1998). Poultry species have different required concentrations of calcium as a result of various production aims. For example, calcium requirements for laying hens are lower at 0 to 6 weeks compared to turkeys, 0.9% versus 1.2%, respectively (National Research Council, 1994). Therefore, calcium concentrations need to be adequate and appropriate for the species; otherwise, calcium deficiency can lead to health problems like rickets and skeletal abnormalities (Akpe et al., 1987).

The calcium source can influence animal performance. Typically, limestone or calcium carbonate is the ideal calcium source to use in poultry diets (Boitumelo, 2004). Other calcium sources have been evaluated to determine the calcium availability and absorption for poultry. Lilburn et al. (1997) supplemented turkey diets with alternative feedstuffs including autoclaved hatchery byproduct meal, bone meal, and limestone looking at various calcium concentrations (0.6, 0.8, 1.0, and 1.2%) for each diet. The results indicated no significant effect on body weight gain and feed efficiency between feedstuffs or calcium concentrations. Additionally, tibia and femur length and width were not different between diets; however, tibia and femur ash were trending to be significant between diets. Moreover, tibia and femur length, width, and ash were found to be significantly different between different Ca concentrations.

Another study conducted with broilers by Ajakaiye et al. (2003) used equal concentrations of calcium (approximately 10 g/kg) from various sources including calcium carbonate, bivalve shell, periwinkle shell, oyster shell, marble dust and snail shell. There were not differences in body weight, feed intake, metabolizable energy of the diet, tibia length and diameter, and apparent and true calcium availability. However, bone ash was significantly greater with bivalve shell compared to oyster shell. Significant differences were found between these sources in calcium biological availability. Two of these sources (bivalve and periwinkle shells) were found to be equal or greater than calcium carbonate in biological availability.

In addition, a broiler study conducted by Applegate et al. (2003) used different calcium sources at two different concentrations in the diets. The calcium sources were calcium carbonate and calcium malate included at 4 or 9 g/kg. The study included three experiments: 1) 4 or 9 g/kg of calcium carbonate from day 7 to 21 with Hubbard × Peterson male chicks; 2) 4 and 9 g/kg calcium carbonate and calcium malate from day 14 to 24 with Ross 308 male chicks; 3) 4 or 9 g/kg of calcium carbonate from day 8 to 22 with Ross 308 or Hubbard × Peterson male chicks. Initially, no significant effect on body weight gain was observed between the inclusion concentrations of calcium carbonate. In the second experiment there was an increase in body weight gain with 4 g/kg compared to 9 g/kg calcium included in the diet for both calcium carbonate and calcium malate. Body weight gain was increased in the second experiment with calcium carbonate compared to calcium malate. Moreover, calcium absorption in the ileum was not affected by the source or concentration of calcium and tibia bone ash did not differ as well. The third experiment showed an increase in the body weight gain with 4 g/kg compare to 9 g/kg calcium.

Therefore, evaluating a new calcium source is not unique; however, evaluating litter ash as the mineral source has not previously been done with poultry, but it has been used with pigs. A study conducted by North Carolina State University Extension (Swine News, 2006) used turkey litter ash in the diet and results indicated no difference between the litter ash and the positive control in growth performance and digestibility. Turkey litter was processed through a gasification procedure by turkey producers in order to obtain turkey litter ash (Pagliari et al., 2010; Strock et al., 2006; Mukhter and Capareda, 2006). However, gasification processes required high temperature and it may reduce mineral bioavailability when processed litter is supplemented in the turkey diet. Additionally, litter ash could be used as an alternative mineral source due to the concentrations of P, Ca, N, K, Mg, and S (Eghball and Barbarick, 2007; Burns and Raman, 2010). Therefore, this study was conducted to evaluate this unique dietary calcium source (turkey litter ash) on the growth performance of turkeys from age 7 to 28 days.

Materials and Methods

Birds and Husbandry

This experiment was conducted at the Michigan State University Poultry Teaching and Research Center and approved by the animal care and use committee of Michigan State University. Four hundreds Hybrid poult were placed in brooder batteries at one day of age and fed a control starter diet that met or exceeded the National Research Council (1994) nutritional requirements. On day seven, poult were individually weighed, tagged, and randomly assigned to 42 experimental pens using an Experimental Animal Allotment Program (EAAP, 2009). The EAAP was used to ensure equal pen weights at the start of the experiment. Each pen consisted of eight poult. Each experimental pen was assigned to one of seven experimental diets for the remainder of the experiment resulting in six replicate pens per diet. At day 12, poult were

moved from the battery brooders to grow-out brooders until the end of the study. Poults were given ad libitum access to feed and water for the duration of the experiment. Temperature was recorded daily and the lighting program followed the Hybrid management guide (Table 10).

Diet formulation

The study consisted of seven experimental diets, four diets with limestone as the Ca source and three diets with litter ash as the Ca source. The limestone diets were formulated and mixed in the following manner: negative control diet (NC); lowest Ca concentration 9.0 g Ca/kg and 109 g Solka Floc/kg; positive control diet (PC); highest Ca concentration 15.1 g Ca/kg and 93 g Solka Floc/kg. The other diets, low limestone Ca (LLC) and medium limestone Ca (MLC), were obtained by blending 66.7% NC diet and 33.3% PC diet and 66.7% PC diet and 33.3% NC diet, respectively. The same procedure was followed for formulating and mixing the litter ash diets: low litter ash calcium (LAC); lowest Ca concentration 9.6 g Ca/kg and 53 g Solka Floc/kg; high litter ash calcium (HAC); highest Ca concentration 12.3 g Ca/kg and no Solka Floc; medium litter ash calcium (MAC); blend of 50% LAC diet and 50% HAC. Phosphorus concentration was formulated to be 9.1 g/kg for all diets except LAC and MAC diets, which were 9.0 g/kg. Chromic oxide was included as an indigestible marker using corn starch as a carrier at 5 g/kg diet. The calculated nutrient values are found in Table 9.

Sample Collection

A sample was taken of each experimental diet and stored at -20°C until further analysis. A partial excreta collection was done from day 27 to day 28. Excreta collected from each pen was scraped from the collection papers into individual zip-lock bags and stored at -20°C for future analysis. Poults were euthanized on day 28 and the ileum, located between Meckel's diverticulum and the ileal-cecal junction was removed (Rutherford et al., 2004). Ileal digesta

from each poult in the experimental pen was collected by flushing the ileum with distilled water. The digesta was stored in plastic containers at -20°C for further analysis. Bone samples (tibia and femurs) from the left leg of each poult were collected, placed into a zip-lock bag by experimental pen and stored at -20°C until further analysis.

Sample Analyses

Microwave Digestion Preparation

A microwave digestion was performed to destroy the organic matter in the determinations of calcium and phosphorus concentrations in feed, ileal, and excreta samples. Following freeze drying of excreta and ileal samples, freeze dried samples and feed samples were ground using a Cyclotec Sample Mill 1093 (FOSS North America, Eden Prairie, MN) with a one mm screen to achieve a uniform grind. Feed ingredients were analyzed prior to formulation and feed samples were ground after diet formulation. Samples were digested in nitric acid using a MARS 5 microwave digestion system (CEM Corporation, Matthews NC) using the following procedure (Spears and Liody, 2001).

All glassware used in the digestion process was washed using 30% nitric acid and distilled de-ionized (dd) H₂O to remove any residuals left from other minerals analyzed previously. Approximately 0.4 g of sample was weighed into a digestion vessel to which 10 mL of 70% nitric acid (Omni Trace, EMD Chemicals, Inc., Gibbstown, NJ) was added and allowed to predigest at room temperature overnight. The next day, vessels were placed in the microwave digester and the digestion program run at 1200 wattage; 30 minute ramp to 160°C under pressure of 190 PSI, hold samples for 10 minutes, and cool down for five minutes. After further cooling in a fume hood for 10 more minutes, 2 ml of 30% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) was added. The sample digest was then transferred to a 25 ml volumetric flask and cooled

completely; dd H₂O was added to bring the volume to 25 ml. Samples were transferred to 50 ml polypropylene tubes and stored at room temperature until calcium and phosphorus analysis.

Calcium determination

Calcium concentration was determined by atomic absorption spectroscopy after dilution in a 1% La solution (from lanthanum chloride, Sigma-Aldrich, St. Louis, MO) as an ionization suppressant to eliminate the phosphate interference, which occurs in an air-acetylene flame. The initial feed ingredients were analyzed using the method of standard additions on a Solar 989 atomic absorption spectrophotometer (Thermo Elemental, Franklin, MA). Other samples (feed, ileal contents, and excreta) were analyzed using an AA7000 Series atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). These samples were analyzed against a five point, matrix matched standard curve (Ca standard source: VWR International, West Chester, PA) ranging in concentration between 1 and 5 µg/ml Ca. A Bovine Liver Standard (NIST, Gaithersburg, MD) was simultaneously analyzed to maintain instrument accuracy.

Phosphorus determination

Phosphorus was analyzed by measuring the phosphate ion concentration. These ions react with two reagents, molybdate and Elon (p-methylaminophenol sulfate), to make a product that can be read in the spectrophotometer called molybdenum blue (Kaplan and Pesce, 1989). The first solution consisted of 2.5 g of molybdate sulfuric (MS) solution suspended in minimal dd H₂O added to 7 ml of sulfuric acid and the volume brought to 500 ml by adding dd H₂O. The second solution was made by dissolving 1.5 g of sodium bisulfate and 0.5 g of Elon into dd H₂O to make a 50 ml volume (Gomori, 1942). The samples were diluted tenfold using 450 ml of dd H₂O and 50 ml of sample. Concentrations of standard samples used for analyzing phosphorus

were 0.0, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg/dl. The standard samples were made by using 15mg/dl phosphorus, ddH₂O, MS, and Elon (Gomori, 1942). Standards and samples were run in duplicate on a 96-well microplate. Each well received 50 µL standard or sample, 250 µL of MS solution and 25 µL of Elon. The plate was placed in a microplate vortex mixer for 45 minutes and read at 700 nm on a SpectraMax 384 (Molecular Devices) plate reader.

Ether extraction assay

Ether extraction was used to determine the amount of fat in feed, ileal, excreta, and bone samples. The procedure utilized filter paper, which was hot weighed after being kept overnight in a drying oven at 100°C. Briefly, 1 g of each sample was weighed out in duplicate on filter paper. Filter papers, including the samples, were placed in the drying oven for approximately 8 hours and then hot weighed again. A round bottom flask (modified soxhlet) was filled 2/3 of the way with ethyl ether and then the extraction vessel was filled with samples (feed, ileal, excreta, or bones). The extraction vessel takes approximately 1.5 to 2 hours to fill and siphon back the ether to the heating flask (one cycle). After about six cycles, the lid was removed from the vessel and samples were unloaded on trays and placed in a fume hood to evaporate the ether. When the ether evaporated completely, the samples were placed in the drying oven at 100°C overnight and hot weighed the following day. The percent fat calculation was determined accordingly:

$$\% \text{Fat} = \left(1 - \left(\frac{\text{DM hot wt.} - \text{Paper hot wt.}}{\text{sample wt.}} \right) \right) \times 100$$

DM (dry matter) hot wt. = the hot weight of the sample + the hot weight of the paper

Paper hot wt. = the hot weight of the filter paper without sample

Sample wt. = the weight of the sample prior to being placed in the drying oven

Dry matter

Two different dry matter protocols were used depending on the amount of sample available. For feed and excreta samples, 1 g of sample was placed in an empty weigh pan. Pans were weighed and dried in a drying oven at 105°C for 24 hr. The following day, samples were weighed and recorded individually. Ileal sample dry matter was obtained by subtracting the paper hot weight and paperclip weight, used to secure sample in filter paper, from the sample.

Energy determination

Bomb calorimetry was used to determine the energy of the feed, ileal, and excreta samples. One g of feed and approximately 0.8 g ileal and excreta samples were weighed out and formed into pellets using the pellet press holder. Pellet weight was recorded with two duplicate pellets per sample. The bomb calorimeter was heated to 150°F. A standard sample, benzoic acid, was run first followed by the samples. Two bombs were used to run samples. Each bomb head has one capsule, where pellets were placed, and a 10 cm fuse wire was attached to each one of the two fuses and to the pellets, away from the capsule side. The bomb head was inserted into the bomb cylinder and secured. Each bomb was filled with oxygen to 32 atmospheres and placed in the calorimeter bucket that was then filled with 2000 ml of dd H₂O. The calorimeter bucket was placed into the bomb calorimeter and the two ignition wires were pushed on the bomb head prior to closing the bomb calorimeter cover. The initial temperature was taken at equilibrium and the second reading was taken approximately six minutes after firing the bomb. The bucket and bomb were removed from the calorimeter and bomb valve opened to release the pressure. The burned wire was measured and used in the calculation with 1 cm of wire equal to 2.3 calories. The bomb was cleaned and dried between samples. Energy was calculated using the following formula:

$$\text{Heat of Combustion (cal/g)} = \frac{(\text{Ft} - \text{It}) \times \text{standard} - (\text{calories of wire burned})}{\text{sample wt. (g)}}$$

Ft = Final temperature

It = Initial temperature

Standard = benzoic acid was used as standard and it has an energy value of approximately 2400 calories

Nitrogen determination

Total nitrogen was analyzed using HACH total nitrogen method (Hach et al., 1987) running all samples in duplicate. The procedure involves weighing approximately (0.07 g) of sample onto weigh paper. Samples were placed into a 100 ml digesdahl flask. Ten ml of sulfuric acid was added to the sample and digested overnight at room temperature. The digesdahl burner was heated to 440°C and the digesdahl flask was placed on it. The vacuum system was attached to the digesdahl burner to suction smoke from the digesdahl flask. After about six minutes all water was evaporated from the liquid sample. Next, 10 ml of 50% H₂O₂ was added to the flask and heated for another six minutes resulting in white smoke production from the boiling acid. Both the digesdahl flask and condenser were removed from the burner when white smoke was no longer being produced indicating that H₂O₂ was removed. After cooling the contents of the flask to room temperature, 100 ml of dd H₂O was added to the sample. Eight hundred µl of the diluted sample was removed from the flask and placed in a centrifuge tube. Twenty ml of 0.1g/l solution of polyvinyl alcohol (PVA) was added to each tube and vortexed to ensure adequate mixing. Each sample tube was analyzed in duplicate on a 96-well microplate. One hundred sixty µl of sample or standard were pipetted into each well, with 32.25 µl of PVA, and 7.75 µl of Nessler reagent. Each plate had 84 samples plus 12 standards (0, 0.01, 0.02, 0.04, 0.06, and 0.08 mg N/ml). The plate was placed on a plate shaker for five minutes and read using a

spectrophotometer at 460 nm wavelength (Hach et al., 1987). Nitrogen percentage was calculated:

$$\% N = \frac{N \left(\frac{\text{mg}}{\text{dl}} \right) \times \text{dilution (100)}}{\text{sample wt. (g)} \times 1000} \times 100$$

$$\% \text{ CP} = \% \text{ N} \times 6.25$$

$$N \left(\frac{\text{mg}}{\text{dl}} \right) = ((\text{average value of N sample read by spectrophotometer} - \text{Y intercept})/\text{slope}) \times 100$$

Sample wt. (g) = the weight of the sample prior to being placed in the drying oven

CP = Crude Protein

6.25 = Consistent correction factor multiplied with N% to obtain crude protein percentage

Chromium determination

All glassware used in this analysis was washed using 30% nitric acid and distilled de-ionized (dd) H₂O to remove any residuals left from the minerals analyzed previously. Duplicate 0.5 g samples from each pen were placed into 150 ml Pyrex glass beaker and ashed at 600°C for 1.5 hours in the ashing oven. After cooling to room temperature, 3 ml of phosphoric acid-manganese sulfate solution and 4 ml of 4.5 %, w/v, potassium bromate solution were added to each beaker. Beakers were covered with watch glasses and digested on a hot plate for seven minutes until the color of the digested sample changed to purple. Beakers were removed from the hot plate and cooled to room temperature. Digested samples were rinsed from the beaker with dd H₂O into a 100 ml volumetric flask. Calcium chloride solution, containing 4000 ppm of calcium, was added to the flask (12.5 ml) and the volume was brought up to 100 ml by adding dd H₂O. Samples were diluted 1/3 (1 part sample, 3 part dd H₂O) for feed samples and 1/7 (1 part sample, 7 part dd H₂O) for ileal and excreta samples. The diluted samples were analyzed using atomic absorption spectrophotometer (SpectrAA 220 FS, Varian Analytical Instruments, Walnut Creek,

CA). Standard samples (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 ppm) were used for analyzing the samples (Williams et al., 1962). Chromium percentage was calculated using the following equation:

$$\% \text{ Cr} = \left(\frac{\left(\frac{\text{Cr ppm/g}}{\text{sample wt. (g)}} \right)}{1000} \right) \times 100$$

Cr ppm/g = chromium value read by the AA multiplied by the correction factor (dilution)

Sample wt. (g) = the weight of the sample prior to being placed in the drying oven

Bone

Bones were removed from the freezer, thawed, and cleaned using a scalpel. The fibula was separated and removed from the tibia. The length and the width of each bone were measured with a measurement tape and Vernier calipers. All the tibias from each pen were wrapped in a piece of gauze that is big enough to hold all the bones together with a wing tag for identification; the same procedure was used for the femurs. The package of bones was ether extracted using the same procedure as stated above. Following ether extraction, the bones were removed from the gauze and placed into a hot weighed crucible. The crucibles were placed in the drying oven 105°C for approximately 12 hours and then hot weighed prior to ashing at 600°C in the ashing oven for at least 12 hours. Following ashing, the crucibles were hot weighed again.

Production Measurements

Body weight and feed intake were measured weekly. The dead birds were weighed and factored in the calculation of body weight gain (BWG) and feed conversion ratio (FCR) per pen. Apparent ileal nutrient digestibility and total tract digestibility were calculated as described below (Adedokun et al., 2007):

$$\text{Apparent ileal nutrient digestibility, (\%)} = [1 - (\text{chromium in diet}/\text{chromium in ileal digesta}) \times (\text{nutrients in ileal digesta}/\text{nutrients in diet})]$$

Total tract nutrient digestibility, (%) = $[1 - (\text{chromium in diet} / \text{chromium in excreta}) \times (\text{nutrients in excreta} / \text{nutrients in diet})]$.

Nutrients = defined as dry matter, nitrogen, calcium, phosphorus, fat, and energy

Statistical analysis

With a randomized complete block design (RCBD), the data were analyzed using the PROC MIXED feature of SAS (SAS 9.2, Cary, NC. USA). Differences were adjusted using the Tukey's method and significance accepted at $P < 0.05$. Pen was used as the experimental unit. Statistical analysis for body weight gain, feed intake, and feed conversion ratio was run for day 7 to 14, 14 to 21, 22 to 28, and 7 to 28 individually. Body weight gain, feed intake, feed conversion ratio, ileal and excreta nutrients digestibility and bones were analyzed using the statistical model $Y_{ij} = \mu + T_i + B_j + E_{ij}$, The observation (Y_{ij}) is equal to the sum of mean (μ), the mean effect of treatments (T_i), fixed effect of block (B_j), and the error term (E_{ij}). Calcium effect between treatments was measured using linear and quadratic contrast based on seven concentrations of analyzed dietary calcium.

Results

Parameters measured

Body weight was measured weekly. There were no differences between treatments during each week or from day 7 to day 28 (Tables 11, 12). The negative control diet (9.2 g/kg Ca) resulted in the lowest body weight and unexpectedly, the medium limestone calcium (11.7 g/kg Ca) had the highest body weight, out gaining the positive control (12.9 g/kg Ca). Overall, linear response for the body weight gain was observed in the limestone diets due to the increase of Ca in the diet ($P \leq 0.03$) while the body weight gain in the ash diets had a trend toward a linear response (Table 11, 12). There was no difference between calcium sources when evaluating body weight gain.

Similar to the observations associated with body weight, no differences in feed intake were observed between the dietary treatments regardless of the calcium source (Table 13). Also, no difference was found between calcium sources in the feed conversion ratio. Feed conversion ratio in the limestone diets showed a linear response trend ($P \leq 0.06$) while in the litter ash diets showed a quadratic response ($P \leq 0.03$; Table 14).

Bone

Femur and tibia bone measurements (length, width, weight, and ash) were determined. No differences were observed in tibia or femur size related to dietary mineral source. However, a difference in femur ash percentages was found between calcium sources ($P \leq 0.05$; Table 17). Femur ash in the limestone diets had a linear response ($P \leq 0.05$) when Ca increases in the diet while no linear or quadratic trend was observed with the litter ash. The opposite was observed with the tibia ash with no linear trend for the limestone diets, but a quadratic response was observed for tibia ash in the litter ash diets ($P \leq 0.006$; Table 17).

Nutrient Digestibility

Nutrient digestibility was measured in the ileum and in the excreta of the poult (Tables 15, 16). No difference was observed in the dry matter or nitrogen between the diets in the ileum or excreta (Tables 15, 16). Ileal calcium digestibility was different between the calcium levels for the low ($P \leq 0.05$) and high ($P \leq 0.003$) diets but no difference was observed between the calcium sources (Table 15). Ileal calcium digestibility with limestone and litter ash diets showed a linear response curve ($P < 0.0001$; $P \leq 0.0006$). Ileal phosphorus digestibility has a linear trend response curve with limestone ($P \leq 0.06$) and litter ash diets ($P \leq 0.008$). The percent fat digestibility in the diet was different between the two mineral sources with ash litter diets having higher values than the limestone diets ($P \leq 0.02$; Table 15).

The major differences observed in the excreta digestibility revolve around calcium ($P \leq 0.003$) and energy ($P \leq 0.0005$). Overall, the difference in total tract calcium digestibility was significant between the calcium sources for the medium ($P \leq 0.02$) and high ($P \leq 0.0001$) diets (Table 16). Total tract calcium digestibility in the limestone diet had a linear response curve ($P \leq 0.0001$) and with a litter ash diet had both linear and quadratic response ($P < 0.0001$; $P \leq 0.02$; Table 16). Energy digestibility was different between the mineral sources for the medium diets ($P \leq 0.0005$). Total tract energy digestibility with the ash diets showing a quadratic response curve ($P \leq 0.002$).

Discussion

Body weight and feed intake

Table 9 presents the diet formulations and analyzed values of diets providing calcium from two different sources. The diets were designed to have calcium increase from 9.0 g/kg to 15.1 g/kg using a standard calcium source (limestone) while the alternative calcium source (litter ash) also increasing from 9.6 g/kg to 12.3 g/kg. Since the calcium source is the only variable that changed, the production aspects measured were anticipated to increase as the calcium levels increased. However, this did not occur. Poults on the standard medium diet had a 41 g higher body weight gain at the end of 21 days compared to the positive control (Table 12). However, low diet of the litter ash had 125 g and 41 g higher body weight gain at the end of 21 days compared to medium and high ash diets respectively (Table 12).

A similar observation was made with feed intake for the limestone and litter ash diets. Sanders et al. (1992) found that increasing calcium levels from limestone in turkey diets do not translate into a linear increase in body weight or feed efficiency when they used Nicholas tom turkeys from 0 to 16 days of age. Similarly, Lilburn et al. (1997) noted that increasing calcium

levels from limestone in turkey diets do not show a linear increase in body weight gain or feed intake of commercial poult 0 to 18 days of age.

. Moreover, similar results have been observed in broilers where body weight gain or feed intake was not affected by the level of calcium in the diet (Walk et al., 2012; Zyla et al., 2000). A study with ducks found that body weight and feed intake did not increase linearly with the increase of calcium levels (Rush et al., 2005). A study published with swine found no differences between limestone and turkey litter ash in body weight gain and feed efficiency (Swine News, 2006). Therefore, no differences observed in body weight gain (Table 11, 12) and feed intake (Table 13) in the present study between calcium sources.

Bone

No significant differences were found between calcium sources related to femur or tibia length, width, or weight (Table 17). Thus, these are similar to results reported by Lilburn et al. (1997) for turkey poult. However, in their study, calcium level did influence the bone measurements (Lilburn et al., 1997). In the current study, the femur ash was statistically different between sources at 28 days of age. Lilburn et al. (1997) reported no significant differences between tibia and femur ash with 10-day-old turkey poult due to calcium source but calcium levels affected tibia and femur ash. Walk et al. (2012) found that with increasing levels of calcium intake, the percentage of tibia ash increased as well. There was a linear response of femur ash with the limestone diets ($P \leq 0.05$) and the tibia ash had a quadratic response with the litter ash diets ($P \leq 0.006$). Calcium and phosphorus influence tibia ash more than body weight and feed intake according to Walk et al. (2012). The differences observed between published research and the present study may stem from the calcium: phosphorus levels in the diet or the availability of calcium from the litter ash.

Nutrient Digestibility

Nutrient digestibility was determined in the ileum and the excreta of turkey poults. There were no differences in ileal digestibility between calcium sources with the exception of fat. Fat digestibility was numerically higher in the litter ash diets compared to the limestone diets leading to the difference between calcium sources ($P \leq 0.02$). The values are lower than what has been reported for broilers (Mountzouris et al., 2010; Gehring et al., 2012) or swine (Johnston et al., 2004). Mountzouris et al. (2010) reported no difference in the ileal dry matter digestibility between different calcium levels in broilers with a dry matter percentage around 63%. Similarly, no difference was observed in the ileal dry matter digestibility between calcium sources in current study; however, low dry matter percentage was found (56%) (Table 15). This could be due to diet composition or turkey versus broiler diets. Nitrogen digestibility was not significantly different between calcium sources and similar in values reported by Gehring et al., 2012.

Ileal calcium digestibility was not different between calcium sources but was different between the levels of calcium. The diets with the lowest levels of calcium (limestone – 10.4 g/kg vs. litter ash – 9.9 g/kg) were different ($P \leq 0.05$) compared to the highest levels of calcium (limestone – 12.9 g/kg vs. litter ash – 12.1 g/kg; $P \leq 0.003$; Table 15). Ileal calcium digestibility values decreased as the dietary calcium levels increased in limestone diets ($P \leq 0.0001$). In the litter ash diets, calcium digestibility increased linearly with dietary calcium levels ($P \leq 0.0006$). Amad et al. (2011) found that ileal calcium digestibility for the control treatment was approximately 38% when broilers age 22 to 42 days were fed calcium at a concentration of 9.9 g/kg from limestone and monocalcium phosphate, which is higher than we observed in this experiment. Johnston et al. (2004) reported that lower calcium concentrations in the diet would increase calcium digestibility in the ileum. However, this was not consistent with the data

presented in Table 15. Therefore, there is an inverse relationship between calcium intake and ileal calcium digestibility; when calcium intake increases the calcium digestibility decreases.

Ileal phosphorus digestibility was trend to be different between the calcium sources ($P \leq 0.06$). The limestone diets were trending toward a negative linear relationship between dietary calcium values and phosphorus digestibility. The litter ash diets had a negative linear response with less than 50% digestibility of phosphorus being found with the highest concentration of calcium from litter ash in the diet (12.1 g/kg). Yi et al. (1996) reported ileal phosphorus digestibility values of 53% while Camden et al. (2001) reported values of 55%. With the exception of the highest concentration of litter ash calcium, all other values were similar to what has been reported in the literature.

Nutrient digestibility in the excreta was similar to ileal digestibility with no differences between calcium source except for calcium and energy digestibility (Table 16). A similar relationship was found with excreta calcium digestibility with calcium decreasing linearly across calcium source diets ($P < 0.0001$). Johnston et al. (2004) reported that reducing calcium intake levels results in an increase in calcium digestibility which is opposite to what was observed in the present study.

Energy digestibility in the excreta was affected by the calcium source ($P \leq 0.0005$). Johnston et al. (2004) indicated the concentrations of calcium and phosphorus supplied in the diet influence the energy digestibility. With the exception of the mid-levels of calcium (limestone – 11.7 g/kg; litter ash 11.0 g/kg), energy levels were found to be similar between the other calcium levels (Table 16). A study in pigs with turkey litter ash reported that energy digestibility was not significantly different between NC and PC diets (Swine News, 2006).

Summary

Using litter ash in the diet of turkey poult resulted in minimal differences compared to the limestone diets when evaluating the calcium source. The results of body weight, feed intake, feed conversion ratio, and tibia and femur measurements indicated no differences between calcium sources or levels of calcium included in the diet on turkey poult performance from 7 to 28 days of age. The exception was the differences in femur ash between calcium sources that may warrant a further investigation into the biological impact on the femur. For example, does the difference in ash relate to the overall strength of the femur or the ability to withstand fracturing?

Calcium digestibility needs to be considered when evaluating the excreta values while looking at the ileal values there is no difference between sources. The data suggest that diets formulated to contain approximately 11 g/kg of calcium from litter ash perform the same as the limestone diet. Therefore, there appears to be no difference between the dietary calcium sources. If the litter ash is priced competitively, turkey diets can be formulated to use it as a calcium source. However, the amount of litter ash is considerably higher, g/kg, compared to limestone so availability could be an issue for diet inclusion.

APPENDIX

Table 9. Diet formulation and nutrient composition of experimental diets (as-fed basis) for calcium

Ingredients, g/kg	Diet (g/kg) ¹						
	NC	LLC	MLC	PC	LAC	MAC	HAC
Corn	264.4	264.4	264.4	264.4	264.4	264.9	265.4
Soybean meal, 48% CP	500	500	500	500	500	500	500
DL-Methionine	2.6	2.6	2.6	2.6	2.6	2.6	2.6
L-threonine	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Lysine HCl	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Soybean oil	50	50	50	50	50	50	50
Monocalcium phosphate ²	31	31	31	31	17	11.5	6
Litter ash	-	-	-	-	77	108.5	140
Limestone (38% Ca)	7	12	18	23	-	-	-
NaCl	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Vitamin-mineral premix ³	3	3	3	3	3	3	3
Solkafloc ⁴	109	104	98	93	53	26.5	0
Chromic oxide premix ⁵	25	25	25	25	25	25	25
Calculated analyses							
ME kcal/kg	2627	2627	2627	2627	2627	2629	2630
CP g/kg	273	273	273	273	273	273	273
Calcium g/kg	9.0	11.0	13.1	15.1	9.6	10.9	12.3
Phosphorus g/kg	9.1	9.1	9.1	9.1	9.0	9.0	9.1
Non-phytate phosphorus g/kg	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Ca:P Ratio	1.0	1.2	1.4	1.7	1.1	1.2	1.4
Determined analyses							
CP g/kg	278	280	282	283	283	282	281
Calcium g/kg	9.2	10.4	11.7	12.9	9.9	11.0	12.1
Phosphorus g/kg	10.8	10.6	10.4	10.2	10.6	10.7	10.8
Ca:P ratio	0.9	1.0	1.1	1.3	0.9	1.0	1.1

¹ Diet abbreviations: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium.

² 16% Ca, 21% P

³ Supplies the following per kg diet: Vit. A, 5484 IU; Vit. D3, 2643 ICU; Vit E, 11 IU; Menadione sodium bisulfite, 4.38 mg; Riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; Niacin, 44.1 mg; Choline chloride, 771 mg; Vit B12, 13.2 ug; Biotin, 55.2 ug; Thiamine mononitrate, 2.2 mg; Folic acid, 990 ug; Pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 ug. Also contains per g of premix: Vit. A, 1828 IU; Vit. D3, 881 ICU; Vit E, 3.67 IU; Menadione sodium bisulfite, 1.46 mg; Riboflavin, 1.83 mg; d-pantothenic acid, 3.67 mg; Niacin, 14.69 mg; Choline chloride, 257 mg; Vit B12, 4.4 ug; Biotin, 18.4 ug; Thiamine mononitrate, 735 ug; Folic acid, 330 ug; Pyridoxine hydrochloride, 1.1 mg; I, 370 ug; Mn, 22.02 mg; Cu, 1.48 mg; Fe, 14.69 mg; Zn, 14.69 mg; Se, 100 ug.

⁴ Purified cellulose, International Fiber Corp., North Tonawanda, NY

⁵ Chromic oxide (Cr2O3) premix added as indigestible marker at a ratio 1:4 of chromic oxide: corn starch.

Table 10. Lighting program used for the 28-day calcium trial¹

Day	Photoperiod	
	Light (hr)	Dark (hr)
1	24	0
2	23	1
3 to 5	20	4
6 to 9	18	6
10 to 28	16	8

¹ Lighting program based on Hybrid turkey management guide

Table 11. Body weight gain (g/bird) for turkey poult fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/bird) ³					
NC	9.2	163.3 ± 10.6	249.7 ± 14.1	350.0 ± 18.3	790.0 ± 24.3
LLC	10.4	186.6 ± 10.6	250.8 ± 14.1	376.3 ± 18.3	813.7 ± 24.3
MLC	11.7	185.7 ± 10.6	302.6 ± 14.1	372.5 ± 18.3	865.5 ± 24.3
PC	12.9	194.8 ± 10.6	282.5 ± 14.1	375.0 ± 18.3	852.3 ± 24.3
LAC	9.9	200.0 ± 10.6	267.5 ± 14.1	385.8 ± 18.3	853.4 ± 24.3
MAC	11.0	191.9 ± 10.6	284.9 ± 14.1	364.2 ± 18.3	846.3 ± 24.3
HAC	12.1	196.6 ± 10.6	277.9 ± 14.1	373.8 ± 18.3	848.3 ± 24.3
Probability					
Contrast					
LLC vs. LAC		0.38	0.41	0.71	0.26
MLC vs. MAC		0.68	0.38	0.75	0.58
PC vs. HAC		0.90	0.82	0.96	0.91
Limestone vs. litter ash		0.42	0.87	1.00	0.78
Source of variation					
Response Curve					
Linear limestone ⁴		0.06	0.02	0.39	0.03
Linear litter ash ⁵		0.09	0.13	0.62	0.17
Quadratic litter ash ⁶		0.16	0.30	0.57	0.22

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 12. Body weight gain (g/pen) for turkey poult fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/pen) ³					
NC	9.2	1,306 ± 85.0	1,937 ± 113.8	2,800 ± 146.1	6,176 ± 222.7
LLC	10.4	1,493 ± 85.0	2,007 ± 113.8	3,010 ± 146.1	6,510 ± 222.7
MLC	11.7	1,485 ± 85.0	2,393 ± 113.8	2,980 ± 146.1	6,859 ± 222.7
PC	12.9	1,558 ± 85.0	2,260 ± 113.8	3,000 ± 146.1	6,818 ± 222.7
LAC	9.9	1,600 ± 85.0	2,140 ± 113.8	3,087 ± 146.1	6,827 ± 222.7
MAC	11.0	1,535 ± 85.0	2,253 ± 113.8	2,913 ± 146.1	6,702 ± 222.7
HAC	12.1	1,573 ± 85.0	2,223 ± 113.8	2,990 ± 146.1	6,786 ± 222.7
Probability					
Contrast					
LLC vs. LAC		0.38	0.41	0.71	0.32
MLC vs. MAC		0.68	0.39	0.75	0.62
PC vs. HAC		0.90	0.82	0.96	0.92
Limestone vs. litter ash		0.42	0.88	1.00	0.82
Source of variation					
Response Curve					
Linear limestone ⁴		0.06	0.01	0.39	0.03
Linear litter ash ⁵		0.09	0.08	0.62	0.13
Quadratic litter ash ⁶		0.16	0.24	0.57	0.23

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 13. Feed intake (g/pen) for turkey poult fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/pen) ³					
NC	9.2	2,070 ± 92.0	3,240 ± 109.1	4,753 ± 190.3	10,063 ± 325.9
LLC	10.4	2,020 ± 92.0	3,257 ± 109.1	4,893 ± 190.3	10,170 ± 325.9
MLC	11.7	2,043 ± 92.0	3,423 ± 109.1	5,230 ± 190.3	10,697 ± 325.9
PC	12.9	2,120 ± 92.0	3,383 ± 109.1	5,160 ± 190.3	10,663 ± 325.9
LAC	9.9	2,157 ± 92.0	3,403 ± 109.1	4,960 ± 190.3	10,520 ± 325.9
MAC	11.0	2,048 ± 92.0	3,370 ± 109.1	4,950 ± 190.3	10,368 ± 325.9
HAC	12.1	2,117 ± 92.0	3,483 ± 109.1	5,020 ± 190.3	10,620 ± 325.9
Probability					
Contrast					
LLC vs. LAC		0.30	0.35	0.81	0.45
MLC vs. MAC		0.97	0.73	0.31	0.48
PC vs. HAC		0.98	0.52	0.61	0.93
Limestone vs. litter ash		0.54	0.47	0.45	0.98
Source of variation					
Response Curve					
Linear limestone ⁴		0.68	0.23	0.07	0.12
Linear litter ash ⁵		0.98	0.18	0.39	0.33
Quadratic litter ash ⁶		0.96	0.82	0.71	0.78

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 14. Feed conversion ratio for turkey poult fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(feed/gain) ³					
NC	9.2	1.84 ± 0.16	1.69 ± 0.06	1.70 ± 0.07	1.63 ± 0.02
LLC	10.4	1.36 ± 0.16	1.65 ± 0.06	1.63 ± 0.07	1.56 ± 0.02
MLC	11.7	1.38 ± 0.16	1.45 ± 0.06	1.82 ± 0.07	1.56 ± 0.02
PC	12.9	1.36 ± 0.16	1.51 ± 0.06	1.73 ± 0.07	1.56 ± 0.02
LAC	9.9	1.34 ± 0.16	1.59 ± 0.06	1.61 ± 0.07	1.54 ± 0.02
MAC	11.0	1.34 ± 0.16	1.51 ± 0.06	1.70 ± 0.07	1.55 ± 0.02
HAC	12.1	1.35 ± 0.16	1.57 ± 0.06	1.68 ± 0.07	1.57 ± 0.02
Probability					
Contrast					
LLC vs. LAC		0.94	0.54	0.89	0.49
MLC vs. MAC		0.88	0.54	0.28	0.76
PC vs. HAC		0.96	0.49	0.68	0.93
Limestone vs. litter ash		0.87	0.69	0.35	0.59
Source of variation					
Response Curve					
Linear limestone ⁴		0.06	0.02	0.41	0.06
Linear litter ash ⁵		0.07	0.17	0.85	0.14
Quadratic litter ash ⁶		0.12	0.17	0.77	0.03

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 15. Ileal digestibility mean and standard error for turkey poultlets fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	DM	N (%)	Ca (%)	P (%)	Fat (%)	Energy (Kcal/g)
NC	9.2	55.77 ± 1.20	81.78 ± 2.19	49.46 ^a ± 3.62	60.04 ± 3.38	37.14 ± 2.89	2851 ± 61.2
LLC	10.4	57.48 ± 1.20	81.49 ± 2.19	39.11 ^{ab} ± 4.35	60.89 ± 3.38	40.08 ± 2.89	2921 ± 61.2
MLC	11.7	55.10 ± 1.20	77.85 ± 2.19	29.74 ^{bc} ± 4.35	51.91 ± 3.38	34.85 ± 2.89	2791 ± 61.2
PC	12.9	54.93 ± 1.20	79.46 ± 2.19	14.04 ^c ± 4.42	53.38 ± 3.38	36.50 ± 2.89	2824 ± 61.2
LAC	9.9	55.99 ± 1.20	78.53 ± 2.19	27.26 ^{bc} ± 3.97	52.61 ± 3.38	42.15 ± 2.89	2776 ± 61.2
MAC	11.0	56.64 ± 1.32	80.09 ± 2.40	34.52 ^{abc} ± 5.39	51.22 ± 3.70	44.57 ± 3.17	2738 ± 61.2
HAC	12.1	57.92 ± 1.20	78.68 ± 2.19	29.35 ^b ± 3.01	45.97 ± 3.38	42.30 ± 2.89	2898 ± 61.2
Probability							
Contrast							
LLC vs. LAC		0.39	0.35	0.05	0.09	0.62	0.10
MLC vs. MAC		0.39	0.50	0.49	0.89	0.03	0.57
PC vs. HAC		0.09	0.80	0.003	0.13	0.17	0.40
Limestone vs. litter ash		0.32	0.78	0.43	0.06	0.02	0.42
Source of variation							
Response Curve							
Linear limestone ³		0.36	0.28	<0.0001	0.06	0.57	0.44
Linear litter ash ⁴		0.19	0.48	0.0006	0.008	0.21	0.61
Quadratic litter ash ⁵		0.75	0.74	0.08	0.67	0.19	0.07

^{a-c} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Includes negative control diet

⁴ Includes negative control diet

⁵ Includes negative control diet

Table 16. Excreta digestibility mean and standard error for turkey poult s fed different dietary calcium sources from 7 to 28 days of age

Treatments ¹	Dietary Ca (g/kg) ²	DM	N (%)	Ca (%)	P (%)	Fat (%)	Energy (Kcal/g)
NC	9.2	57.78 ± 0.66	57.18 ± 2.44	55.08 ^a ± 1.97	43.94 ± 1.89	48.16 ± 4.00	3124 ^a ± 26.1
LLC	10.4	59.99 ± 0.66	58.49 ± 2.44	49.78 ^{ab} ± 1.97	47.35 ± 1.89	45.27 ± 4.00	3184 ^a ± 26.1
MLC	11.7	58.57 ± 0.66	59.36 ± 2.44	33.21 ^{cde} ± 1.97	41.15 ± 1.89	47.72 ± 4.00	3131 ^a ± 26.1
PC	12.9	59.01 ± 0.66	58.89 ± 2.44	26.07 ^e ± 1.97	43.08 ± 1.89	46.99 ± 4.00	3156 ^a ± 26.1
LAC	9.9	58.69 ± 0.66	55.66 ± 2.44	46.19 ^{ad} ± 1.97	41.52 ± 1.89	47.40 ± 4.00	3111 ^a ± 26.1
MAC	11.0	58.76 ± 0.66	59.18 ± 2.44	40.10 ^{cd} ± 1.97	43.99 ± 1.89	38.26 ± 4.00	2990 ^b ± 26.1
HAC	12.1	59.12 ± 0.66	55.12 ± 2.44	38.38 ^d ± 1.97	42.60 ± 1.89	39.38 ± 4.00	3127 ^a ± 26.1
Probability							
Contrast							
LLC vs. LAC		0.17	0.42	0.21	0.04	0.71	0.06
MLC vs. MAC		0.84	0.96	0.02	0.30	0.10	0.0005
PC vs. HAC		0.91	0.28	<0.0001	0.86	0.19	0.44
Limestone vs. litter ash		0.54	0.26	0.003	0.46	0.14	0.0005
Source of variation							
Response Curve							
Linear limestone ³		0.47	0.59	<0.0001	0.29	0.96	0.73
Linear litter ash ⁴		0.19	0.81	<0.0001	0.91	0.05	0.42
Quadratic litter ash ⁵		0.65	0.50	0.02	0.91	0.57	0.002

^{a-e} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Includes negative control diet

⁴ Includes negative control diet

⁵ Includes negative control diet

Table 17. Bone measurements, ash, and dry weight for turkey poult fed different dietary calcium sources from 7 to 28 days of age

Trt ¹	Dietary Ca ² (g/kg)	Length (cm)		Width (mm)		Ash (%)		Dry bone wt. (g) ³	
		Femur	Tibia	Femur	Tibia	Femur	Tibia	Femur	Tibia
NC	9.2	6.98 ± 0.07	9.37 ± 0.13	6.34 ± 0.10	6.33 ± 0.08	43.03 ± 0.53	50.86 ± 1.45	18.69 ± 0.84	22.89 ± 1.52
LLC	10.4	6.92 ± 0.07	9.28 ± 0.13	6.14 ± 0.10	6.27 ± 0.08	42.53 ± 0.53	49.83 ± 1.45	19.35 ± 0.84	23.95 ± 1.52
MLC	11.7	6.96 ± 0.07	9.39 ± 0.13	6.32 ± 0.10	6.39 ± 0.08	43.42 ± 0.53	49.96 ± 1.45	19.58 ± 0.84	24.21 ± 1.52
PC	12.9	7.03 ± 0.07	9.54 ± 0.13	6.34 ± 0.10	6.46 ± 0.08	44.35 ± 0.53	50.08 ± 1.45	20.59 ± 0.84	25.93 ± 1.52
LAC	9.9	6.94 ± 0.07	9.41 ± 0.13	6.30 ± 0.10	6.43 ± 0.08	42.03 ± 0.53	47.42 ± 1.45	19.15 ± 0.84	24.48 ± 1.52
MAC	11.0	6.83 ± 0.07	9.60 ± 0.13	6.11 ± 0.10	6.36 ± 0.08	43.24 ± 0.53	46.01 ± 1.45	17.45 ± 0.84	24.98 ± 1.52
HAC	12.1	6.93 ± 0.07	9.20 ± 0.13	6.29 ± 0.10	6.37 ± 0.08	42.42 ± 0.53	50.88 ± 1.45	19.04 ± 0.84	22.77 ± 1.52
Probability									
Contrast									
LLC vs. LAC		0.79	0.50	0.26	0.15	0.51	0.25	0.87	0.81
MLC vs. MAC		0.22	0.28	0.16	0.80	0.81	0.06	0.08	0.72
PC vs. HAC		0.36	0.09	0.70	0.41	0.02	0.70	0.20	0.15
Limestone vs. ash		0.28	0.99	0.70	0.82	0.05	0.13	0.07	0.62
Source of variation									
Response Curve ⁴									
Linear limestone		0.59	0.32	0.68	0.14	0.05	0.74	0.13	0.18
Linear ash		0.46	0.53	0.47	0.94	0.86	0.98	0.86	0.93
Quadratic ash		0.26	0.09	0.21	0.60	0.92	0.006	0.37	0.21

¹ Diets: NC = negative control, LLC = low limestone calcium, MLC = medium limestone calcium, PC = positive control, LAC = low litter ash calcium, MAC = medium litter ash calcium, HAC = high litter ash calcium

² Analyzed value

³ Dry bone wt. = ether extracted bone weight.

⁴ Includes negative control diets for linear and quadratic calculations

REFERENCES

REFERENCES

- Applegate, T. J., R. Angel, and H. L. Classen 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. *Poult. Sci.* 82:1140-1148.
- Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. *Poult. Sci.* 86:2598-2607.
- Akpe, M. P., P. E. Waibel, K. Larntz, A. L. Metz, S. L. Noll, and M. M. Walser. 1987. Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. *Poult. Sci.* 66:713-720.
- Ajakaiye, A., J.O. Atteh, and S. Leeson. 2003. Biological availability of calcium in broiler chicks from different calcium sources found in Nigeria. *Animal Feed Sci. and Technol.* 104:209-214.
- Amad, A. A., K. Manner, K. R. Wendler, K. Neumann, and J. Zentek, 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poult. Sci.* 90:2811-2816.
- Boitumelo, P. T., 2004. Influence of limestone particle size in layer diets on shell characteristics at peak production. M.Sc. Thesis, University of the Free State, Bloemfontein, South Africa.
- Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soybean diets for broilers. *Anim. Sci.* 73:289-297.
- Elaroussi, M. A., L. R. Forte, S.L. Eber, and H.V. Biellier, 1994. Calcium homeostasis in laying hen. 1. Age and dietary calcium effects, Columbia, USA. *Poult. Sci.* 73:1581-1589.
- Gomori G. 1942. A modification of the colorimetric phosphorus determination for use with a photoelectric colorimeter. *J Lab. Clin. Med.* 27:955-957.
- Gehring, C. K., M. R. Bedford, A. J. Cowieson, and W. A. Dozier. 2012. Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. *Poult. Sci.* 91:1908-1914.
- Highfill, C. 1998. Calcium, phosphorus and vitamin D3 in your birds diet. *Winged Wston Pet Bird Magazine*. April Magazine.
- Hach, C. C., B. K. Bowden, A. B. Kopelove, and S. V. Brayton 1987. More powerful peroxide kjeldahl digestion method. *J. Assoc. Off. Anal. Chem.* 70(5):783-787.

- Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. *J. Anim. Sci.* 82:705-714.
- Klasing, K. C. 1998. *Comparative avian nutrition*. Cab International.
- Kaplan, L. A. and A. J. Pesce. 1989. *Clinical Chemistry: Theory, Analysis and Correlation*. (2nd ed.), C.V. Mosby, St. Louis. 974-983.
- Lilburn, M. S., G. W. Barbour, R. Nemasetoni, C. Coy, M. Werling, and A. G. Yersin. 1997. Protein quality and calcium availability from extruded and autoclaved turkey hatchery residue. *Poult. Sci.* 76:841-848.
- Mussehl, F. E. and C. W. Ackerson. 1935. Calcium and phosphorus requirements of growing turkeys. *Poult. Sci.* 14:147-151.
- Mountzouris, K. C., P. Tsitsrikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poult. Sci.* 89:58-67.
- NRC, National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Rush, J. K., C. R. Angel, K. M. Banks, K. L. Thompson, and T. J. Applegate. 2005. Effect of dietary calcium and vitamin d3 on calcium and phosphorus retention in white pekin ducklings. *Poult. Sci.* 84:561-570.
- Sanders, A.M., H.M. E. JR, and G.N. Rowland 1992. Calcium and phosphorus requirements of the very young turkey as determined by response surface analysis. *Br. J. Nutr.*, 67:421-435.
- Siebrits, F.K., 1993. Mineral and vitamins in pig diets. In: E.H. Kemm (Ed.). *Pig Production in South Africa*. Agricultural Research Council Bulletin 427.
- Spears, J. W., and M. Lioyd. 2001. Personal communication, North Carolina State University.
- Swine news. 2006. Combustion Ash can serve as a Mineral Supplement in Swine Diets. *NCSU Extension Swine Husbandry*, 29:03.
- Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometer. *J. Agric. Sci.* 59:381-385.
- Walk, C. L., M. R. Bedford, and A. P. McElroy. 2012. Influence of limestone and phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility. *Poult. Sci.* 91:1371-1378.

Yi, Z., E. T. Kornegay, and D. M. Denbow. 1996. Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poult fed corn-soybean meal diets. *Poult. Sci.* 75(8):979-990.

Zyla, K., J. Koreleski, S. Swiatkiewicz, A. Wikiera, M. Kujawski, J. Piironen, and D. R. Ledoux. 2000. Effects of phosphorolytic and cell wall-degrading enzymes on the performance of growing broilers fed wheat-based diets containing different calcium levels. *Poult. Sci.* 79(1):66-76.

Chapter Three

Phosphorus

Introduction

Phosphorus is an important element for poultry production (Powell et al., 2008). Applegate and Angel (2008) reported that phosphorus is important for animal growth and bone development and the amount of phosphorus consumed will increase as long as animals are growing. Moreover, phosphorus is important for the animal's blood, enzymes and cells and is used in the synthesis of the high-energy chemicals ADP and ATP (Bell and Weaver, 2002; Pond et al., 1995). Phosphorus concentrations have to be sufficient in poultry diets; otherwise, health problems, such as rickets, may develop (Waibel et al., 1984). Phosphorus digestion and absorption occurs in the ileum and the absorption of phosphorus is increased when dietary phosphorus intake increases and is decreased with increased calcium intake (Hurwitz et al., 1978). However, Powell et al. (2011) found that phosphorus digestibility increased as calcium concentration increased. Moreover, phosphorus digestibility is affected by microbial phytase due to the improvement of phosphorus digestibility with the addition of microbial phytase in the diet (Rutherford et al. 2004).

Phosphorus availability and digestibility are other important factors for animal growth and they need to be measured to determine the phosphorus requirement. The bone ash method was initially used to determine phosphorus availability from different phosphorus sources in turkey diets (Wilcox et al. 1954, 1955). In addition, body growth is another measure of the availability of phosphorus and it provides similar results to bone ash (Patrick and Bacon 1957; Summers et al., 1959). Moreover, Akpe et al. (1987) found that bone ash and bone densitometry

could be used for measuring phosphorus availability from different phosphorus sources for poult; however, bone densitometry was faster and more accurate than bone ash.

There are many sources of phosphorus used in poultry diets and the effect of these sources varies between animals. Monocalcium and dicalcium phosphate are the most standard phosphorus sources used with poultry. Wilcox et al. (1954) found that phosphorus from monocalcium phosphate and dicalcium phosphate were equally effective in terms of turkey growth; however, phosphorus from monocalcium phosphate increased tibia ash compared to dicalcium phosphate. Scott et al. (1962) evaluated anhydrous dicalcium phosphate (CaHPO_4) and found that adding soybean meal to a diet with this source of phosphorus would increase bone ash. Other researchers demonstrated that anhydrous dicalcium phosphate (CaHPO_4) was a poor phosphorus source compared to hydrated dicalcium phosphate $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Gillis et al., 1962; Scott et al., 1962). Therefore, different phosphorus sources have been evaluated to determine their efficacy in poultry nutrition.

As new byproducts become available in the poultry industry, there is potential to include them within the diet. However, depending on the cost and nutrient availability not all byproducts are considered. Turkey producers are processing turkey litter through a gasification procedure (Pagliari et al., 2010; Strock et al., 2006; Mukhter and Capareda, 2006). The byproduct, litter ash, could be used as an alternative mineral source due to the concentrations of P, Ca, N, K, Mg, and S (Eghball and Barbarick, 2007; Burns and Raman, 2010). However, the high temperatures during the gasification process may reduce the bioavailability of these minerals when fed in a turkey diet. Therefore, a study was conducted to evaluate phosphorus availability from a unique dietary mineral source (turkey litter ash) on the performance of turkeys from 7 to 28 days of age.

Materials and Methods

Birds and Husbandry

This experiment was conducted at the Michigan State University Poultry Science Teaching and Research Center and approved by the animal care and use committee of Michigan State University. Three hundred eighty-five Hybrid poultts were placed in brooder batteries at one day of age and fed a control starter diet that met or exceeded the National Research Council (1994) nutritional requirements. On day seven, poultts were individually weighed, tagged, and randomly assigned to 42 experimental pens using an Experimental Animal Allotment Program (EAAP, 2009). The EAAP was used to ensure equal pen weights at the start of the experiment. Each pen consisted of eight poultts. Each experimental pen was assigned to one of seven different experimental diets for the remainder of the experiment resulting in six replicate pens per diet. At day 12, poultts were moved from the battery brooders to grow-out brooders until the end of the study. Poultts were given ad libitum access to feed and water for the duration of the experiment. Temperature was recorded daily and the lighting program followed the Hybrid management guide (Table 19).

Diet formulation

Diets were formulated and mixed at Purdue University and transported to Michigan State University. The study consisted of experimental diets, four diets with monosodium phosphate as the P source and three diets with litter ash as the P source. The monosodium phosphate diets were formulated and mixed in the following manner: Negative control diet (NC); lowest P concentration 5.3 g P/kg and 90 g Solka Floc/kg; positive control diet (PC); highest P concentration of 9.2 g P/kg and 72 g Solka Floc/kg. The other diets, low monosodium phosphate (LMP) and medium monosodium phosphate (MMP), were obtained by blending 66.7% NC diet

and 33.3% PC diet and 66.7% PC diet and 33.3% NC diet, respectively. The same procedure was followed for formulating and mixing the litter ash diets: low litter ash phosphorus (LAP); lowest P concentration 5.5 g P/kg and 41 g Solka Floc/kg; high litter ash phosphorus (HAP); highest P concentration of 7.3 g P/kg and no Solka Floc; medium litter ash phosphorus (MAP); blend of 50% LAP diet and 50% HAP. The calcium concentration was formulated to be 15.1 g/kg for all diets. Chromic oxide was included as an indigestible marker using corn starch as a carrier at 5 g/kg diet. The calculated nutrient values are found in Table 18.

Sample Collection

A sample of each experimental diet was taken and stored at - 20°C until further analysis. A partial excreta collection was done from day 27 to day 28. Excreta collected from each pen was scraped from the collection papers into individual zip-lock bags and stored at -20°C for future analysis. Poult were euthanized on day 28 and the ileum, located between Meckel's diverticulum and the ileal-cecal junction was removed (Rutherford et al., 2004). Ileal digesta from each poult in the experimental pen was collected by flushing the ileum with distilled water and storing the digesta in plastic containers at - 20°C for subsequent analysis. Bone samples (tibia and femurs) from the left leg of each poult were collected placed into a zip-lock bag by experimental pen and stored at - 20°C until further analysis.

Sample Analyses

Microwave Digestion Preparation

A microwave digestion was performed to destroy the organic matter in the determinations of calcium and phosphorus concentrations in feed, ileal, and excreta samples. Following freeze drying of excreta and ileal samples, freeze dried samples and feed samples were ground using a Cyclotec Sample Mill 1093 (FOSS North America, Eden Prairie, MN) with a one mm screen to

achieve a uniform grind. Feed ingredients were analyzed prior to formulation and feed samples were ground after diet formulation. Samples were digested in nitric acid using a MARS 5 microwave digestion system (CEM Corporation, Matthews NC) using the following procedure (Spears and Lioyd, 2001).

All glassware used in the digestion process was washed using 30% nitric acid and distilled de-ionized (dd) H₂O in order to remove any residuals left from other minerals analyzed previously. Approximately 0.4 g of sample was weighed into a digestion vessel to which 10 mL of 70% nitric acid (Omni Trace, EMD Chemicals, Inc., Gibbstown, NJ) was added and allowed to predigest at room temperature overnight. The next day, vessels were placed in the microwave digester and the digestion program run at 1200 wattage; 30 minute ramp to 160°C under pressure of 190 PSI, hold samples for 10 minutes, and cool down for five minutes. After further cooling in a fume hood for 10 more minutes, 2 ml of 30% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) was added. Sample digest was then transferred to a 25 ml volumetric flask and cooled completely; dd H₂O was added to bring the volume to 25 ml. Samples were transferred to 50 ml polypropylene tubes and stored at room temperature until analyses of calcium and phosphorus.

Calcium determination

Calcium concentration was determined by atomic absorption spectroscopy after dilution in a 1% La solution (from lanthanum chloride, Sigma-Aldrich, St. Louis, MO) as an ionization suppressant to eliminate the phosphate interference, which occurs in an air-acetylene flame. The initial feed ingredients were analyzed using the method of standard additions on a Solar 989 atomic absorption spectrophotometer (Thermo Elemental, Franklin, MA). Other samples (feed, ileal contents, and excreta) were analyzed using an AA7000 Series atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). These samples were

analyzed against a five point, matrix-matched standard curve (Ca standard source: VWR International, West Chester, PA) ranging in concentration from 1 to 5 µg/ml Ca. A Bovine Liver Standard (NIST, Gaithersburg, MD) was simultaneously analyzed to maintain instrument accuracy.

Phosphorus determination

Phosphorus was analyzed by measuring the phosphate ion concentration. These ions react with two reagents, molybdate and Elon (p-methylaminophenol sulfate), to make a product that can be read in the spectrophotometer called molybdenum blue (Kaplan and Pesce, 1989). The first solution consisted of 2.5 g of molybdate sulfuric acid (MS) suspended in minimal dd H₂O added to 7 ml of sulfuric acid and the volume brought to 500 ml by adding dd H₂O. The second solution was made by dissolving 1.5 g of sodium bisulfate and 0.5 g of Elon in dd H₂O to make a 50 ml volume (Gomori, 1942). The samples were diluted tenfold using 450 ml of dd H₂O and 50 ml of sample. Standard sample concentrations used for analyzing phosphorus were 0.0, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg/dl. The standard samples were made by using 15 mg/dl phosphorus, dd H₂O, MS, and Elon (Gomori, 1942). Standards and samples were run in duplicate on a 96-well microplate. Each well received 50 µL standard or sample, 250 µL of MS solution and 25 µL of Elon. The plate was placed in a microplate vortex mixer for 45 minutes and read at 700 nm on a SpectraMax 384 (Molecular Devices) plate reader.

Ether extraction assay

Ether extraction was used to determine the amount of fat in feed, ileal, excreta, and bone samples. The procedure utilizes filter papers, which were hot weighed after keeping them overnight in the drying oven at 100°C. Briefly, 1 g of sample was weighed out in duplicate on filter paper. Filter papers, including the samples, were placed in the drying oven for

approximately eight hours and then hot weighed again. A round bottom flask (modified soxhlet) was filled with ethyl ether two thirds of the way and then the extraction vessel was filled with samples (feed, ileal, excreta, or bones). The extraction vessel took approximately 1.5 to 2 hours to fill and to siphon the ether back into the heating flask (one cycle). After about six cycles, the lid was removed from the vessel and samples were unloaded on trays and placed in a fume hood to evaporate the ether. When the ether evaporated completely, the samples were placed in the drying oven at 100°C overnight and hot weighed the following day. The percent fat was determined accordingly:

$$\% \text{Fat} = \left(1 - \left(\frac{\text{DM hot wt.} - \text{Paper hot wt.}}{\text{sample wt.}} \right) \right) \times 100$$

DM (dry matter) hot wt. = the hot weight of the sample + the hot weight of the paper

Paper hot wt. = the hot weight of the filter paper without sample

Sample wt. = the weight of the sample prior to being placed in the drying oven

Dry matter

Two different dry matter protocols were used depending on the amount of sample available. For feed and excreta samples, 1 g of sample was placed in an empty weigh pan. Pans were weighed and dried in a drying oven at 105°C for 24 hr. The following day, samples were weighed and recorded individually. Ileal sample dry matter was obtained by subtracting the paper hot weight and paperclip weight (used to secure sample in filter paper) from the sample.

Energy determination

Bomb calorimetry was used to determine the energy of the feed, ileal, and excreta samples. One g of feed and approximately 0.8 g of ileal and excreta samples were weighed out and formed into pellets using the pellet press holder. Pellet weight was recorded with two duplicate pellets per sample. The bomb calorimeter was heated to 150°F. A standard sample,

benzoic acid, was run first followed by the samples. Two bombs were used to run samples. Each bomb head had one capsule, where pellets were placed, and a 10 cm fuse wire was attached to each one of the two fuses and to the pellets, away from the capsule side. The bomb head was inserted into the bomb cylinder and secured. Each bomb was filled with oxygen to 32 atmospheres and placed in the calorimeter bucket that was then filled with 2000 ml of dd H₂O. The calorimeter bucket was placed into the bomb calorimeter and the two ignition wires were pushed on the bomb head prior to closing the bomb calorimeter cover. The initial temperature was taken at equilibrium and the second reading was taken approximately six minutes after firing the bomb. The bucket and bomb were removed from the calorimeter and bomb valve opened to release the pressure. The burned wire was measured and used in the calculation where 1 cm wire equaled 2.3 calories. The bomb was cleaned and dried between samples. Energy was calculated using the following formula:

$$\text{Heat of Combustion (cal/g)} = \frac{(\text{Ft} - \text{It}) \times \text{standard} - (\text{calories of wire burned})}{\text{sample wt. (g)}}$$

Ft = Final temperature

It = Initial temperature

Standard = benzoic acid was used as standard and it has an energy value of approximately 2400 calories

Nitrogen determination

Total nitrogen was analyzed using HACH total nitrogen method (Hach et al., 1987) running all samples in duplicate. The procedure involves weighing approximately 0.07 g of sample onto a type of paper. Samples were placed into a 100 ml digesdahl flask. Ten ml of sulfuric acid was added to the sample and digested overnight at room temperature. The digesdahl burner was heated to 440°C and the digesdahl flask was placed on it. The vacuum system was

attached to the digesdahl burner to suction smoke from the digesdahl flask. After about six minutes all the water was evaporated from the liquid sample. Next, 10 ml of 50% H₂O₂ was added to the flask and heated for another six minutes resulting in white smoke production resulting from the boiling acid. Both digesdahl flask and condenser were removed from the burner when white smoke was no longer being produced indicating that H₂O₂ was removed. After cooling the contents of the flask to room temperature, 100 ml of dd H₂O was added to the sample. Eight hundred µl of the diluted sample was removed from the flask and placed in a centrifuge tube. Twenty ml of 0.1g/l solution of polyvinyl alcohol (PVA) was added to each tube and vortexed to ensure adequate mixing. Each sample tube was analyzed in duplicate on a 96-well microplate. One hundred sixty µl of sample or standard was pipetted into each well, with 32.25 µl of PVA, and 7.75 µl of Nessler reagent. Each plate had 84 samples plus 12 standards (0.0, 0.01, 0.02, 0.04, 0.06, and 0.08 mg N/ml). The plate was placed on a plate shaker for 5 minutes and read using a spectrophotometer at 460 nm wavelength (Hach et al., 1987). Nitrogen percentage was calculated:

$$\% N = \frac{N \left(\frac{\text{mg}}{\text{dl}} \right) \times \text{dilution (100)}}{\text{sample wt. (g)} \times 1000} \times 100$$

$$\% CP = \% N \times 6.25$$

$$N \left(\frac{\text{mg}}{\text{dl}} \right) = ((\text{average value of N sample read by spectrophotometer} - \text{Y intercept})/\text{slope}) \times 100$$

Sample wt. (g) = the weight of the sample prior to being placed in the drying oven

CP = Crude Protein

6.25 = Consistent correction factor multiplied with N% to obtain crude protein percentage

Chromium determination

All glassware used in this analysis was washed using 30% nitric acid and distilled de-ionized (dd) H₂O to remove any residuals left from the minerals analyzed previously. Duplicate 0.5 g samples from each pen were placed into 150 ml Pyrex glass beaker and ashed at 600°C for 1.5 hours in the ashing oven. After cooling to room temperature, 3 ml of phosphoric acid-manganese sulfate solution and 4 ml of 4.5 %, w/v, potassium bromate solution were added to each beaker. Beakers were covered with watch glasses and the samples were digested on a hot plate for seven minutes until the color of the digested sample changed to purple. Beakers were removed from the hot plate and cooled to room temperature. Digested samples were rinsed from the beaker with dd H₂O into a 100 ml volumetric flask. Calcium chloride solution, containing 4000 ppm of calcium, was added to the flask (12.5 ml) and the volume was brought up to 100 ml by adding dd H₂O. Samples were diluted 1/3 (1 part sample, 3 parts dd H₂O) for feed samples and 1/7 (1 part sample, 7 parts dd H₂O) for ileal and excreta samples. The diluted samples were analyzed using atomic absorption spectrophotometer (SpectrAA 220 FS, Varian Analytical Instruments, Walnut Creek, CA). Standard samples (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 ppm) were used for analyzing the samples (Williams et al., 1962). Chromium percentage was calculated using the following equation:

$$\% \text{ Cr} = \left(\frac{\left(\frac{\text{Cr ppm/g}}{\text{sample wt. (g)}} \right)}{1000} \right) \times 100$$

Cr ppm/g = chromium value read by the AA multiplied by the correction factor (dilution)

Sample wt. (g) = the weight of the sample prior to being placed in the drying oven

Bone

Bones were removed from the freezer, thawed, and cleaned using a scalpel. The fibula was separated and removed from the tibia. The length and the width of each bone were measured with Stainless steel tape. All the tibias from each pen were wrapped in a piece of gauze held together with a wing tag for identification; the same procedure was used for the femurs. The package of bones was either extracted using the same procedure as stated above. Following ether extraction, the bones were removed from the gauze and placed into a hot weighed crucible. The crucibles were placed in the drying oven 105°C for approximately 12 hours and then hot weighed prior to ashing at 600°C in the ashing oven for at least 12 hours. Following ashing, the crucibles were hot weighed again.

Production Measurements

Body weight and feed intake were determined weekly. The dead birds were weighed and factored in the calculation of body weight gain and feed conversion ratio per pen. Apparent ileal nutrient digestibility and total tract digestibility were calculated as described below (Adedokun et al., 2007):

Apparent ileal nutrient digestibility (%) = $[1 - (\text{chromium in diet} / \text{chromium in ileal digesta}) \times (\text{nutrients in ileal digesta} / \text{nutrients in diet})]$

Total tract nutrient digestibility (%) = $[1 - (\text{chromium in diet} / \text{chromium in excreta}) \times (\text{nutrients in excreta} / \text{nutrients in diet})]$.

Nutrients = defined as dry matter, nitrogen, calcium, phosphorus, fat, and energy

Statistical analysis

With a randomized complete block design (RCBD), the data were analyzed using the PROC MIXED feature of SAS (SAS 9.2, Cary, NC. USA). Differences were adjusted using the Tukey's method and significance accepted at $P < 0.05$. Pen was used as the experimental unit.

Statistical analysis for body weight gain, feed intake, and feed conversion ratio was run for day 7 to 14, 14 to 21, 22 to 28, and 7 to 28 individually. Body weight gain, feed intake, feed conversion ratio, ileal and excreta nutrient digestibility and bones were assessed using the statistical model $Y_{ij} = \mu + T_i + B_j + E_{ij}$ where the observation (Y_{ij}) is equal to the sum of mean (μ), the mean effect of treatments (T_i), fixed effect of block (B_j), and the error term (E_{ij}). Phosphorus effect between treatments was measured using linear and quadratic contrast based on seven concentrations of analyzed dietary phosphorus.

Results

Parameters measured

Body weight and feed intake were measured on a weekly basis from day 7 to 28. Body weight was different between phosphorus sources ($P \leq 0.01$; Tables 20, 21). Body weight gain for monosodium phosphate diets had a negative linear trend ($P \leq 0.01$) while the litter ash diets had a positive linear trend ($P \leq 0.01$; Table 20). The same was observed when evaluating the body weight gain on a pen level (Table 21). The inversely related linear curves resulted in differences between the high (12.4 g/kg – standard vs. 9.3 g/kg – litter ash) phosphorus levels in the diet ($P \leq 0.002$) during the 21-day period (Tables 20, 21).

Differences in body weight gain are also reflected in feed intake (Table 22) and feed conversion ratio (Table 23). Feed intake was influenced by phosphorus source with consumption linearly decreasing as dietary phosphorus concentrations increased with the standard phosphorus diets ($P \leq 0.02$; Table 22). While not significant, there was a trend for poult on the litter ash diets to consume more feed with increasing phosphorus concentrations. The feed conversion ratio was not affected by phosphorus source, but it had a positive linear response ($P \leq 0.03$) to

increasing phosphorus concentrations in the standard diet while no linear or quadratic response was observed with the litter ash diets (Table 23).

Bone

Bone parameters analyzed in the study were femur and tibia length, width, percent ash, and bone weight (Table 26). Femur and tibia length were not different between treatments ($P > 0.05$). Femur width is influenced by phosphorus source ($P \leq 0.05$) while no influence was observed with the tibia. Femur width had a positive linear response as the phosphorus levels increased in litter ash diets. Femur and tibia ash were highly influenced by phosphorus source ($P \leq 0.0002$, $P < 0.0001$ respectively). Both femur and tibia ash had a positive linear response ($P \leq 0.004$, $P < 0.0008$ respectively) and quadratic response ($P \leq 0.04$, $P < 0.003$ respectively) with litter ash diets whereas the monosodium phosphate diets resulted in no linear or quadratic trend. Bone weight was not influenced by the phosphorus source but the femur had a negative linear response to the increasing phosphorus concentrations in the monosodium phosphate diets ($P \leq 0.009$). However, there was a strong positive linear response ($P < 0.0002$) for the femur (Table 26) with the litter ash diets. No linear or quadratic trends were found for tibia bone weight.

Nutrient digestibility

Nutrient digestibility was measured in the ileum and excreta from the turkey poult at day 28. The apparent ileal digestibility (Table 24) indicated that phosphorus source influenced nitrogen ($P \leq 0.04$), calcium ($P \leq 0.0004$), fat ($P \leq 0.03$), and energy ($P \leq 0.0004$). The nitrogen values from the litter ash source had a quadratic response curve ($P \leq 0.03$). The difference in the ileal nitrogen digestibility was noted between the low diets of monosodium phosphate and litter ash. The apparent digestibility of calcium had a strong negative linear relationship to the dietary phosphorus concentrations in the standard diets ($P \leq 0.0007$; Table 24). While differences were

observed in the fat and energy values related to phosphorus source, no linear or quadratic lines can be used to explain the response values.

Total gastrointestinal tract digestibility is reported in Table 25. The phosphorus source had an impact on dry matter digestibility with values associated with the standard diets being numerically higher than those associated with the litter ash diets ($P \leq 0.006$; Table 25). The percent nitrogen was different between phosphorus sources ($P \leq 0.003$) with a negative linear trend associated with the litter ash diets ($P \leq 0.002$). Although, calcium differences existed between the mineral sources ($P \leq 0.02$), the values could not be explained by linear or quadratic equations. Percent phosphorus digestibility in the standard diet was greatly affected by litter ash ($P < 0.0001$) having a negative linear response to increasing phosphorus levels with monosodium phosphate diets ($P < 0.0001$), while the litter ash had a quadratic response ($P \leq 0.02$). Finally, the energy value was different between the phosphorus sources ($P < 0.0001$). No linear response was found for the monosodium phosphate source, but the litter ash energy values had a negative linear response to the increasing phosphorus levels ($P < 0.0001$; Table 25).

Discussion

Body Weight and Feed Intake

The diets of this study contained two different phosphorus sources (monosodium phosphate and litter ash). Monosodium phosphate was the standard source used with phosphorus concentrations of 7.9, 9.4, 10.9 and 12.4 g/kg respectively. The test phosphorus source was litter ash at concentrations of 7.4, 8.4, and 9.3 g/kg respectively. The phosphorus concentrations in all diets were formulated to increase from low to high concentrations while maintaining a consistent amount of calcium across dietary treatments (15.1 g/kg). Potter (1988) reported that 4.4 g of total phosphorus was needed to obtain good body weight gain for turkeys 0 to 4 weeks of age.

Waldroup et al. (2000) indicated that the phosphorus requirements for broiler body weight gain is about 5.7 g for 0 to 3 wk of age but can change depending on the calcium and nonphytate phosphorus concentrations in the diet. The 1994 NRC recommendation for phosphorus was 6 g/kg from 0 to 4 wks of age. Those values are considerably lower than the negative control diet (7.9 g/kg). The study was conducted to determine the availability of phosphorus from litter ash to be used in turkey diet formulations.

Body weight gain was found to be significant between treatments from d 7 to 28 ($P < 0.01$; Table 20 and 21). Body weight, feed intake and feed conversion ratio had a negative linear response to phosphorus concentrations of the monosodium phosphate diets (Tables 20-23). The opposite was observed with the litter ash diets with a positive linear response trending toward significance. Potchanakorn and Potter (1987) and Potter (1988) used increasing concentrations of phosphorus from different sources in turkey diets to three weeks of age and found that body weight gain increased linearly with all phosphorus sources. Diets with increased concentrations of phosphorus and a consistent concentration of calcium resulted in a linear increase in body weight and feed intake (Driver et al., 2005). This is similar to what was observed with the litter ash diets. The negative linear response with the monosodium phosphate diets may be due to the calcium: phosphorus ratio and nonphytate phosphorus concentrations in the diets.

Bone

Bone characteristics including femur and tibia length, width, ash, and weight were evaluated (Table 26). Femur and tibia length were not influenced by the phosphorus source, however, femur width was affected. It is unclear why the femur width was affected by the phosphorus source but it could be related to the available phosphorus for bone growth. The percent of ash in the femur and tibia was highly affected by phosphorus source ($P \leq 0.0002$; $P \leq$

0.0001) with a positive linear trend observed with the litter ash treatments in both bones ($P \leq 0.004$; $P \leq 0.0008$; Table 26). No difference was observed between tibia and femur ash with the monosodium phosphate treatments similar to results reported by Roberson et al. (2004). Roberson et al. (2004) found an increase in the bone ash values with the increase of calcium and nonphytate phosphorus concentrations. Bone ash was a good factor for evaluating phosphorus availability compared to body weight (Nelson and Walker, 1964).

Nutrient digestibility

Apparent ileal digestibility was evaluated using the different phosphorus sources (Table 24). Apparent ileal dry matter digestibility was not different between phosphorus sources. De Coca-Sinova et al. (2008) and Adedokun et al. (2007) reported dry matter digestibility values similar to those in the present study. A difference between nitrogen digestibility existed with phosphorus source ($P \leq 0.04$) with the values reported being similar to values in the literature (De Coca-Sinova et al., 2008). The differences observed in the calcium digestibility may be due to the extremely low (5.54%) and negative (-4.31%) values found at the highest concentrations of phosphorus in the monosodium phosphate diet. Ileal phosphorus digestibility values did not vary between sources or have a linear relationship to calculated values. However, the litter ash diets had a quadratic relationship with phosphorus digestibility at the highest and lowest dietary phosphorus concentrations being similar while phosphorus digestibility at the the middle phosphorus concentration was 4 to 6% higher ($P \leq 0.04$). Rodehutscord et al. (2012) found the phosphorus digestibility did not change with increased concentrations of phosphorus similar to the monosodium phosphate diets of this study. The ileal fat digestibility and energy values were different between the litter ash and monosodium phosphate diets ($P \leq 0.03$, $P \leq 0.0004$). Fat

digestibility was found to be approximately similar to the digestibility of fat reported by Mountzouris et al. (2010).

Total tract nutrient digestibility was analyzed and reported in Table 25. Total tract dry matter digestibility was different between the phosphorus sources ($P \leq 0.006$). Yi et al. (1996) found that dry matter total tract digestibility decreased with the increase of phosphorus concentrations in the diet. Nitrogen digestibility was different between standard phosphorus diets and litter ash treatments ($P \leq 0.003$). The total tract nitrogen digestibility decreased linearly with the increase of phosphorus concentrations in the litter ash treatments. However, Johnston et al. (2004) reported that nitrogen, dry matter, and energy total tract digestibility was not affected by lowering the concentration of phosphorus in pig diets. Calcium digestibility was different between the two phosphorus sources ($P \leq 0.02$) but no linear or quadratic trends were observed within the dietary sources. However, Johnston et al. (2004) indicated that total tract calcium digestibility in pigs increased when the concentration of phosphorus decreased in the diet. The phosphorus digestibility was different between the phosphorus sources ($P < 0.0001$). Phosphorus digestibility in birds fed the monosodium phosphate diets had a negative linear response ($P < 0.0001$) to increasing dietary phosphorus while phosphorus digestibility in birds fed the litter ash diets had a quadratic response ($P \leq 0.02$) with an increase and then decrease in percent phosphorus digestible as the dietary phosphorus increased in the diet. Johnston et al., (2004) observed that lowering phosphorus concentration in the diet reduced total tract phosphorus digestibility.

Although there were no differences between sources of phosphorus for fat digestibility, fat digestibility had a negative linear response ($P \leq 0.04$) to increasing phosphorus in both the monosodium phosphate diets and litter ash diets. Energy total tract digestibility was different

between phosphorus sources ($P < 0.0001$) and there was a negative linear response ($P < 0.0001$) to the increasing dietary phosphorus levels in the litter ash diets only. The literature is sparse on data related to nutrient digestibility of ash being fed to poultry. However, a study that involved feeding turkey litter ash to swine found no differences in digestibility compared to the control diets (Swine News, 2006).

Summary

Body weight gain and feed intake were influenced by the source of phosphorus resulting in differences between the monosodium phosphate and litter ash diets. Feed conversion ratio was not different between the phosphorus sources in the study. Within the femur and tibia measurements, the percent ash was greatly influenced by the sources with litter ash having values lower than the monosodium phosphate diets. The ramifications of these differences are unknown but could lead to skeletal weakness or have a negative influence on femur and tibia strength later in life. Most of the nutrients digested in the ileum and total tract showed differences between the two sources. The most important parameters to further evaluate would be calcium and phosphorus digestibility. However, the data support the use of litter ash at the concentration of 8.4 g/kg to provide the phosphorus needed for the turkey poult from day 7 to 28.

APPENDIX

Table 18. Diet formulation and nutrient composition of experimental diets (as-fed basis) for phosphorus

Ingredients, g/kg	Diet (g/kg) ¹						
	NC	LMP	MMP	PC	LAP	MAP	HAP
Corn	273.4	273.4	273.4	273.4	271.8	272.7	273.6
Soybean meal, 48% CP	500	500	500	500	500	500	500
DL-Methionine	2.6	2.6	2.6	2.6	2.6	2.6	2.6
L-Threonine	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Lysine HCl	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Soybean oil	50	50	50	50	50	50	50
Monosodium phosphate ²	12	18	24	30	-	-	-
Litter ash	-	-	-	-	77	101	125
Limestone (38% Ca)	36	36	36	36	21.6	17.2	12.8
NaCl	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Vitamin-mineral premix ³	3	3	3	3	3	3	3
Solkafloc ⁴	90	84	78	72	41	20.5	0
Chromic oxide premix ⁵	25	25	25	25	25	25	25
Calculated analyses							
ME, kcal/kg	2658	2658	2658	2658	2653	2656	2659
CP, g/kg	274	274	274	274	274	274	274
Calcium, g/kg	15.1	15.1	15.1	15.1	15.1	15.1	15.1
Phosphorus, g/kg	5.3	6.6	7.9	9.2	5.5	6.4	7.3
Nonphytate phosphorus, g/kg	4.0	5.3	6.6	7.9	4.2	5.1	6.0
Ca:P Ratio	2.9	2.5	2.1	1.6	2.7	2.4	2.1
Determined analyses							
CP, g/kg	283	285	286	287	281	281	282
Calcium, g/kg	15.2	14.8	14.4	14.0	14.9	15.2	15.5
Phosphorus, g/kg	7.9	9.4	10.9	12.4	7.4	8.4	9.3
Ca:P Ratio	1.9	1.6	1.3	1.1	2.0	1.8	1.7

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP= low litter ash, MAP = medium litter ash, HAP = high litter ash

² Made by Prince Agri Products Quincy, IL and it has 26% P, 19% Na, and 1500 ppm fluorine.

³ Supplies the following per kg DIET: Vit. A, 5484 IU; Vit. D3, 2643 ICU; Vit E, 11 IU; Menadione sodium bisulfite, 4.38 mg; Riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; Niacin, 44.1 mg; Choline chloride, 771 mg; Vit B12, 13.2 ug; Biotin, 55.2 ug; Thiamine mononitrate, 2.2 mg; Folic acid, 990 ug; Pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 ug. Also contains per g of premix: Vit. A, 1828 IU; Vit. D3, 881 ICU; Vit E, 3.67 IU; Menadione sodium bisulfite, 1.46 mg; Riboflavin, 1.83 mg; d-pantothenic acid, 3.67 mg; Niacin, 14.69 mg; Choline chloride, 257 mg; Vit B12, 4.4 ug; Biotin, 18.4 ug; Thiamine mononitrate, 735 ug; Folic acid, 330 ug; Pyridoxine hydrochloride, 1.1 mg; I, 370 ug; Mn, 22.02 mg; Cu, 1.48 mg; Fe, 14.69 mg; Zn, 14.69 mg; Se, 100 ug.

⁴ Purified cellulose. International Fiber Corp., North Tonawanda, NY

⁵ Chromic oxide (Cr₂O₃) premix added as indigestible marker at a ratio 1:4 of chromic oxide:corn starch.

Table 19. Lighting program used for the 28 day phosphorus trial¹

Day	Photoperiods	
	Light (hr)	Dark (hr)
1	24	0
2	23	1
3 to 5	20	4
6 to 9	18	6
10 to 28	16	8

¹ Lighting program based on Hybrid guide

Table 20. Body weight gain (g/bird) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/bird) ³					
NC	7.9	146.9 ± 3.5	279.2 ± 7.5	373.8 ± 13.8	799.8 ^{ab} ± 22.4
LMP	9.4	153.6 ± 3.5	277.1 ± 7.5	363.8 ± 13.8	794.4 ^{ab} ± 22.4
MMP	10.9	146.5 ± 3.5	258.8 ± 7.5	338.3 ± 13.8	739.5 ^{ab} ± 22.4
PC	12.4	137.4 ± 3.5	257.5 ± 7.5	325.4 ± 13.8	720.4 ^b ± 22.4
LAP	7.4	146.6 ± 3.5	277.1 ± 7.5	345.0 ± 13.8	768.7 ^{ab} ± 22.4
MAP	8.4	159.3 ± 3.5	292.5 ± 7.5	345.8 ± 13.8	797.6 ^{ab} ± 22.4
HAP	9.3	158.7 ± 3.5	296.7 ± 7.5	374.2 ± 13.8	829.5 ^a ± 22.4
Probability					
Contrast					
LMP vs. LAP		0.16	1	0.34	0.42
MMP vs. MAP		0.01	0.003	0.70	0.08
PC vs. HAP		0.0001	0.0008	0.02	0.002
Monosodium phosphate vs. Litter ash		0.003	0.0004	0.28	0.01
Source of variation					
Response curve					
Linear monosodium phosphate ⁴		0.03	0.02	0.009	0.006
Linear litter ash ⁵		0.005	0.04	0.29	0.08
Quadratic litter ash ⁶		0.40	0.78	0.90	0.88

^{a,b} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 21. Body weight gain (g/pen) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/pen) ³					
NC	7.9	1,175 ± 29.5	2,233 ± 60.3	2,990 ± 110.6	6,399 ^{ab} ± 179.3
LMP	9.4	1,229 ± 29.5	2,217 ± 60.3	2,910 ± 110.6	6,355 ^{ab} ± 179.3
MMP	10.9	1,160 ± 29.5	2,070 ± 60.3	2,707 ± 110.6	5,916 ^{ab} ± 179.3
PC	12.4	1,100 ± 29.5	2,060 ± 60.3	2,603 ± 110.6	5,763 ^b ± 179.3
LAP	7.4	1,173 ± 29.5	2,217 ± 60.3	2,760 ± 110.6	6,150 ^{ab} ± 179.3
MAP	8.4	1,274 ± 29.5	2,340 ± 60.3	2,767 ± 110.6	6,381 ^{ab} ± 179.3
HAP	9.3	1,269 ± 29.5	2,373 ± 60.3	2,993 ± 110.6	6,636 ^a ± 179.3
Probability					
Contrast					
LMP vs. LAP		0.19	1	0.34	0.42
MMP vs. MAP		0.009	0.003	0.70	0.08
PC vs. HAP		0.0003	0.0008	0.02	0.002
Monosodium phosphate vs. Litter ash		0.003	0.0004	0.28	0.01
Source of variation					
Response curve					
Linear monosodium phosphate ⁴		0.03	0.02	0.009	0.006
Linear litter ash ⁵		0.008	0.04	0.29	0.08
Quadratic litter ash ⁶		0.43	0.78	0.90	0.88

^{a,b} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 22. Feed intake (g/pen) for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(g/pen) ³					
NC	7.9	1,907 ± 32.9	3,250 ± 68.2	4,843 ± 124.2	10,000 ^{ab} ± 211.2
LMP	9.4	1,943 ± 32.9	3,267 ± 68.2	4,793 ± 124.2	10,003 ^{ab} ± 211.2
MMP	10.9	1,863 ± 32.9	3,117 ± 68.2	4,573 ± 124.2	9,553 ^{ab} ± 211.2
PC	12.4	1,827 ± 32.9	3,043 ± 68.2	4,413 ± 124.2	9,283 ^b ± 211.2
LAP	7.4	1,880 ± 32.9	3,160 ± 68.2	4,673 ± 124.2	9,713 ^{ab} ± 211.2
MAP	8.4	1,983 ± 32.9	3,383 ± 68.2	4,803 ± 124.2	10,170 ^{ab} ± 211.2
HAP	9.3	1,933 ± 32.9	3,340 ± 68.2	4,987 ± 124.2	10,260 ^a ± 211.2
Probability					
Contrast					
LMP vs. LAP		0.18	0.28	0.50	0.34
MMP vs. MAP		0.01	0.009	0.20	0.05
PC vs. HAP		0.03	0.004	0.002	0.002
Monosodium phosphate vs. Litter ash		0.05	0.01	0.03	0.02
Source of variation					
Response curve					
Linear monosodium phosphate ⁴		0.04	0.02	0.01	0.009
Linear litter ash ⁵		0.19	0.05	0.11	0.08
Quadratic litter ash ⁶		0.13	0.17	0.95	0.47

^{a,b} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 23. Feed conversion ratio for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	d7 to 14	d15 to 21	d22 to 28	d7 to 28
(feed/gain) ³					
NC	7.9	1.62 ± 0.02	1.46 ± 0.02	1.62 ± 0.04	1.56 ± 0.02
LMP	9.4	1.58 ± 0.02	1.48 ± 0.02	1.65 ± 0.04	1.58 ± 0.02
MMP	10.9	1.61 ± 0.02	1.51 ± 0.02	1.69 ± 0.04	1.62 ± 0.02
PC	12.4	1.66 ± 0.02	1.48 ± 0.02	1.71 ± 0.04	1.61 ± 0.02
LAP	7.4	1.60 ± 0.02	1.43 ± 0.02	1.70 ± 0.04	1.58 ± 0.02
MAP	8.4	1.56 ± 0.02	1.45 ± 0.02	1.74 ± 0.04	1.60 ± 0.02
HAP	9.3	1.52 ± 0.02	1.41 ± 0.02	1.67 ± 0.04	1.55 ± 0.02
Probability					
Contrast					
LMP vs. LAP		0.42	0.12	0.35	0.83
MMP vs. MAP		0.06	0.05	0.36	0.40
PC vs. HAP		<0.0001	0.02	0.48	0.02
Monosodium phosphate vs. Litter ash		0.001	0.001	0.51	0.09
Source of variation					
Response curve					
Linear monosodium phosphate ⁴		0.11	0.28	0.09	0.03
Linear litter ash ⁵		0.001	0.36	0.98	0.32
Quadratic litter ash ⁶		0.56	0.17	0.72	0.39

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Means represent 6 pens of 8 birds per pen.

⁴ Includes negative control diet

⁵ Includes negative control diet

⁶ Includes negative control diet

Table 24. Ileal digestibility mean and standard error for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	DM	N (%)	Ca (%)	P (%)	Fat (%)	Energy (Kcal/g)
NC	7.9	62.96 ^{ab} ± 0.69	89.19 ± 0.96	21.72 ± 5.35	61.01 ± 2.80	51.72 ± 2.95	3107 ^{bc} ± 32.4
LMP	9.4	63.96 ^{ab} ± 0.69	89.09 ± 0.96	16.66 ± 5.35	58.02 ± 2.80	49.78 ± 2.95	3259 ^a ± 32.4
MMP	10.9	64.29 ^{ab} ± 0.69	88.82 ± 0.96	5.54 ± 5.35	58.62 ± 2.80	53.24 ± 2.95	3227 ^{ab} ± 32.4
PC	12.4	63.80 ^{ab} ± 0.69	87.41 ± 0.96	-4.31 ± 5.35	56.82 ± 2.80	56.18 ± 2.95	3166 ^{abc} ± 32.4
LAP	7.4	61.74 ^b ± 0.69	86.06 ± 0.96	20.24 ± 5.35	51.70 ± 2.80	55.95 ± 2.95	3072 ^c ± 32.4
MAP	8.4	64.97 ^a ± 0.69	87.95 ± 0.96	26.80 ± 5.35	57.00 ± 2.80	59.34 ± 2.95	3133 ^{abc} ± 32.4
HAP	9.3	65.03 ^a ± 0.69	86.20 ± 0.96	22.49 ± 5.35	53.24 ± 2.80	60.53 ± 2.95	3138 ^{abc} ± 32.4
Probability							
Contrast							
LMP vs. LAP		0.03	0.03	0.64	0.12	0.15	0.0002
MMP vs. MAP		0.50	0.53	0.008	0.68	0.15	0.05
PC vs. HAP		0.22	0.38	0.001	0.37	0.31	0.55
Monosodium phosphate vs. Litter ash		0.85	0.04	0.0004	0.10	0.03	0.0004
Source of variation							
Response curve							
Linear monosodium phosphate ³		0.36	0.20	0.0007	0.35	0.21	0.33
Linear litter ash ⁴		0.001	0.62	0.71	0.83	0.11	0.16
Quadratic litter ash ⁵		0.13	0.03	0.49	0.04	0.67	0.49

^{a-c} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Includes negative control diet

⁴ Includes negative control diet

⁵ Includes negative control diet

Table 25. Excreta digestibility mean and standard error for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Treatments ¹	Dietary P (g/kg) ²	DM	N (%)	Ca (%)	P (%)	Fat (%)	Energy (Kcal/g)
NC	7.9	64.10 ± 0.51	63.07 ^a ± 1.38	40.84 ± 2.53	62.73 ^a ± 2.30	61.49 ± 2.01	3233 ^{bc} ± 16.0
LMP	9.4	64.20 ± 0.51	62.83 ^a ± 1.38	34.74 ± 2.53	52.92 ^a ± 2.30	55.58 ± 2.01	3375 ^a ± 16.0
MMP	10.9	63.26 ± 0.51	61.28 ^{ab} ± 1.38	31.45 ± 2.53	41.16 ^b ± 2.30	56.81 ± 2.01	3300 ^b ± 16.0
PC	12.4	64.06 ± 0.51	62.63 ^a ± 1.38	36.51 ± 2.53	39.78 ^b ± 2.30	54.61 ± 2.01	3280 ^b ± 16.0
LAP	7.4	62.37 ± 0.51	61.40 ^{ab} ± 1.38	40.19 ± 2.53	53.80 ^a ± 2.30	59.77 ± 2.01	3211 ^{cd} ± 16.0
MAP	8.4	63.06 ± 0.51	58.56 ^{ab} ± 1.38	41.19 ± 2.53	60.35 ^a ± 2.30	57.22 ± 2.01	3148 ^{de} ± 16.0
HAP	9.3	62.44 ± 0.51	56.09 ^{bc} ± 1.38	35.61 ± 2.53	58.08 ^a ± 2.30	55.12 ± 2.01	3107 ^e ± 16.0
Probability							
Contrast							
LMP vs. LAP		0.02	0.47	0.14	0.79	0.15	<0.0001
MMP vs. MAP		0.77	0.17	0.006	<0.0001	0.89	<0.0001
PC vs. HAP		0.03	0.002	0.80	<0.0001	0.86	<0.0001
Monosodium phosphate vs. Litter ash		0.006	0.003	0.02	<0.0001	0.31	<0.0001
Source of variation							
Response curve							
Linear monosodium phosphate ³		0.64	0.65	0.16	<0.0001	0.04	0.36
Linear litter ash ⁴		0.56	0.002	0.19	0.47	0.04	<0.0001
Quadratic litter ash ⁵		0.06	0.46	0.21	0.02	0.64	0.46

^{a-e} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Includes negative control diet

⁴ Includes negative control diet

⁵ Includes negative control diet

Table 26. Bone measurements, ash, and dry weight for turkey poult fed different dietary phosphorus sources from 7 to 28 days of age

Trt ¹	Dietary P (g/kg) ²	Length (cm)		Width (mm)		Ash (%)		Dry bone wt. (g) ³	
		Femur	Tibia	Femur	Tibia	Femur	Tibia	Femur	Tibia
NC	7.9	6.85 ± 0.50	9.08 ± 0.11	6.16 ± 0.10	6.17 ^{ab} ± 0.15	43.54 ^a ± 0.78	52.85 ^{ab} ± 1.05	18.34 ^{ab} ± 0.51	21.37 ± 0.91
LMP	9.4	6.87 ± 0.50	9.15 ± 0.11	6.12 ± 0.10	6.01 ^{ab} ± 0.15	43.84 ^a ± 0.78	52.35 ^{ab} ± 1.05	19.18 ^a ± 0.51	22.73 ± 0.91
MMP	10.9	8.11 ± 0.50	8.83 ± 0.11	6.14 ± 0.10	6.19 ^a ± 0.15	42.40 ^a ± 0.78	55.25 ^a ± 1.05	18.06 ^{ab} ± 0.51	19.45 ± 0.91
PC	12.4	6.68 ± 0.50	8.85 ± 0.11	5.95 ± 0.10	6.10 ^{ab} ± 0.15	43.93 ^a ± 0.78	53.07 ^{ab} ± 1.05	16.59 ^{bc} ± 0.51	19.84 ± 0.91
LAP	7.4	6.60 ± 0.50	9.06 ± 0.11	5.74 ± 0.10	5.50 ^b ± 0.15	38.29 ^b ± 0.78	43.61 ^c ± 1.05	15.22 ^c ± 0.51	19.47 ± 0.91
MAP	8.4	6.66 ± 0.50	8.94 ± 0.11	5.87 ± 0.10	5.79 ^{ab} ± 0.15	41.14 ^{ab} ± 0.78	48.96 ^b ± 1.05	17.57 ^{ab} ± 0.51	20.83 ± 0.91
HAP	9.3	6.91 ± 0.50	9.09 ± 0.11	6.13 ± 0.10	6.30 ^a ± 0.15	42.83 ^a ± 0.78	51.10 ^{ab} ± 1.05	18.79 ^{ab} ± 0.51	22.32 ± 0.91
Probability									
Contrast									
LMP vs. LAP		0.71	0.59	0.007	0.02	<0.0001	<0.0001	<0.0001	0.02
MMP vs. MAP		0.05	0.49	0.05	0.07	0.26	0.0002	0.51	0.29
PC vs. HAP		0.74	0.13	0.19	0.35	0.32	0.19	0.005	0.06
NaH ₂ PO ₄ vs. ash ⁴		0.24	0.33	0.05	0.07	0.0002	<0.0001	0.08	0.79
Source of variation									
Response curve ⁵									
Linear NaH ₂ PO ₄		0.75	0.05	0.16	0.94	0.94	0.45	0.009	0.06
Linear ash		0.72	0.97	0.04	0.005	0.004	0.0008	0.0002	0.06
Quadratic ash		0.98	0.46	0.53	0.62	0.04	0.003	0.05	0.74

^{a-c} Means within the same column, not sharing a common superscript, are significantly different ($P \leq 0.05$).

¹ Diet abbreviations: NC = negative control, LMP = low monosodium phosphate, MMP = medium monosodium phosphate, PC = positive control, LAP = low litter ash, MAP = medium litter ash, HAP = high litter ash

² Analyzed value

³ Dry bone wt. = ether extracted bone weight.

⁴ Monosodium phosphate (NaH₂PO₄); ash = litter ash

⁵ Includes negative control diets for linear and quadratic calculations

REFERENCES

REFERENCES

- Applegate, T. J. and R. Angel. 2008. Phosphorus requirements for poultry. Purdue Univ. and Univ. Maryland, College Park.
- Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate, 2007. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. *Poult. Sci.* 86:2598-2607.
- Akpe, M. P., P. E. Waibel, K. Larntz, A. L. Metz, S. L. Noll, and M. M. Walser. 1987. Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. *Poult. Sci.* 66:713-720.
- Bell, D. D., and W. D. Weaver (eds.). 2002. Commercial chicken meat and egg production (5th ed.). Page 384 - 385 in *Vitamins, Minerals, and Trace Ingredients*. Craig N. Coon. Kluwer Academic Pub. Norwell, Mass. MA, USA.
- Burns, R.T. and D. R. Raman. 2010. Animal Waste: Utilization. *In Encyclopedia of Agricultural, Food, and Biological Engineering*, Second Edition. Taylor and Francis: New York, 65-66.
- De Coca-Sinova, A., D. G. Valencia, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2008. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. *Poult. sci.* 87(12):2613-2623.
- Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2005. Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poult. sci.* 84(9):1406-1417.
- Eghball, B. and K. A. Barbarick. 2007. Manure, compost and biosolids. *Encyclopedia of Soil Science*, Second Edition. Taylor and Francis: New York, 1049-1052.
- Gillis, M. B., H. M. Edwards, and R. J. Young, 1962. Studies on the availability of calcium orthophosphates to chickens and turkeys. *J. Nutr.* 78(2):155-161.
- Gomori G. 1942. A modification of the colorimetric phosphorus determination for use with a photoelectric colorimeter. *J Lab. Clin. Med.* 27:955-957.
- Hurwitz, S., D. Dubrov, U. Eisner, G. Risenfeld and A. Bar. 1978. Phosphate absorption and excretion in the young turkey, as influenced by calcium intake. *J. Nutr.* 108:1329-1335.
- Hach, C. C., B. K. Bowden, A. B. Kopelove, and S. V. Brayton. 1987. More powerful peroxide kjeldahl digestion method. *J. Assoc. Off. Anal. Chem.* 70 (5):783-787.

- Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. *J. Anim. Sci.* 82:705-714.
- Kaplan, L. A. and A. J. Pesce. 1989. *Clinical Chemistry: Theory, Analysis and Correlation*. (2nd ed.), C.V. Mosby, St. Louis. 974-983.
- Mountzouris, K. C., P. Tsitsrikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poult. Sci.* 89:58-67.
- Mukhter, S. and S. Capareda. 2006. *Manure to energy: Understanding Processes, Principles and Jargon*. Texas Cooperative Extension, The Texas A&M University System.
- NRC, 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(1):94-98.
- Patrick, H., and J. A. Bacon, 1957. Relation of protein source to biological value of phosphates. *J. Biol. Chem.*, 228:569-572.
- Pagliari, P., C. Rosen, J. Strock, and M. Russelle, 2010. Phosphorus availability and early corn growth response in soil amended with turkey manure ash. *Communications in Soil Science and Plant Analysis*, 41(11):1369-1382.
- Powell, S., S. Johnston, L. Gaston, and L. L. Southern. 2008. The Effect of Dietary Phosphorus Level and Phytase Supplementation on Growth Performance, Bone-Breaking Strength, and Litter Phosphorus Concentration in Broilers. *Poult. Sci.* 87:949-957.
- Powell, S., T. D. Bidner, and L. L. Southern. 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. *Poult. Sci.* 90(3):604-608.
- Potter, L. M. 1988. Bioavailability of phosphorus from various phosphates based on body weight and toe ash measurements. *Poult. Sci.* 67:96-102.
- Potchanakorn, M. and L. M. Potter. 1987. Biological values of phosphorus from various sources for young turkeys. *Poult. Sci.* 66:505-513.
- Pond, W. G., D. C. Church, and K. R. Pond. 1995. Basic animal nutrition and feeding (4th ed.). Page 175-177 in *Inorganic Mineral Elements*. John Wiley and Sons, New York.

Rutherford, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on the ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low phosphorus diet for broilers. *Poult. Sci.* 83:61-68.

Roberson, K. D., M. W. Klunzinger, and R. A. Charbeneau. 2004. Benefit of feeding dietary calcium and nonphytate phosphorus levels above National Research Council recommendations to tom turkeys in the growing-finishing phases. *Poult. sci.* 83(4):689-695.

Rodehutsord, 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(4):965-971.

Summers, J. D., S. J. Slinger, W. F. Pepper, I. Motzok, and G. C. Ashton. 1959. Availability of phosphorus in soft phosphate and phosphoric acid and the effect of acidulation of soft phosphate. *Poult. Sci.* 38(5):1168-1179.

Scott, M. L., H. E. Butters, and G. O. Ranit. 1962. Studies on the requirements of young poult for available phosphorus. *J. Nutr.* 78(2):223-230.

Strock, J., C. Rosen, and P. Pagliari. 2006. Turkey Manure Ash Progress Report. Univ. Minnesota.

Waibel, P. E., N. A. Nahorniak, H. E. Dziuk, M. M. Walser, and W. G. Olson. 1984. Bioavailability of phosphorus in commercial phosphate supplements for turkeys. *Poult. Sci.* 63:730-737.

Wilcox, R. A., C. W. Carlson, W. Kohlmeyer, and G. F. Gastler. 1954. The availability of phosphorus from different sources for poult fed purified diets. *Poult. Sci.* 33(5):1010-1014.

Wilcox, R. A., C. W. Carlson, W. Kohlmeyer, and G. F. Gastler. 1955. The availability of phosphorus from different sources for poult fed practical-type diets. *Poult. Sci.* 34(5):1017-1023.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, C. A. Fritts, F. Yan, H. L. Stilborn, R. C. Crum Jr, and V. Raboy. 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poult. Sci.* 79(10):1451-1459.

Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometer. *J. Agric. Sci.* 59:381-385.

Yi, Z., E. T. Kornegay, V. Ravindran, M. D. Lindemann, and J. H. Wilson. 1996. Effectiveness of Natuphos phytase in improving the bioavailabilities of phosphorus and other nutrients in soybean meal-based semipurified diets for young pigs *J. Anim. Sci.* 74(7):1601-1611

Chapter Four

Overall Conclusion

The hypothesis of this study is gasification will result in minimal calcium and phosphorus availability due to non-specific binding to other minerals. The objective is to evaluate calcium and phosphorus bioavailability from turkey litter ash and the effect on the growth performance of turkeys. Turkey litter ash was used as a calcium and phosphorus source in the studies. Different levels of the litter ash were compared to various levels of calcium (limestone) and phosphorus (monosodium phosphate) standard sources. The first experiment evaluated the availability of calcium from litter ash and the second experiment looked at phosphorus availability. Each study evaluated body weight, feed intake, feed conversion ratio, bone characteristics, and ileal and total tract nutrient digestibility.

In the first experiment there were no significant differences between turkey litter ash and limestone for body weight gain, feed intake, feed conversion ratio, and tibia and femur measurements with different calcium levels during the study period. However, femur ash was significantly different between the two calcium sources. No difference was observed between calcium sources; however, significant difference was found between calcium levels in the ileal digestibility of calcium, specifically between low limestone 10.4 g/kg and low litter ash 9.9 g/kg and between high limestone 12.9 g/kg (PC) and high litter ash 12.1 g/kg. Also, ileal fat digestibility was found to be different between calcium sources. Similarly, total tract calcium digestibility was noted to be different between sources. Additionally, no differences were found between the source and the level of calcium on the ileal and total tract dry matter, nitrogen, phosphorus, fat, and energy digestibility. Therefore, the results of this experiment suggest that

using approximately 11 g/kg calcium from turkey litter ash in the diet will result in performance similar to the standard limestone diet.

Phosphorus source in the second experiment had an effect on the body weight gain and feed intake. However, feed conversion ratio was not different between monosodium phosphate and turkey litter ash. There were no differences observed between sources or levels of phosphorus on tibia and femur length. No difference was found between phosphorus sources on tibia width and weight; however, there was a difference between phosphorus levels, specifically between low monosodium phosphate diet 9.4 g/kg and low litter ash diet 7.4 g/kg on tibia width. Differences were also found between phosphorus sources on femur width and femur and tibia ash. Additionally, litter ash had decreased tibia and femur ash compared to monosodium phosphate sources. Litter ash and monosodium phosphate phosphorus sources had an effect on most of the ileum and total tract nutrient digestibility, specifically nitrogen, calcium, fat, and energy with ileum and dry matter, nitrogen, calcium, phosphorus, and energy with the total tract. Therefore, the data of this experiment found that using 8.4 g/kg phosphorus from the litter ash in the diet resulted in performance similar to the monosodium phosphate diet.

The price of litter ash may be cheaper than the standard calcium and phosphorus sources. However, the availability and inclusion level of the litter ash can limit the use within turkey diets. Additional, studies should be conducted to evaluate the impact of using litter ash for an entire grow-out period using the availability values from this project. Another aspect that needs to be evaluated is the inclusion of a phytase and the impact it might have on phosphorus within the litter ash.