

PHOSPHORUS BIOAVAILABILITY AND DIGESTIBILITY IN CANOLA MEALS
DETERMINED BY DIFFERENT METHODS

BY

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THESIS

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ABSTRACT

Six experiments were conducted to determine P bioavailability for a new test high-protein canola meal (TCM), a conventional canola meal (CCM) and dehulled soybean meal (SBM) using different types of animal assays. Experiment 1 was a chick-growth bioassay conducted to determine relative P bioavailability in the TCM, CCM, and SBM relative to KH_2PO_4 . A phosphorus-deficient cornstarch-dextrose-SBM basal diet was fed as Diet 1. Diets 2 and 3 had 0.05% or 0.10% P added from KH_2PO_4 , respectively. The remaining diets had 12.5% or 25% TCM, CCM, or SBM added in place of cornstarch and dextrose. Bioavailability of P was estimated using the multiple-regression slope-ratio method where tibia ash was regressed on supplemental P intake. A linear increase in tibia ash was observed as the P level was increased by addition of KH_2PO_4 , CCM, TCM, or SBM. Mean bioavailability values of P in the TCM, CCM, and SBM relative to KH_2PO_4 were 18, 15, and 34%, respectively. It was concluded that the bioavailable P content of the new TCM was statistically equal to the CCM. Experiment 2, an additional chick bioassay, was conducted to determine the effect of phytase enzyme (Optiphos, Huvepharma, Sofia, Bulgaria) on bioavailability of the P in the CMs. Diet 1 was a phosphorus-deficient TCM-cornstarch-dextrose diet, with the TCM as the only source of dietary P. Diets 2-4 had 0.05%, 0.10%, or 0.15% P added from KH_2PO_4 , respectively. Diets 5 and 6 were the same as Diet 1 with 125 or 250 units phytase added per kg of diets, respectively. Diets 7-14 were the same as Diets 1-7 except that CCM was the CM source. Multiple-regression was used to regress bone ash on supplemental P intake and the slope-ratio method was used to determine P release by phytase. It was estimated that the addition of 125 or 250 units/kg of phytase greatly increased the bioavailable P content of the TCM by 0.05 and 0.10%, respectively, and the response to phytase was slightly lower for the CCM than TCM. The results indicated that phytase greatly and similarly increased the bioavailability of P in the TCM and CCM. Experiment 3 was an ad-

libitum-fed chick experiment which evaluated the effect of phytase on P digestibility and (ileal) retention (excreta) values for CCM based on ileal and excreta contents, respectively. The chicks were fed a P - deficient cornstarch - dextrose CM basal diet (.13% available P) as Diet 1. Diets 2 and 3 were the basal diet plus 125 or 250 FTU/kg of phytase, respectively. On Day 22, the ileal digesta and excreta were collected and analyzed for P. Ileal P digestibility was 38.0%, 44.8%, and 46.6% for birds fed Diets 1-3, respectively. The P retention values were determined to be 38.7%, 47.3%, and 51.0% for Diets 1-3, respectively. Experiment 4 was a precision - fed chick assay conducted to determine the ileal P digestibility of CCM. The chicks were fed a nutritionally complete corn - SBM starter diet from Days 1 - 20. On Day 21, after fasting for 9 hours overnight, the chicks were tube fed 3, 6, or 9 g for CCM. Ileal digesta were collected four hours after feeding. Mean ileal P digestibility was determined to be 47.5% in chicks fed 6 g and 40% in chicks fed 9 g of CCM. Experiment 5 was conducted using a precision-fed rooster assay to determine P retention values for CCM. After withdrawal of feed for 24 hours, the roosters were tube-fed 8, 16, or 24 g of CCM. All excreta (feces + urine) were collected 48 hours after feeding. Phosphorus standardized retention values were 34.6%, 28.5%, and 23.1% for birds tube-fed 8, 16, and 24 g of CCM, respectively. Experiment 6 was an ad libitum-fed chick assay to determine the ileal P digestibility and retention of CCM with and without increasing levels of dietary supplemental Ca and Ca: P ratio. The chicks were fed a P deficient - dextrose - CCM basal diet (0.039% available P, 13.50% CCM) as Diet 1. Diets 2-4 contained increasing levels of 27%, 40.50%, or 54% added CCM, respectively. The Ca: available P ratio was maintained at a 2:1 ratio in Diets 1-4. Diets 5-8 were the same as Diets 1-4 but supplemental calcium was added so that the Ca: available P ratio was maintained at a 6:1 ratio. Diets were fed from Days 15-21 posthatch and excreta were collected on Days 21 and 22 and ileal digesta on Day 22. Phosphorus

digestibility decreased with each increase in dietary CCM and also with increased Ca: P ratio.

Phosphorus retention values also generally decreased with increased Ca: P ratio. The results of Experiments 3-6 indicated the P digestibility and retention values for CCM varied among balance methods and sometimes among levels of CCM fed.

DEDICATION

This thesis is dedicated to my father and mother, Larry and Debbie Hanna. Everything I am, or hope to be, I owe to my parents. It is also dedicated to my darling husband and my hero, Benjamin.

“Maybe my limit isn’t where I thought it was, maybe I could do considerably more.”

- Commander Keith Davids, USN

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CHAPTER 1

INTRODUCTION

Since the 1970s, canola has become one of the world's most important oilseed crops and was developed by removing a large percentage of antinutritional factors from rapeseed. To be deemed, "canola," the oil of the seed must contain less than 2% erucic acid within its fatty acid profile and the solid portion of the seed can have no greater than 30 μmol of any combination of glucosinolates (Canola Council, 2014). Canola is a plant that originated in Canada and is the product of plant breeders' efforts to make rapeseed more economically competitive and nutritionally useful (Bell, 1993). A great benefit of canola is that it flourishes in cooler, northern areas where soybeans and other crops cannot thrive. However, there is great variability in these climates where canola is grown that may affect its concentrations of fat, protein, amino acids, and carbohydrates. Canola is a member of the Brassica species and produces a yellow – flowering plant (Barthet and Duan, 2011; Newkirk, 2011).

Canola ranks second in global production among oilseed crops, second to soybeans (USDA, 2012). It is often an economically viable substitute for some soybean meal (SBM) in poultry diets (Mushtaq et al., 2007). However, until recent years canola meal, which is the ingredient produced after the oil is removed from canola seeds, has had limited inclusion in poultry diets primarily because of anti – nutritional factors. One of those factors is phytate, which binds to phosphorus and renders it largely unavailable to the animal as well as increases the phosphorus concentration in excreta. Although canola meal has a higher percentage of phosphorus than SBM (NRC, 2012), it also contains a greater amount of phytate, a reason that it has not surpassed or equaled the use of SBM in livestock feeds. In addition to phytate and glucosinolates and erucic acid, tannins and sinapine are also antinutritional factors in canola meal, which can reduce feed intake and nutrient digestibility (Bell, 1993).

It has been stated that if canola meal had more digestible energy, greater protein content, less fiber, and fewer glucosinolates, its inclusion in non-ruminant diets and monetary value could be greatly increased (Bell, 1993). In an effort to make these traits a possibility in canola meal, breeding programs have been established to reduce glucosinolates, increase protein, and decrease the fiber content of canola to make canola meal more competitive with SBM (Khajali and Slominski, 2012). Along with breeding programs aimed at increasing canola's nutritional value, phytase supplementation has been successful in improving phosphorus digestibility in poultry diets and decreasing phosphorus levels in excreta (Selle and Ravindran, 2007).

Plant breeding genetic selection programs have produced a new yellow – seeded canola that has a larger seed and thinner hull than conventional black – seeded varieties (Thacker, 1990; Slominski et al., 1994; Khajali and Slominski, 2012). Because of the thinner hull, a reduction in fiber has occurred which is expected to contribute to the meal having a greater nutritional value. The larger embryo in proportion to the total seed also resulted in an increased level of protein in the canola meal from these new breeds of canola (Khajali and Slominski, 2012). More recent research has shown that other high – protein reduced – fiber canola meals have increased true metabolizable energy (TME_n) and digestible amino acids than conventional canola meal (Chen et al., 2015). There has not yet been enough research done to evaluate the availability of the phosphorus in the new high - protein, reduced fiber canola meal and the effect of phytase enzyme on the availability of the phosphorus. Thus, that is the primary objective of this thesis.

LITERATURE CITED

- Barthet, V. J., and J. K. Duan. 2011. Seed morphology, composition, and quality. Pages 125-145 in *Canola: Chemistry, Production, Processing, and Utilization*. J. K. Duan, N. A. M.
- Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. *Can. J. Anim. Sci.* 73:679-697.
- Canola Council of Canada. 2014. What is Canola? <http://www.canolacouncil.org/oil-and-meal/what-is-canola/>.
- Chen X., C. Parr, P. Utterback, and C. M. Parsons. 2015. Nutritional evaluation of canola meals produced from new varieties of canola seeds for poultry. *Poult Sci.* 94:984-991.
- Khajali, F., and B. A. Slominski. 2012. Review: Factors that affect the nutritive value of canola meal for poultry. *Poult. Sci.* 91:2564-2575.
- Mushtaq, T., M. Sarwar, G. Ahmad, M. A. Mirza, H. Nawaz, M. M. Haroon Mushtaq, and U. Noreen. 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. *Poult. Sci.* 86:2144-2151.
- Newkirk, R. 2011. Meal nutrient composition. Pages 229-244 in *Canola: Chemistry, Production, Processing, and Utilization*. J. K. Duan, N. A. M. Eskin, D. Hickling, eds. AOCS Press, Urbana, IL.
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition: Review. *Anim. Feed Sci. Technol.* 135:1-41.
- Slominski, B. A., L. D. Campbell, and W. Guenter. 1994. Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. *J. Agric. Food Chem.* 42:704-707.
- Thacker, P. A. 1990. Canola meal. In: P. A. Thacker and R. N. Kirkwood, editors, *Nontraditional feed sources for use in swine production*. Butterworth Publishers, Stoneham, MA. p. 69-78.

United States Department of Agriculture. 2012. Soybeans and oil crops: Canola. <http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx>. (Accessed 12 March 2015.)

CHAPTER 2

THE NUTRITIONAL VALUE OF CONVENTIONAL AND HIGH-PROTEIN CANOLA MEAL FED TO POULTRY: A LITERATURE REVIEW

INTRODUCTION

Canola meal is a protein source commonly used in livestock diets. Through significant breeding programs, the nutritional quality of rapeseed has been improved, in part by reducing anti – nutritional factors, making it a more competitive and valuable feed ingredient for livestock. Rapeseed (*Brassica Rapa*) has been known to contain 25 to 45 % erucic acid and 50 to 100 $\mu\text{mol/g}$ glucosinolates (Bell, 1993). Erucic acid can lead to development of fatty deposits on the heart and skeletal muscles and can retard growth (Przybylski and Eskin, 2011). Glucosinolates are the most concerning of the anti-nutritional factors, although by themselves, they are inactive molecules and are not toxic. Myrosinase is an enzyme that is always present, along with glucosinolates, in rapeseed. When moisture is added and rupture of the seed occurs, myrosinase hydrolyzes the glucosinolates, producing unstable aglucones (Fenwick, 1982). These compounds then break down further to yield isothiocyanates, nitriles, thiocyanates, or oxazolidithione (Tripathi and Mishra, 2007) which cause malfunctioning of the thyroid gland, reduced growth performance, liver hemorrhages, kidney enlargement, taint in brown eggs, and perosis in chicks (Fenwick and Curtis, 1980; Bell, 1993).

Although breeding programs have achieved erucic acid levels below 2% and glucosinolates to levels below 30 $\mu\text{mol/g}$, resulting in “double-low” rapeseed, coined “canola” in North America, other anti-nutritional factors persist (Bell, 1993). Tannins, sinapine, and phytic acid also exist in canola, all of which can have a negative effect on animal growth and reproduction (Bell, 1993; Khajali and Slominski, 2012). Tannins are water-soluble polyphenolic plant metabolites, present in the seed hull, that have the ability to precipitate gelatin and other

proteins from aqueous solutions (Haslam, 1981; Buter, 1989; Khajali and Slominski, 2012). Their negative nutritional effects are thought to be caused by their ability to form complexes with protein, thus lowering protein digestibility and energy digestibility (Buter, 1989). Tannins comprise 1.5 to 3% of canola meal (Bell, 1993), 70-96% are insoluble, and they cause the dark color in the meal (Khajali and Slominski, 2012). In a study done by Mansoori and Acamovic (2007), increased endogenous losses of Met, His, and Lys were observed when tannic acid was added to the diet. The presence of tannins in canola meal is one factor that may economically prevent high inclusion of the meal in poultry diets because Met and Lys are the first limiting amino acids for poultry.

Canola meal may contain as much as 0.6 to 1.8% sinapine (Thacker, 1990) and is the cause of the bitter taste of the feed ingredient (Kozłowska et al., 1990). A compound called trimethylamine can be produced from sinapine or from choline in the small intestine (Khajali and Slominski, 2012). This causes the fishy taint in brown layer eggs because the hens lack the enzyme trimethylamine oxidase, which is necessary to remove trimethylamine. Consequently, trimethylamine is deposited into eggs, leaving an unpleasant odor (Ward et al., 2009).

Phytic acid is the principal storage form for phosphorus (P) and its adverse effects are manifested through reduced P absorption and utilization (Erdman, 1979). In addition to reducing the absorption of P, phytate also has the ability to bind to Ca, K, Zn, Fe, Mn, and Mg and therefore also reduce their bioavailability (Nwokolo and Bragg, 1977). At low intestinal pH levels, phytate may also be able to bind to the amino acids Arg, Lys, and His, which results in insoluble phytate – protein complexes and reduced availability (Anderson, 1985). The amount of phytate P contained in Brassica meals ranges from 36 (Broz and Ward, 2007) to greater than 70% of total P (Summers et al., 1983). The proportion of phytate bound P to non-phytate P in

canola meal is 66.4% as reported by Selle and Ravindran (2007). Adding phytase, a naturally occurring enzyme, to poultry diets will hydrolyze phosphates from the phytate molecule, resulting in more available P and Ca for the animal (Simons et al., 1990; Selle et al., 2009). Canola meal has increased levels of fiber compared to SBM because the hulls remain with the seeds during processing. Like phytate, fiber also binds minerals such as P, Ca, Mg, Mn, Zn and Cu, rendering them less available. Lastly, fiber is poorly digested by poultry and decreases the energy value of canola meal (Nwokolo and Bragg, 1977; Bell, 1993).

Canola contains between 40 and 50% oil and yields canola meal that contains approximately 38% protein by weight on average (Fenwick and Curtis, 1980). Ninety - seven to 99% of that oil is removed by solvent prepress extraction to create the meal (Barthet and Duan, 2011). The resulting oil has the lowest amount of saturated fatty acids of any vegetable oil, with 50% less than corn and soybean oil, and is marketed for human consumption (Aukema and Campbell, 2011). The neutral detergent fiber (NDF) content of canola meal is 22 to 26% compared with SBM at 8 to 12% respectively (Slominski et al., 1994; Khajali and Slominski, 2012; NRC, 2012).

In an attempt to improve the nutritive value of canola meals, several approaches have been implemented. Feeding value of canola meal has been increased by using heat and mechanical treatments such as flaking, steam pelleting, and extrusion (Shen et al., 1983; Salmon et al., 1988). In an experiment carried out by Woyengo et al. (2010), broiler chicks were fed expeller-extracted canola meal with the outcome indicating that the standardized ileal digestible amino acid and AME_n values of expeller – extracted meal were higher than solvent – extracted meal. Barret et al. (1998) compared weight gain of chicks fed alkaline heated canola meal, untreated canola meal and SBM. The chicks fed SBM and heat treated canola meal experienced

weight gains that did not differ significantly from each other. However, chicks fed untreated canola meal gained less weight than those fed the other ingredients, suggesting that alkaline heating of canola meal can reduce its negative effects observed in chicks. The majority of attempts at improving canola meal quality have concentrated on processing techniques, however breeding programs are making efforts to produce a nutritionally superior canola seed.

Yellow-seeded varieties of canola have been the product of genetic modification attempts to create canola that is of a higher nutritional quality than conventional black seeded canola. The yellow seed color is due to a thinner seed coat, which makes the embryo inside the coat visible, unlike black seeds (Rahman, 2001). Due to their thinner hull and a larger seed, yellow – seeded canola has lower fiber content and an increased crude protein content when compared to dark varieties (Downey and Bell, 1990; Thacker, 1990; Khajali and Slominski, 2012). Yellow-seeded canola is reported to contain a total dietary fiber (TDF) concentration of about 24% while conventional black-seeded varieties commonly contains about 30% (Khajali and Slominski, 2012). Because of their larger seed, yellow canola varieties possess a larger embryo which results in a higher percentage of protein than conventional canola seeds. Meal from yellow-seeded cultivars on average contains 44.5% protein versus 42.7 % for meal from dark seeded cultivars (Simbaya et al., 1995) and has higher AA concentration than dark seeds (Slominski et al., 2012). It is also of importance to note that canola meal produced from yellow-seeded canola seeds has 10 $\mu\text{mol/g}$ less glucosinolates than canola meal from black-seeded breeds (Duan and DeClerq, 1988; Slominski et al., 2012). The new yellow-seeded types of canola are of great interest both economically and nutritionally but limited research has been conducted concerning their inclusion in poultry diets. Unfortunately, the new yellow – seeded breeds of canola have not performed as well agronomically as conventional breeds. Rashid and Rakow (1995) tested

the yield performance of sixteen different yellow-seeded lines of canola. None of the new yellow-seeded varieties reached the seed yield of the control canola. Additionally, the yellow seed color of the new varieties was negatively influenced by the environment, and only in hot and dry conditions was a favorable yellow seed produced. Under those conditions, the lowest yields were obtained and under cool and wet conditions the best plant yield but worst seed color was observed. This is another issue which is concerning to those developing the new yellow seeds.

Recently, new test high protein canola seeds have also been developed. Fourteen increased protein, reduced fiber canola meal samples, produced from the new canola seeds were evaluated by Chen et al. (2015) at the University of Illinois at Urbana - Champaign and were determined to have an average protein content of 47.9%. The six samples of conventional canola meal in their study had an average protein concentration of 40.5%. The neutral detergent fiber (NDF) of the test meals was 19.07% on average and the acid detergent fiber (ADF) was 13.89%. For the conventional canola meals, an average of 28.05% NDF was reported along with an average of 20.46% ADF. Five different experiments were conducted using precision-fed rooster assays to determine the TME_n and AA digestibility for the CM, as described by Kim et al. (2010). In summary, there was increased CP and amino acid concentrations as well as reduced fiber content as measured by NDF and ADF for all test canola meals compared to conventional canola meal. Most of the test canola meals also had significantly higher TME_n values than did the conventional canola meal samples. In Experiments 1, 2, and 4, the test canola meals had higher amino acid (AA) digestibility values than the conventional meal. The results indicated that the genetically modified canola meals are of greater nutritional value than conventional meals because of their increased digestible AA levels and increased TME_n (Chen et al., 2015).

EFFECTS OF CONVENTIONAL AND HIGH PROTEIN CANOLA MEAL FED TO BROILER CHICKENS

It has been reported that canola meal can be included in broiler starter diets up to 10% of the total diet and included in the broiler grower/finisher diets up to 20% of the total diet without negatively affecting growth performance (Canola Council of Canada, 2014). Leeson et al. (1987) tested this theory by replacing 0%, 25%, 50%, 75%, and 100% of SBM in poultry diets with conventional canola meal with the highest inclusion of canola meal accounting for 38% of the diets for broilers. From weeks 0-3 of age, only slight numerical decreases in body weight gain were seen in birds fed 0%, 9.47%, 18.95%, 28.42% and 37.89% canola meal when compared with birds fed 0% canola meal. Increasing the dietary levels of canola meal led to no significant effect on feed intake or weight gain. Likewise, replacing the SBM in the diet with up to 100% canola meal resulted in no significant effect on metabolizable energy of the diets, bone ash, bone calcium, phosphorus or magnesium content (Leeson et al., 1987).

Mushtaq et al., (2007) conducted a similar growth trial; however different results were reported. The experiment was divided into two phases, starter (day 2 – 21) and grower (day 22-42) using broiler chickens and either 20% or 30% canola meal was included in the diets. Although the meal was obtained from an unidentified species, the meal was analyzed and contained 38.8% CP, 7.17% CF, 0.58% fat, 6.74% ash, and no report of glucosinolates was reported. During the starter phase, when canola meal was included in the diet at 30%, a significant decrease (9.40%) in body weight was observed compared to chicks fed 20% canola meal. At an inclusion level of 30% canola meal, the mortality rate was 4.27% versus 2.29% for 20% inclusion. It was concluded that diets containing 30% canola meal were suitable for broilers only during the grower phase of production (Mushtaq et al., 2007).

The results of experiments evaluating feeding high or increasing levels of canola meal are extremely variable and Gorski, (2015) reported results comparable with Mushtaq et al. (2007). Experiment 1 used 200 Ross 308 males from days 2 – 21 of age and chicks were fed 0, 10, 20, 30, or 40% conventional canola meal in the starter diets. During the grower phase, from days 21 – 37, the birds were then fed diets containing 0, 10, 20, or 30% conventional canola meal. At inclusion levels greater than 10% canola meal in the diet, the chicks experienced significantly reduced weight gain and feed intake during the starter phase. In the grower phase, there was no significant difference in weight gain, feed intake, or gain:feed among dietary treatments. Experiment 2 used 280 Ross 308 males from days 2 – 42 of age and randomly assigned one of seven dietary treatments. In the starter phase (2 – 19 d), either 0 or 8% high – protein, low – fiber test or conventional canola meal was included in the diets. In the grower phase (20 – 44 d), either 0, 8, 16, or 24% test or conventional canola meal was included in the diets. At the 8% inclusion level, no adverse effects on growth performance were observed for either the test or conventional canola meal during the starter phase. Additionally, during the grower phase no significant differences were observed from feeding 8, 16, or 24% test or conventional canola meal.

Slominski et al. (1999) conducted a two week growth experiment with the objective of comparing brown and yellow – seeded varieties of *Brassica napus*. From 4 – 18 days of age, the broiler chicks were fed two treatment diets. One treatment consisted of yellow – seeded canola meal and the other of brown – seeded meal. The yellow – seeded meal accounted for 29.7% of the experimental diet and the brown accounted for 29.5%. The CP and glucosinolate content of the meals did not differ. The yellow – seeded canola meal had substantially less fiber and fat than the brown – seeded canola meal. The weight gain was consistent between the two groups of

broilers but the feed to gain ratio of the birds fed diets containing yellow - seeded meal was improved over the birds fed brown – seeded meal. Although feed intake was not disclosed, it can be calculated that feed intake was less for birds fed the yellow – seeded canola meal. The conclusion was drawn that the yellow – seeded canola meal was superior to the brown – seeded variety of *Brassica napus* (Slominski et al., 1999).

McNaughton et al., (2014) studied broiler growth performance of birds fed a genetically modified, a near – isogenetic but not genetically modified, and a commercial canola meal that was not genetically modified. The experiment was conducted from days 1 – 42 of age and the different canola meals were included at 10% and 20% of the starter and grower diet, respectively. The genetically modified canola seeds produced meals that had an average of 48.6% CP, the near isogenetic but non – genetically modified canola meal contained an average of 44.3% CP, and the commercial canola meal contained 42.9% CP on average. The crude fiber content of the genetically modified seed was 10.1% as compared to 12.0%, and 13.5% respectively, in the meal from non-genetically modified but near – isogenetic seeds and commercial seeds. The diets fed in the starter and grower phases were both isocaloric and formulated to be equal in digestible amino acids. The results for body weight gain, feed intake, and mortality among the treatments did not differ during either segment of the experiment. It was concluded that the meal produced from genetically modified canola seeds and the meal from the non-genetically modified, near isogenetic and the non-genetically modified commercial seeds were all of equal nutritional value.

PHOSPHORUS IN CANOLA MEAL

Phosphorus is the second most abundant mineral in the body and is extremely important for bone mineralization, phospholipid support of membranes, and energy storage as ATP (Lisegang et al., 2002; Viveros et al., 2002). Conventional black-seeded solvent extracted canola meal consists of 1.08% total phosphorus according to the Swine NRC (2012) and 1.17% according to the Poultry NRC (1994). Simons et al. (1990) reported 1.22% total phosphorus in canola meal. Slominski et al. (2012) reported a higher phosphorus content than others at 1.30% in black – seeded conventional canola meal and 1.24% for high protein yellow – seeded canola meal. The phytate – phosphorus content of the two meals was similar, suggesting that the phosphorus digestibility of the yellow – seeded meal may be slightly less than that of the black – seeded meal (Slominski et al., 2012). Very few values have been published regarding the phosphorus content of yellow – seeded varieties of canola meal. About two-thirds of phosphorus in canola meal is stored as phytic acid (Simons et al., 1990). This makes the mineral largely unavailable to poultry because they lack sufficient endogenous amounts of the necessary enzyme, phytase, to hydrolyze the phytate-phosphorus complex (Selle and Ravindran, 2007; Akinmusire and Adeola, 2009).

Phosphorus Digestibility and Relative Bioavailability in Canola Meal for Broilers

The total phosphorus and nonphytate phosphorus levels reported in the Poultry NRC (1994) for canola meal are 1.17 and 0.30, respectively. Thus, the ratio of 0.30 to 1.17 is 25.6%, suggesting that this is the approximate phosphorus digestibility of canola meal for poultry.

Mutucamarana et al. (2014) conducted a trial to determine ileal phosphorus digestibility and excreta retention of phosphorus in canola meal when fed to broiler chickens using diets formulated to contain increasing concentrations of phosphorus. Canola meal was the only source of phosphorus in the four test diets. The canola meal used in this study contained 0.970% phosphorus and diets 1-4 contained 1.36, 2.60, 3.90, and 5.29% respectively. True ileal phosphorus digestibility and excreta retention coefficients were calculated using a linear regression method. The coefficients for phosphorus retention decreased linearly from 0.70 - 0.54 in the birds with increasing dietary phosphorus and canola meal concentration. However, apparent ileal phosphorus digestibility was not influenced by canola meal and phosphorus inclusion level. The authors found the total phosphorus to be 46.9% digestible and 48.6% retainable in canola meal. In contrast, the nonphytate phosphorus: total phosphorus ratio was 29.0%. It was calculated that 25.2% of the phytate – phosphorus in the canola meal was digested and absorbed. It has been reported that the utilization of phytate – phosphorus can range from 0 – 75% among feed ingredients and a wide array of factors influence this phenomenon (Angel et al., 2002). In the study by Mutucamarana et al. (2014), calcium levels were maintained at a low level to maintain a 2:1 calcium to phosphorus ratio and the low dietary calcium level may have contributed to the high phosphorus utilization estimations. That is, feeding lower than recommended calcium levels in broiler diets (9g/kg) (Ross 308 Broiler Nutrition Specification, 2007) may have resulted in the chicks hydrolyzing more phytate – phosphorus than if they had been fed recommended or higher calcium levels. Mohammed et al. (1991) found that chicks fed diets containing 5 g/kg of calcium had a 15% increased phytate – phosphorus utilization over chicks fed a diet with 10 g/kg of calcium. Nevertheless, the authors of the study concluded that the regression method can be used to successfully measure true phosphorus digestibility in feed

ingredients and that ileal digestibility and retention coefficients are suitable to assess phosphorus availability in broilers (Mutucamarana et al., 2014). More work needs to be done to assess accuracy of the mineral's digestibility in canola meal, particularly since the 47-49% digestible/retainable phosphorus values are higher than expected on the nonphytate: phytate phosphorus levels in this ingredient.

Parr (2014) conducted an experiment to determine the relative phosphorus bioavailability in the meals from two new test high – protein canola seeds (CMA or CMB), a conventional canola seed (CCM) and a control SBM using a chick growth tibia ash assay. A phosphorus-deficient cornstarch-dextrose SBM basal diet was fed as Diet 1. Diets 2 and 3 had 0.05 and 0.10% phosphorus added from KH_2PO_4 , respectively. Diets 4-11 contained 12.5 or 25% added test CMA, CMB, CCM or SBM and were fed to 320 New Hampshire x Columbian male chicks from days 8 - 21 of age. Bioavailability of P in the feed ingredients, relative to KH_2PO_4 , was estimated using the slope – ratio method (Finney, 1978). The CMA contained 1.26%, CMB contained 1.16%, and the CCM 1.16% total phosphorus. Using the multiple regressions of tibia ash (mg/tibia and %) on supplemental phosphorus intake, relative phosphorus bioavailability values for the samples were found to be 15.1%, 20.0%, and 13.0%, for CMA, CMB, and CCM, respectively. The control SBM was found to have less total phosphorus at 0.57% but greater relative bioavailability at 41.6% (Parr, 2014). In a different study than the one previously discussed, conducted by Mutucumarana et al. (2014), SBM was found to contain 0.65% total phosphorus, 33.3% of which was nonphytate phosphorus, and 79.8% true digestible phosphorus, supporting the Parr (2014) SBM findings that although canola meal contains a higher amount of phosphorus than SBM, the phosphorus is less digestible.

Effect of Phytase Enzyme on Phosphorus Digestibility or Availability

Although there are few studies that have determined phosphorus (P) digestibility in canola meal specifically, there are many studies concerning dietary addition of phytase which increases bioavailable phosphorus of oilseeds and reduces phosphorus excretion by poultry (Selle and Ravindran, 2007). Phytase hydrolyzes phytic acid and in addition to phosphorus, it may release Ca, Mg, Cu, Zn, Fe, and K which are often phytate – bound minerals (Sebastian et al., 1996a). One unit of phytase (FTU) is defined as the amount of the enzyme required to released 1 μmol of phosphorus from sodium phytate at 37° C (Augspurger et al., 2007). The efficacy of microbial phytase is influenced by the dietary Ca:P ratio (Liu et al., 1998), Ca bioavailability, inorganic phosphorus supplements, age of the bird (Kornegay, 1996), and the level of phytase supplemented in the diet (Shirley and Edwards, 2003). When poultry manure is spread over soil, phosphorus is leached into ground water, streams, lakes, rivers, and oceans leading to eutrophication and eventual mortality of marine life (Ryden et al., 1973). Phytase reduces the excretion of phosphorus in manure and reduces the need for supplemental phosphorus levels in the diet. Phosphorus is the third most expensive nutrient in the diet of nonruminant animals (Augspurger et al., 2003).

Simons et al. (1990) demonstrated the ability of phytase to reduce phosphorus excretion and increase bioavailability for broiler chickens. It was found that 1500 FTU/kg, when combined with decreased phosphorus (7.5 versus 4.5 g/kg) and calcium (9.0 versus 6 g/kg) levels in the diet decreased the amount of phosphorus in excreta by 45%. Phosphorus bioavailability in the corn-SBM diet was increased to over 60% by using phytase.

Paik (2003) conducted two broiler trials to determine the effect of phytase supplementation on P excretion. When 600 FTU/kg were supplemented to corn – SBM diets,

decreasing the nonphytate – phosphorus by 0.2%, did not affect growth performance. In addition, dietary phosphorus availability was increased by up to 14.8% and availability of some minerals by up to 30% and phosphorus excretion was decreased by up to 60%. Sebastian et al. (1996) conducted a study where phytase was added to a low – phosphorus corn – SBM diet and an increased retention of P, Ca, Cu, and Zn (12.4, 12.2, 19.3, and 62.3% respectively) was observed. A 13.2% increase in body weight gain was also reported.

Another study on the use of phytase in poultry diets was been conducted by Zhang et al. (2000b). Zhang et al. (2000b) fed corn-soybean meal-based diets containing 0.46% total P, 0.21% nonphytate P, and 0.92% Ca to 7 day old broilers until 28 days of age. Diets were supplemented with 250, 500, and 2,500 FTU/kg. Over the total 28-day trial, compared with the basal diet (0.19% nonphytate P and 0.46% total P), supplemental phytase of 250, 500, or 2,500 U/kg, averaged across sources, increased BW gain 5.7, 8.1, or 17.1%; gain:feed 3.9, 6.8, or 11.8%; and feed intake 1.2, 3.9, or 5.6%, respectively. Increased BW gain was a result of both an increase in feed intake and an improvement in gain:feed ratio. Results also showed that the response of birds to phytase appeared after 2 weeks after the experiment began and lasted until the end of the trial (Table 3). Simons et al. (1990), Saylor et al. (1991), and Kornegay et al. (1997) have reported improvements in feed efficiency when phytase was supplemented to low P broiler diets. In contrast, other researchers reported that gain:feed ratios of broilers were unaffected by phytase supplementation (Schoner et al., 1991; Denbow et al., 1995).

Parr (2014) evaluated the effects of phytase on relative phosphorus bioavailability for canola meal using a chick – growth tibia ash assay. In that study, 0.05 and 0.10% KH_2PO_4 , or 250, or 500 FTU/kg were added to a basal diet that contained test CMA as the only source of phosphorus. A simple linear regression of tibia ash on the 0.05 and 0.10% supplemental

phosphorus from KH_2PO_4 were calculated to create a standard curve. The weight gain and tibia ash for the 250 and 500 FTU/kg treatments were greater than expected and exceeded the range of the KH_2PO_4 standard curve. By extrapolation to extend the range of the KH_2PO_4 standard curve, it was estimated that 0.13% and 0.19% phosphorus were released from 250 and 500 FTU/kg, respectively. Parr (2014) suggested that the amount of phosphorus released from 250 FTU/kg was likely accurate based on previous research conducted in the same laboratory by Augspurger et al (2003), but the only certain conclusion that could be made was that both 250 and 500 FTU/kg released more than 0.1% phosphorus.

CONCLUSIONS

It has been indicated that canola meals produced from new varieties of canola seeds contain greater concentrations of CP, decreased fiber and glucosinolates compared to conventional canola meal. This can improve the feeding value for poultry and increase canola meal inclusion in poultry diets. Little research has been done to determine the phosphorus bioavailability in conventional or new canola meal varieties. A large proportion of phosphorus is bound to phytate, limiting its digestibility and absorption. Even less research has been done to determine the phosphorus availability in the new, high-protein canola meal for poultry. Yellow-seeded canola meal may have decreased phosphorus digestibility because it has more phosphorus bound to phytate than black, conventional meals. However, by including microbial phytase in diets containing canola meal, increased hydrolysis of phytate occurs, improving phosphorus digestibility. More research is needed to evaluate the phosphorus bioavailability of conventional and high protein canola meals for broilers. Research is also needed to further determine the effect of microbial phytase inclusion on phosphorus availability in conventional and high protein canola meals for broiler chickens. The objective of this thesis was to evaluate the phosphorus bioavailability in conventional and new, high-protein, reduced fiber canola meal as well as the effect on phosphorus availability when microbial phytase is added to canola meal diets for poultry.

LITERATURE CITED

- Akinmusire, A. S., and O. Adeola. 2009. True digestibility of phosphorus in canola and soybean meals for growing pigs: Influence of microbial phytase. *J. Anim. Sci.* 87:977-983.
- Anderson, P. A. 1985. Interactions between proteins and constituents that affect protein quality. Pages 31-46 *in: Digestibility and Amino Acid Availability in Cereals and Oilseeds*. G. W. Finley and D. T. Hopkins, ed. American Association of Cereal Chemists, St. Paul, MN.
- Angel, R., A. S. Dhandu, and T.J. Applegate. 2002. Phosphorus requirements of broilers and the impact of exogenous phytases. *Proc. Arkansas Poultry Nutr. Conf.*
- Aukema, H., and L. Campbell. 2011. Oil nutrition and utilization. In: J. K. Duan, N. A. M. Eskin, and D. Hickling, editors, *Canola: Chemistry, production, processing, and utilization*. AOCS Press, Urbana, IL. p. 245-280.
- Augspurger, N. R., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *J. Anim. Sci.* 81:474-483.
- Augspurger, N. R., D. M. Websel, and D. H. Baker. 2007. An *Escherichia coli* phytase expressed in yeast effectively replaces inorganic phosphorus for finishing pigs and laying hens. *J. Anim. Sci.* 85:1192-1198.
- Barrett, J. E., C. F. Klopfenstein, and H.W. Leipold. 1998. Alkaline heating of canola and rapeseed meals reduces toxicity for chicks. *Plant Foods for Human Nutrition*. 52: 9-15.

- Barthet, V. J., and J. K. Duan. 2011. Seed Morphology, composition, and quality. In: J. K. Duan, N. A. M. Eskin, and D. Hickling, editors, *Canola: Chemistry, production, processing, and utilization*. AOCS Press, Urbana, IL. p. 119-162.
- Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. *Can. J. Anim. Sci.* 73:679-697.
- Broz, J., N. E. Ward. 2007. The role of vitamins and feed enzymes in combating metabolic challenges and disorders. *J. Appl. Poult. Res.* 16:150-159.
- Burel, C., T. A. Boujard, A. M. Escaffre, S. J. Kaushik, G. Boeuf, K. A. Mol, S. Van der Geysen, and E. R. Kuhn. 2000. Dietary low glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). *Br. J. Nutr.* 83:653-664.
- Butler, L.G. 1989, Sorghum polyphenols, in *Toxicants of Plant Origin*, vol. 4, Cheeke, P.R., Ed., CRC Press, Boca Raton, FL, 95-121.
- Canola Council of Canada. 2014. Poultry diets using canola meal. www.canolacouncil.org/oil-and-meal/canola-meal/poultry-diets-using-canola-meal/
- Chen X., C. Parr, P. Utterback, and C. M. Parsons. 2015. Nutritional evaluation of canola meals produced from new varieties of canola seeds for poultry. *Poult Sci.* 94:984-991.
- Denbow, D.M., V. Ravindran, E.T. Kornegay, Z. Yi and R.M. Hulet, 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. *Poult. Sci.*, 74: 1831-1842.
- Duan, J. K., and D. R. DeClercq. 1988. Quality of yellow and dark seed in *Brassica campestris* canola varieties Candle and Tobin. *J. Am. Oil Chem. Soc.* 65:122-126.

- Downey R. K., and J. M. Bell. 1990. New developments in canola research. In: F. Shahidi, editor, *Canola and rapeseed: Production, chemistry, nutrition and processing technology*. Springer US, New York. p. 37-46.
- Erdman, J. W., Jr., 1979. Oilseed phytates: nutritional implications. *J. Am. Oil Chem. Soc.* 56:736-741.
- Fenwick, G. R., and R. F. Curtis. 1980. Rapeseed meal and its use in poultry diets. A review. *Anim. Feed Sci. Technol.* 5:255-298.
- Fenwick, G. R., 1982. The assessment of a new protein source—Rapeseed. *Proceedings of the Nutrition Society*, 41, pp 277-288. doi:10.1079/PNS19820043.
- Finney, D. J. 1978. *Statistical Method in Biological Assay*. 3rd. ed. Charles Griffin and Company, Ltd., High Wycombe, Buckinghamshire, UK.
- Gorski, M. F., 2015. Nutritional evaluation of canola meal produced from a new variety of canola seeds in broiler chickens and laying hens. (Master's defense). University of Illinois at Urbana-Champaign.
- Haslam, E. 1981. Vegetable tannins, in *Secondary Plant Products* (E. E. Conn, ed.), Vol. 7 of *The Biochemistry of Plants* (P. K. Stumpf and E. E. Conn, eds.), 527-556, Academic Press, NY.
- Khajali, F., and B. A. Slominski. 2012. Review: Factors that affect the nutritive value of canola meal for poultry. *Poult. Sci.* 91:2564-2575.
- Kies, A. K., L. H. De Jonge, P. A. Kemme, and A. W. Jongbloed. 2006. Interaction between protein, phytate, and microbial phytase. In vitro studies. *J. Agric. Food Chem.* 54:1753-1758.

- Kim, E. J., C. M. Parsons, R. Srinivasan, and V. Singh. 2010. Nutritional composition, nitrogen-corrected true metabolizable energy, and amino acid digestibilities of new corn distillers dried grains with solubles produced by new fractionation processes. *J. Poult. Sci.* 89:44-51.
- Kornegay, E. T., E. M. Denbow, and Z. Zhang, 1997. Phytase supplementation of corn-soybean meal diets from three to seven week of age. *Poultry Sci.* 76(Suppl. 1):6. (Abstr.)
- Kozłowska, H., M. Naczka, F. Shahidi, and R. Zadernowski. 1990. Phenolic acids and tannins in rapeseed and canola. Page 193-210 in *Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology*. F. Shahidi, ed. Van Nostrand Reinhold, New York, NY.
- Leeson, S., J. O. Atteh, and J. D. Summers. 1987. The replacement value of canola meal for soybean meal in poultry diets. *Can. J. Anim. Sci.* 67:151-158.
- Liesegang, A., R. Ursprung, J. Gasser, M.-L. Sassi, J. Risteli, J. L. Riond, and M. Wanner. 2002. Influence of dietary phosphorus deficiency with or without addition of fumaric acid to a diet in pigs on bone parameters. *J. Anim. Physiol. A. Nutr.* 86:1-16.
- Liu, J., D. W. Bollinger, D. R. Ledoux, and T. I Veum. 1998. Lowering the dietary calcium to phosphorus ration increases phosphorus utilization in low phosphorus corn-soybean meal diets supplemented with microbial phytase for growing finishing pigs. *J. Anim. Sci.* 76:808-813.
- Mansoori, B., and T. Acamovic. 2007. The effect of tannic acid on the excretion of endogenous methionine, histidine and lysine with broilers. *Anim. Feed Sci. Technol.* 134:198-210.
- McNaughton, J., M. Roberts, D. Rice, B. Smith, B. Hong, B. Delaney, and C. Iiams. 2014. Comparison of broiler performance and carcass yields when fed diets containing

- genetically modified canola meal from event DP-Ø73496-4, near-isogenic canola meal, or commercial canola meals. *J. Poult. Sci.* 93:1-11.
- 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 phytate-P by the chick. *Br. J. Nutr.* 66:251-259
- Mushtaq, T., M. Sarwar, G. Ahmad, M. A. Mirza, H. Nawaz, M. M. Haroon Mushtaq, and U. Noreen. 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. *Poult. Sci.* 86:2144-2151.
- Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. *Poult. Sci.* 93:412-419.
- NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Nwokolo, E. N., and D. B. Bragg. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. *Can. J. Anim. Sci.* 57:475-477.
- Paik, I. 2003. Application of phytase, microbial or plant origin, to reduce phosphorus excretion in poultry production. *Asian-Aust. J. Anim. Sci.* 16:124-135.
- Parr, C. K., 2014. Evaluation of high-protein canola meals fed to growing chicks and weanling pigs. (Master's defense). University of Illinois at Urbana-Champaign.

- Przybylski, R., and N. A. M. Eskin. 2011. Oil composition and properties. In: J. K. Duan, N. A. M. Eskin, and D. Hickling, editors, *Canola: Chemistry, production, processing, and utilization*. AOCS Press, Urbana, IL. p. 189-227.
- Rahman, M. H., 2001. Production of yellow-seeded *Brassica napus* through interspecific crosses. *Plant Breeding* 120, 463-472.
- Rashid, A. and Rakow, G. 1995. Seed quality improvements in yellow seeded *Brassica napus*. *Proceedings of the 9th International Rapeseed Congress, Cambridge, UK, Vol. 4:* 1144-1146.
- Salmon, R. E., V. I. Stevens, and B. A. Ladbroke. 1988. Full-fat canola seed as a feedstuff for Turkeys. *Poult. Sci.* 67:1731-1742.
- Saylor, W.W., A. Bartnikowski and T. L. Spencer, 1991. Improved performance of broiler chicks fed diets containing phytase. *Poult. Sci.* 70 (Suppl. 1): 104: (Abstr.).
- Schoner, F. J., P. P. Hoppe and G. Schwarz, 1991. Comparative effects of microbial phytase and inorganic phosphorus on performance and retentions of phosphorus, calcium and crude ash in broilers. *J. Anim. Physiol. Anim. Nutr.*, 66: 248-255.
- Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996a. 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.
- Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition: Review. *Anim. Feed Sci. Technol.* 135:1-41.
- Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livest. Sci.* 124:126-141.

- Shen, H., J. D. Summers, and S. Leeson. 1983. The influence of steam pelleting and grinding on the nutritive value of canola rapeseed for poultry. *Anim. Feed Sci. Technol.* 8:303-311.
- Shirley, R. B., and H. M. Edwards, Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. *Poult. Sci.* 82:671-680.
- Simbaya, J., B. A. Slominski, G. Rakow, L. D. Campbell, R. K. Downey, and J. M. Bell. 1995. Quality characteristics of yellow-seeded *Brassica* seed meals: protein, carbohydrates, and dietary fiber components. *J. Agric. Food Chem.* 43:2062-2066.
- Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.* 64:525-540.
- Slominski, B. A., L. D. Campbell, and W. Guenter. 1994. Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. *J. Agric. Food Chem.* 42:704-707.
- Slominski, B. A., J. Simbaya, L. D. Campbell, G. Rakow, and W. Guenter. 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. *Anim. Feed Sci. Technol.* 78:249-262.
- Slominski, B. A., W. Jia, A. Rogiewicz, C. M. Nyachoti, and D. Hickling. 2012. Low-fiber canola. Part 1. Chemical and nutritive composition of the meal. *J. Agric. Food Chem.* 60:12225-12230.
- Summers, J. D., B. D. Lee, and S. Leeson. 1983. Sodium, potassium and phosphorus in canola and soybean meal. *Nut. Rep. Int.* 28:955-963.
- Thacker, P. A. 1990. Canola meal. In: P. A. Thacker and R. N. Kirkwood, editors, *Nontraditional feed sources for use in swine production*. Butterworth Publishers, Stoneham, MA. p. 69-78.

- Thacker, P. A., and D. Petri. 2011. Nutritional evaluation of canola protein concentrate for broiler chickens. *Asian-australas. J. Anim. Sci.* 24:1607-1614
- Tripathi, M. K., and A. S. Mishra. 2007. Glucosinolates in animal nutrition: A review. *Anim. Feed Sci. Technol.* 132:1-27.
- Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poult. Sci.* 81:1172-1183.
- Ward, A. K., H. L. Classen, and F. C. Buchanan. 2009. Fishy-egg tainting is recessively inherited when brown-shelled layers are fed canola meal. *Poult. Sci.* 88:714-721.
- Woyengo, T.A., Kiarie, and C. M. Nyachoti. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. *Poult Sci.* 89: 1182-1189.

CHAPTER 3

PHOSPHORUS BIOAVAILABILITY IN HIGH-PROTEIN CANOLA MEAL, CONVENTIONAL CANOLA MEAL, AND SOYBEAN MEAL FED TO CHICKS

ABSTRACT

A chick bioassay (Experiment 1) was conducted to determine relative P bioavailability in a new test canola meal (TCM) containing increased protein and reduced fiber, a conventional canola meal (CCM), and a dehulled soybean meal (SBM). A phosphorus-deficient cornstarch-dextrose-SBM basal diet was fed as Diet 1. Diets 2 and 3 had 0.05% or 0.10% P added from KH_2PO_4 , respectively. The remaining diets had 12.5% or 25% TCM, CCM, or SBM added in place of cornstarch and dextrose. A total of 225 female New Hampshire x Columbian chicks were weighed, wing banded, and allotted to the 9 dietary treatments via a completely randomized design, with each pen having a similar mean initial body weight. There were 5 chicks per pen and 5 replicate pens per treatment. Chicks were fed the experimental diets from Days 8-21 posthatch. An additional chick bioassay (Experiment 2) was conducted to determine the effect of phytase enzyme (Optiphos, Huvepharma, Sofia, Bulgaria) on bioavailability of the P in the CMs. Diet 1 was a phosphorus-deficient TCM-cornstarch-dextrose diet, with the TCM as the only source of dietary P. Diets 2-4 had 0.05%, 0.10%, or 0.15% P added from KH_2PO_4 , respectively. Diets 5 and 6 were the same as Diet 1 with 125 or 250 units phytase added per kg of diets, respectively. Diets 7-14 were the same as Diets 1-6 except that CCM was the CM source. Chicks were fed the experimental diets from days 8 to 21 posthatch. Bioavailability of P was estimated using the multiple-regression slope-ratio method where tibia ash was regressed on

supplemental P intake. A linear increase in tibia ash was observed as the P level was increased by addition of KH_2PO_4 , CCM, TCMs or SBM in Experiment 1. Mean bioavailability values of P in the TCM, CCM, and SBM relative to KH_2PO_4 were 18, 15, and 34%, respectively. A linear increase in tibia ash was also observed with addition of KH_2PO_4 or phytase in Experiment 2. It was estimated that the addition of 125 or 250 units/kg of phytase greatly increased the bioavailable P content of the TCM by 0.05 and 0.10%, respectively and the response to phytase was slightly lower for the CCM than TCM. In conclusion, the bioavailable P content of the new TCM was statistically equal to that of the CCM. Phytase greatly and similarly increased the bioavailability of P in both the TCM and CCM.

Key words: canola meal, chick, digestibility, phosphorus availability, fiber

INTRODUCTION

Canola meal (CM) inclusion in poultry diets has traditionally been limited in the past. High levels of anti-nutritional factors such as fiber and glucosinolates, which reduce the nutritional value of the meal for poultry, are the major causes. Fiber is known to reduce the digestibility of some minerals and decrease the energy content of a feed ingredient (Nwokolo and Bragg, 1977; Bell, 1993). Glucosinolates in poultry diets have been shown to cause increased mortality, perosis, reduced growth and feed intake, and thyroid enlargement (Fenwick and Curtis, 1980; Khajali and Slominski, 2012). As a result, breeding programs have been working towards developing new, higher protein, lower fiber CMs that also have a reduced glucosinolate concentration. Chen et al. (2015) at the University of Illinois at Urbana-Champaign evaluated the TME_n and AA digestibility for 14 increased protein, reduced fiber test CM samples compared to CCM and SBM. Five different precision-fed rooster assays were performed and in

each experiment the test canola meals had a higher concentration of digestible AA than the conventional CM. In the 14 test CMs, the range of TME_n was from 2.2 – 2.7 kcal/g DM and the conventional canola meals had a range of 2.0 – 2.4 kcal/g DM. Almost all of the test CMs had significantly higher TME_n values than the 6 conventional CMs also included in the study. Sibbald (1986) reported similar values for the TME_n of conventional canola meal fed to roosters at 2.054 – 2.271 kcal/g DM. The average protein content of the test CMs was 47.9% compared to 40.5% in the CCMs. The average NDF of the test meals was 19.05% and the ADF was 13.89%. The average NDF and ADF of the CCMs was 28.05% and 20.46%, respectively. The researchers concluded that the new increased protein, reduced fiber CM varieties are nutritionally superior to conventional CMs (Chen et al., 2015).

Little research has been done to determine the phosphorus bioavailability in conventional or new CM varieties. Approximately two-thirds of the total P content in many plant products is bound to phytate, which makes it mostly unavailable to chickens. Because chickens have little endogenous phytase to degrade phytate – P complexes, exogenous phytase can be added to diets to increase the P digestibility, as well as other minerals (Simons et al., 1990). More research is needed to evaluate the phosphorus bioavailability of conventional and high protein canola meals for broilers. Research is also needed to further determine the effect of microbial phytase inclusion on phosphorus availability in conventional and high protein canola meals for broiler chickens. Therefore, the first objective of this study was to evaluate the P bioavailability of a new high-protein, reduced fiber CM compared to CCM and SBM. The second objective was to determine the effect of phytase enzyme on the P bioavailability of the CMs.

MATERIALS AND METHODS

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

Ingredients and Nutrient Analysis

A sample of increased-protein, reduced-fiber test canola meal (TCM), conventional canola meal (CCM) and dehulled soybean meal (SBM) were obtained from commercial sources. Bomb calorimetry (Model 6300; Parr Instruments, Moline, IL) was used to analyze ingredients for GE, CP by combustion (Method 990.03; AOAC International, 2007) using a Rapid N Cube (Elementar Americas Inc, Mt. Laurel, NJ) with Asp as the standard, and ash (Method 942.05; AOAC International, 2007). Ingredients were also analyzed for amino acids (Method 982.30 E [a, b, and c]; AOAC International, 2007), ADF (Method 973.18, AOAC International, 2007), and NDF (Holst, 1973). Ingredient samples were also analyzed for DM by forced air oven drying for 2 hours at 135°C (Method 930.15; AOAC International, 2007). Calcium and P were determined by using inductively coupled plasma spectroscopy (Method 985.01 A, B, and C; AOAC International, 2007) after wet ashing (Method 975.03 B[b]; AOAC International, 2007).

Diets and Experimental Design

In Experiment 1, 1 TCM, 1 CCM, and a control SBM were evaluated for P bioavailability. A P-deficient cornstarch-dextrose-SBM basal diet was fed as Diet 1 (Table 3.1). Diets 2 and 3 had 0.05 or .10% P added from KH_2PO_4 , respectively. Diets 4 – 9 had 12.5 or 25% added canola meal from the TCM, CCM, or the SBM added in place of cornstarch and dextrose (2:1; Table 3.1).

In Experiment 1, a total of 225 New Hampshire x Columbian female chicks were fed a nutritionally complete corn and SBM starter diet for 7 days. On day 7 of age, chicks were fasted overnight prior to being placed on experiment. On day 8, chicks were weighed, wing banded, and allotted to 9 dietary treatments via a complete randomized design so that mean body weight was similar across treatments. There were 5 chicks per pen and 5 replicate pens per each of the 9 treatments. For the duration of the experiment, the chicks were in an environmentally controlled room with continuous lighting and housed in thermostatically-controlled Petersime starter batteries with raised-wire flooring. From days 8 to 21 of age, the experimental diets and water were available ad libitum. On day 22 of age, feed intake per pen was recorded and final body weight of each chick recorded.

In Experiment 2, 1 TCM and 1 CCM were evaluated to determine the amount of P released by phytase from the CM. Diet 1 was a P-deficient TCM – cornstarch-dextrose basal diet in which the only source of dietary P was TCM (Table 3.2). Diets 2, 3, and 4 were as Diet 1 with 0.05, 0.10 or 0.15% P added from KH_2PO_4 , respectively. Diets 5 and 6 were the same as Diet 1 with 125 or 250 FTU/kg of phytase added, respectively. Diet 7 was another P– deficient, cornstarch-dextrose basal diet, with CCM, where CCM was the only dietary source of P (Table 3.3). Diets 8, 9, and 10 were the same as Diet 1 with 0.05, 0.10, or 0.15% P added from KH_2PO_4 , respectively. Diets 11 and 12 were the same as Diet 7 with 125 or 250 FTU/kg of phytase added, respectively.

In Experiment 2, a total of 300 New Hampshire x Columbian male chicks were fed a nutritionally complete corn and SBM starter diet for 7 days. On day 7 of age the chicks were

fasted overnight prior to being placed on experiment. On day 8, chicks were weighed, wing banded, and allotted to 12 dietary treatments via a complete randomized design so that mean body weight was similar across treatments. There were 5 chicks per pen and 5 replicate pens per each of the 12 treatments. For the duration of the experiment, the chicks were in an environmentally controlled room with continuous lighting and housed in thermostatically-controlled Petersime starter batteries with raised-wire flooring. From days 8 to 21 of age, the experimental diets and water were available ad libitum. On day 22 of age, feed intake per pen was recorded and final body weight of each chick recorded.

At the conclusion of both trials, chicks were euthanized via CO₂ inhalation and right tibia bones were collected to determine bone ash content. Data were summarized to calculate weight gain, feed intake, and gain:feed ratio. The tibia bones were pooled within pen, autoclaved, and tissue removed with the aid of cheesecloth. The bones were then dried for 24 hours at 100 degrees C, weighed, and then dry-ashed in a muffle furnace for 24 hours at 600 degrees C. Ash weight was represented as milligrams per tibia and as a percentage of dry bone weight (Chung and Baker, 1990).

Statistical Analysis

For Experiments 1 and 2, data for growth performance and bone ash were initially analyzed using PROC ANOVA of SAS (SAS Institute. Inc., Cary, NC) with pen as the experimental unit. Differences among treatment means were assessed using the least significant difference test. Multiple linear regression (GLM procedure of SAS) was conducted by regressing tibia ash (mg/tibia) and tibia ash percent on supplemental P intake (mg/chick) from the KH₂PO₄ or the CM or SBM samples. The slope-ratio method (Finney, 1978) was used to estimate the bioavailability of P in the TCM, or CCM or SBM relative to KH₂PO₄. In

Experiment 2, the slope-ratio method was also used to determine P release by phytase in the TCM and CCM.

RESULTS AND DISCUSSION

Nutrient Composition

Nutrient compositions of the canola meal and SBM are presented in Table 3.4. The TCM contained a greater CP content than the CCM, as expected, but was less than the SBM. The CP in the CCM and TCM were both higher than the value of 38.0% reported by the Poultry NRC (1994). Both canola meals also contained AA values that were higher than previously published, except for arginine in the CCM (NRC, 2012). The TCM contained higher levels of all AA than the CCM due to its increased CP content. Levels of Met and Cys were higher in the TCM than SBM and most other AA values were similar between the two ingredients. The 15.10% NDF and 9.22% ADF in the TCM was less than the 18.88% and 14.32% in the CCM. However, the TCM still had considerably greater NDF and ADF than SBM.

The total P and calculated available P were the same between the TCM and CCM at 1.20% and 0.40%, respectively. Both are greater than the total and available P values reported by the NRC (1994) at 1.17% and 0.30%, respectively. The values of the CMs used herein are less than what Slominski et al. (2012) reported for total P content at 1.30% and .52% available phosphorus. Values for Ca content for the TCM and CCM were the same and are similar to previously published values (Bell, 1993; Khajali and Slominski, 2012).

Phosphorus Bioavailability (Experiment 1)

When compared to Diet 1, weight gain was increased with inclusions of KH_2PO_4 , canola meal, or SBM (Table 3.5). No consistent effects on feed intake or gain to feed ratio among

treatments were observed. However, a linear increase in tibia ash (mg/tibia and %) was observed as P increased with addition of KH_2PO_4 , CM, or SBM. This increase in tibia ash is in agreement with previous research conducted by Kim et al. (2008). The multiple regression of tibia ash (mg/tibia and %) on supplemental P intake was highly significant. (R^2 values of 0.84 and 0.77 respectively; $P < 0.001$). The estimated P bioavailability values relative to KH_2PO_4 and the calculated bioavailable P concentrations in the TCM, CCM, and SBM are shown in Table 3.6. The bioavailability estimates for the TCM and for the CCM did not differ significantly from each other but both were significantly less than the SBM. When the bioavailability values were multiplied by the total P content, the amount of bioavailable P in the TCM was 15% higher than the CCM, with SBM being intermediate. Thus the bioavailability of the P and the concentration of bioavailable P in TCM are similar to or greater than for CCM.

By dividing the available P, or non-phytate bound P, by the total P content, expected values for bioavailability of P in CM and SBM can be estimated. Based on these calculations, the expected P bioavailability for CM listed in the Swine NRC (2012) is 40%. Using the same method, P bioavailability is calculated to be 25.6% in CCM and 41.5% in SBM according to the Poultry NRC (1994). The P bioavailability values for TCM, CCM and SBM based on tibia ash % herein were similar to the values in the Poultry NRC (1994) with values based on tibia ash (mg) being slightly lower. It is most important to note that the bioavailability of the P in the new TCM was statistically equal to or greater than that of the CCM.

Effect of Phytase on Bioavailability of P (Experiment 2)

In Experiment 2, a linear increase in weight gain and tibia ash was observed with each addition of KH_2PO_4 or phytase to the TCM or CCM basal diet (Table 3.7). These findings are in agreement with previous research (Nelson et al., 1971; Mitchell and Edwards, 1996; Green,

2011). There were several significant differences ($P < 0.05$) between TCM diet treatments (1-6) and CCM diet treatments (7-12) containing the same respective levels of added P from KH_2PO_4 or phytase for bone ash. Multiple regressions of tibia ash (mg/tibia and %) on supplemental P intake from KH_2PO_4 and phytase in the TCM and CCM were highly significant (R^2 values were 0.91 and 0.93, 0.90 and 0.92, respectively; $P < .001$). The amount of P released by phytase for TCM and CCM was estimated using a two-step procedure. First, the slope ratio method was used to determine the amount of P released from the TCM and CCM by each unit of phytase enzyme. Secondly, the values were then multiplied by the 125 and 250 FTU/kg levels of added phytase. Using this method, it was calculated that 0.050% and 0.100% P for tibia ash (mg/tibia) was released and 0.054% and 0.108% for tibia ash (%) was released by 125 and 250 FTU/kg, respectively, in the TCM. In the CCM, 0.041% and 0.082% P were determined to be released based on tibia ash (mg/tibia) and 0.045% and 0.089% P were released based on tibia ash (%) by 125 and 250 FTU/kg, respectively (Table 3.8). These values are similar to values previously obtained by Pillai et al. (2006) of 0.11 - 0.12% P release when corn – SBM diets were supplemented with 250 FTU/kg of phytase.

In conclusion, the results of Experiment 2 indicate that the bioavailability of the P in TCM and CCM is increased by microbial phytase. These results indicate that phytase increases the bioavailability of P in CM and the effect of phytase on TCM is equal to or greater than that of CCM. The observed significant difference in bone ash between several TCM and CCM dietary treatments containing the same level of supplemental P or phytase suggests that the TCM contained a slightly greater concentration of bioavailable P than CCM.

LITERATURE CITED

- AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.
- Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. *Can. J. Anim. Sci.* 73:679-697.
- Chen X., C. Parr, P. Utterback, and C. M. Parsons. 2015. Nutritional evaluation of canola meals produced from new varieties of canola seeds for poultry. *Poult Sci.* 94:984-991.
- Chung, T. K., and D. H. Baker. 1990. Phosphorus utilization in chicks fed hydrated sodium calcium aluminosilicate. *J. Anim. Sci.* 68:1992-1998.
- Fenwick, G. R., and R. F. Curtis. 1980. Rapeseed meal and its use in poultry diets. A review. *Anim. Feed Sci. Technol.* 5:255-298.
- Finney, D. J. 1978. *Statistical Method in Biological Assay*. 3rd. ed. Charles Griffin and Company, Ltd., High Wycombe, Buckinghamshire, UK.
- Green, J. 2011. The effect of feeding high concentrations of cholecalciferol, phytase, or the combination on broiler chicks and laying hens fed various concentrations of nonphytate phosphorus. M. Sc. Thesis. Iowa State University, Ames.
- Holst, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. *J. AOAC Int.* 56:1352-1356.
- Khajali, F., and B. A. Slominski. 2012. Review: Factors that affect the nutritive value of canola meal for poultry. *Poult. Sci.* 91:2564-2575.
- Kim, E. J., C. M. Amexcua, P. L. Utterback, and C. M. Parsons. 2008. Phosphorus bioavailability, true metabolizable energy, and amino acid digestibilities of high protein corn distillers dried grains and dehydrated corn germ. *Poult. Sci.* 87:700-705.

- Mitchell, R. D., and H. M. Edwards, Jr. 1996. Additive effects of 1, 25-Dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chickens. *Poult. Sci.* 75:111-119.
- Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. *J. Nutr.* 101:1289-1293.
- NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Nwokolo, E. N., and D. B. Bragg. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. *Can. J. Anim. Sci.* 57:475-477.
- Pillai, P. B., T. O'Connor-Dennie, C. M. Owens, and J. L. Emmert. 2006. Efficacy of an *Escherichia coli* Phytase in Broilers Fed Adequate or Reduced Phosphorus Diets and Its
- SAS Institute, 2010. SAS® User's Guide: Statistics. Version 9.2 Edition. SAS Institute, Inc., Cary, NC.
- Sibbald, I. R., 1986. The TME system of feed evaluation: methodology, feed composition data and bibliography. *Tech. Bull.* 4 E. Anim. Res. Centre, Res. Branch, Agricultural. Ottawa, Ontario, Canada.
- Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.* 64:525-540.
- Slominski, B. A., W. Jia, A. Rogiewicz, C. M. Nyachoti, and D. Hickling. 2012. Low-fiber canola. Part 1. Chemical and nutritive composition of the meal. *J. Agric. Food Chem.* 60:12225-12230.

Zhang, Z. B., E. T. Kornegay, J. S. Radcliffe, D. M. Denbow, H. P. Veit, and C. T. Larson.

2000b. Comparison of genetically engineered microbial and plant phytase for young broilers. *Poult. Sci.* 79:709-717.

TABLES

Table 3.1. Ingredient composition of experimental diets in Experiment 1 (as-fed basis).

Ingredient, %	Dietary treatments								
	P- deficient control ¹	KH ₂ PO ₄		Test canola meal		Soybean meal		Conventional canola meal	
	1	2	3	4	5	6	7	8	9
Dextrose	16.68	16.61	16.54	12.52	8.35	12.52	8.35	12.52	8.35
Cornstarch	33.38	33.22	33.07	25.04	16.71	25.04	16.71	25.04	16.71
Test or conventional canola	-	-	-	12.50	25.00	-	-	12.50	25.00
Soybean meal	42.00	42.00	42.00	42.00	42.00	54.50	67.00	42.00	42.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Dicalcium phosphate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table 3.1 (cont.)

Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
KH ₂ PO ₄	-	0.23	0.45	-	-	-	-	-	-
Bacitracin-BMD premix ⁴	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
DL-Met	.30	.30	.30	.30	.30	.30	.30	.30	.30

¹The P-deficient control diet was calculated to contain 3.328 kcal of ME/kg, 20.5% CP, 0.11% available P and 0.76% Ca.

²Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³Provided as milligrams per kilogram of diet: manganese, 75 from MnSO₄·H₂O; iron, 75 from FeSO₄·H₂O; zinc, 75 mg from ZnO; copper, 5 mg from CuO₄·5H₂O; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from Na₂SeO₃.

⁴Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

Table 3.2. Ingredient composition of Diets 1-6 in Experiment 2 (as-fed basis).

Ingredient, %	Dietary Treatments					
	TCM control ¹	KH ₂ PO ₄			Phytase	
	1	2	3	4	5	6
Dextrose	15.57	15.50	15.42	15.35	15.57	15.57
Cornstarch	31.15	30.90	30.85	30.69	31.14	31.13
TCM	45.00	45.00	45.00	45.00	45.00	45.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.34	1.34	1.34	1.34	1.34	1.34
Dicalcium phosphate	-	-	-	-	-	-
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.13	0.13	0.13	0.13	0.13	0.13
L-Arg	0.15	0.15	0.15	0.15	0.15	0.15

Table 3.2 cont.

L-Thr	0.12	0.12	0.12	0.12	0.12	0.12
L-Ile	0.15	0.15	0.15	0.15	0.15	0.15
TiO ₂	0.40	0.40	0.40	0.40	0.40	0.40
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10
KH ₂ PO ₄	-	0.23	0.45	0.68	-	-
Phytase ⁴	-	-	-	-	0.006	0.013
Bacitracin-BMD premix ⁵	0.04	0.04	0.04	0.04	0.04	0.04

¹The P-deficient test canola meal (TCM) control diet was calculated to contain 3,061 kcal of ME/kg, 21% CP, 0.149% available P and 0.77% Ca.

²Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³Provided as milligrams per kilogram of diet: manganese, 75 from MnSO₄·H₂O; iron, 75 from FeSO₄·H₂O; zinc, 75 mg from ZnO; copper, 5 mg from CuO₄·5H₂O; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from Na₂SeO₃.

⁴The 0.006 and 0.013% phytase premix supplied 125 and 250 FTU/kg of phytase, respectively.

⁵Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

Table 3.3. Ingredient composition of Diets 7-12 in Experiment 2 (as-fed basis).

Ingredient, %	Dietary Treatments					
	CCM control ¹	KH ₂ PO ₄			Conventional canola meal	
	7	8	9	10	11	12
Dextrose	15.40	15.33	15.25	15.18	15.40	15.40
Cornstarch	30.81	30.65	30.51	30.35	30.80	30.80
CCM	45.00	45.00	45.00	45.00	45.00	45.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.34	1.34	1.34	1.34	1.34	1.34
Dicalcium phosphate	-	-	-	-	-	-
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl	0.13	0.13	0.13	0.13	0.13	0.13

Table 3.3 cont.

L-Arg	0.15	0.15	0.15	0.15	0.15	0.15
L-Ile	0.15	0.15	0.15	0.15	0.15	0.15
TiO ₂	0.40	0.40	0.40	0.40	0.40	0.40
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10
KH ₂ PO ₄	-	0.23	0.45	0.68	-	-
Phytase ⁴	-	-	-	-	0.006	0.013
Bacitracin-BMD premix ⁵	0.040	0.040	0.040	0.040	0.40	0.40

¹The P-deficient conventional canola meal (CCM) control diet was calculated to contain 3,042 kcal of ME/kg, 19% CP, 0.131% available P and 0.78% Ca.

²Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³Provided as milligrams per kilogram of diet: manganese, 75 from MnSO₄·H₂O; iron, 75 from FeSO₄·H₂O; zinc, 75 mg from ZnO; copper, 5 mg from CuO₄·5H₂O; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from Na₂SeO₃.

⁴The 0.006 and 0.013% phytase premix supplied 125 and 250 FTU/kg of phytase, respectively

⁵Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

TABLE 3.4. Analyzed nutrient composition of ingredients used in Experiments 1 and 2 (as-fed basis).

Item	Test canola meal	Conventional canola meal	Soybean meal
ME _n ¹ , kcal/kg	2,200	2,010	2,450
DM, %	89.4	88.9	89.0
CP, %	45.40	40.25	49.00
NDF ² , %	15.10	18.88	6.74
ADF ² , %	9.22	14.32	3.83
Ca, %	0.60	0.60	0.29
Total P ² , %	1.20	1.20	0.62
Available P ³ , %	0.40	0.40	0.19
Total AA ² , %			
Arg	2.79	1.66	3.54
His	1.12	1.01	1.24
Ile	1.78	1.57	2.24
Leu	2.84	2.67	3.65
Lys	2.48	2.17	2.99
Met	0.92	0.79	0.68
Phe	1.63	1.56	1.80
Thr	1.83	1.66	1.93
Trp	0.58	0.52	0.70
Val	2.32	2.02	2.35
Cys	1.28	1.04	0.69
Ileal Digestible AA ¹ , %			
Arg	2.59	2.34	3.32
His	1.12	0.80	1.15
Ile	1.55	1.34	2.03
Leu	2.78	2.14	3.30
Lys	2.06	1.75	2.56
Met	0.84	0.71	0.62
Phe	1.58	1.17	2.21
Thr	1.52	1.36	1.68
Trp	0.57	0.51	0.68
Val	1.94	1.65	2.02
Cys	1.11	0.89	0.58

¹ME_n value calculated from TME_n conventional roosters in a precision-fed rooster assay. Ileal digestible AA values determined using cecectomized roosters in a precision-fed rooster assay.

²NDF = neutral detergent fiber; ADF= acid detergent fiber; P = phosphorus; AA= amino acids.

³Calculated assuming that 30% of the total P was available

Table 3.5. Growth performance and tibia ash for chicks in Experiment 1 from 8-21 d of age.¹

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Bone ash ² (mg/tibia)	Bone ash ³ (%)
1. Basal Diet (B) ⁴	208 ^c	436 ^c	476 ^{cd}	248 ^f	31.3 ^d
2. B + 0.05% P ⁵	249 ^a	496 ^a	503 ^{abc}	343 ^b	37.4 ^b
3. B + 0.10% P ⁵	235 ^{abc}	496 ^a	474 ^{cd}	385 ^a	40.2 ^a
4. B + 12.5% TCM ⁶	247 ^a	465 ^{abc}	531 ^a	294 ^e	34.7 ^c
5. B + 25% TCM ⁶	244 ^{ab}	453 ^{bc}	538 ^a	329 ^{bc}	38.5 ^{ab}
6. B + 12.5% CCM ⁶	249 ^a	474 ^{ab}	525 ^{ab}	300 ^{de}	35.1 ^c
7. B + 25% CCM ⁶	240 ^{ab}	481 ^{ab}	500 ^{abc}	316 ^{cde}	37.4 ^b
8. B + 12.5% SBM ⁶	227 ^{abc}	465 ^{abc}	487 ^{bcd}	301 ^{de}	35.3 ^c
9. B + 25% SBM ⁶	234 ^{abc}	452 ^{bc}	518 ^{abc}	323 ^{bcd}	37.6 ^b
Pooled SEM	9.80	11.6	14.8	8.00	0.64

^{a-f}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹Means represent 5 pens of 5 chicks per treatment; average initial BW was 100 g.

²Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), TCM (X_2), CCM (X_3), and SBM (X_4) yielded the equation: $Y = 260.5 + 281.0 \pm 19.8X_1 + 50.0 \pm 6.93X_2 + 41.9 \pm 6.56X_3 + 94.7 \pm 13.5X_4$ ($R^2 = 0.835$). The (\pm) values are standard errors of the regression coefficients.

³Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), TCM (X_2), CCM (X_3), and SBM (X_4) yielded the equation: $Y = 31.93 + 18.49 \pm 1.68X_1 + 4.66 \pm .589X_2 + 3.95 \pm 0.558X_3 + 8.34 \pm 1.15X_4$ ($R^2 = 0.765$). The (\pm) values are standard errors of the regression coefficients.

⁴P-deficient control diet in Table 3.1.

⁵From KH_2PO_4 .

⁶TCM= test canola meal, CCM= conventional canola meal, SBM= soybean meal.

Table 3.6. Relative bioavailability of the P in canola meals and soybean meal for Experiment 1.

Sample	Total P (%)	Bioavailability values ¹ (%)		Bioavailable content ² (%)	
		Tibia ash (mg)	Tibia ash (%)	Tibia ash (mg)	Tibia ash (%)
Test canola meal	1.20	17.8 ^b	25.2 ^b	0.21	0.30
Conventional canola meal	1.20	14.9 ^b	21.4 ^b	0.18	0.26
Soybean meal	0.62	33.7 ^a	45.1 ^a	0.21	0.28

^{a-b}Values within a column with no common superscript are significantly different ($P < 0.05$) as determined using the regression coefficients and standard errors in the multiple regression equations in footnotes 2 and 3 of Table 3.5

¹Calculated by the slope-ratio method using the multiple regression equations in footnotes 2 and 3 of Table 3.5. These are bioavailability values relative to the P in KH_2PO_4 which was set at 100%.

²Bioavailable content = total P \times bioavailability value.

Table 3.7. Growth performance and tibia ash of chicks in Experiment 2 from 8-21 d of age.¹

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Bone ash ² (mg/tibia)	Bone ash ³ (%)
1. TCM basal diet (B) ^{4,5}	265 ^{cd}	476 ^{ef}	557 ^{bc}	329 ^e	34.6 ^f
2. As 1 + 0.05% P ⁶	289 ^{ab}	521 ^{abcd}	557 ^{bc}	396 ^{cd}	36.7 ^f
3. As 1 + 0.10% P ⁶	263 ^{cd}	547 ^{ab}	482 ^d	466 ^b	41.2 ^{bc}
4. As 1 + 0.15% P ⁶	308 ^a	467 ^f	663 ^a	529 ^a	42.6 ^{ab}
5. As 1 + 125 FTU/kg ⁷	286 ^{abc}	500 ^{def}	572 ^b	382 ^d	37.9 ^{ef}
6. As 1 + 250 FTU/kg ⁷	286 ^{abc}	510 ^{bcde}	562 ^{bc}	467 ^b	41.0 ^{bc}
7. CCM basal diet ^{4,5}	250 ^d	477 ^{ef}	525 ^{bcd}	298 ^f	32.8 ^h
8. As 7 + 0.05% P ⁶	266 ^{bcd}	508 ^{cde}	525 ^{bcd}	343 ^e	36.6 ^f
9. As 7 + 0.10% P ⁶	274 ^{bcd}	540 ^{abc}	508 ^{cd}	457 ^b	40.5 ^{cd}
10. As 7 + 0.15% P ⁶	284 ^{abc}	548 ^a	520 ^{bcd}	535 ^a	43.2 ^a
11. As 7 + 125 FTU/kg ⁷	263 ^{cd}	534 ^{abcd}	493 ^d	350 ^e	36.7 ^f
12. As 7 + 250 FTU/kg ⁷	285 ^{abc}	531 ^{abcd}	540 ^{bcd}	420 ^c	39.1 ^{de}
Pooled SEM	8.50	13.4	21.2	9.54	0.56

^{a-h}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹Means represent 5 pens of 5 chicks per treatment; average initial BW was 93.4 g.

²For TCM (Diets 1-6), multiple regression of tibia ash (Y; mg) on supplemental P (%) from KH_2PO_4 (X_1), and supplemental phytase FTU/kg (X_2) yielded the equation: $Y = 325 + 1375 \pm 84.7X_1 + .546 \pm 0.051X_2$ ($R^2 = 0.91$). For CCM (Diets 7-12), multiple regression of tibia ash (Y; mg) on supplemental P (%) from KH_2PO_4 (X_1), and supplemental phytase FTU/kg (X_2) yielded the equation: $Y = 284 + 1656 \pm 87.2X_1 + .54 \pm 0.053X_2$ ($R^2 = 0.93$). The (\pm) values are standard errors of the regression coefficients.

³For TCM (Diets 1-6) multiple regression of tibia ash using TCM (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), FTU/kg (X_2) yielded the equation: $Y = 35.0 + 54.6 \pm 3.60X_1 + .0023 \pm 0.0022X_2$ ($R^2 = 0.90$). For CCM (Diets 7-12) multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), FTU/kg (X_2) yielded the equation: $Y = 33.1 + 69.2 \pm 3.87X_1 + .025 \pm 0.0023X_2$ ($R^2 = 0.92$). The (\pm) values are standard errors of the regression coefficients.

⁴P-deficient TCM or CCM control diet in Table 3.2.

Table 3.7. cont.

⁵TCM = test canola meal, CCM= conventional canola meal, SBM= soybean meal.

⁶From KH_2PO_4 .

⁷Level of phytase provided from Optiphos®, Huvepharma, Sofia, Bulgaria.

Table 3.8. Calculated amount of phosphorus released from test canola meal and Conventional canola meal by phytase enzyme in Experiment 2 using the slope ratio method.

Canola meal source	Phytase (FTU/kg)	Amount of P released (%) ¹	
		Bone Ash (mg/tibia)	Bone Ash (%)
Test	125	.050	.054
Test	250	.100	.108
Conventional	125	.041	.045
Conventional	250	.082	.089

¹Calculated using the multiple regression equations and slopes in footnotes 2 and 3 of Table 3.7. For example for the test canola meal, the ratio of the slopes (.546/1375) was .000397 (Table 3.7), indicating that .000397% P was released by each FTU/kg of phytase. Multiplying .000397 by 125 and 250 FTU/kg of phytase yielded estimates of .050 and .100% P release, respectively.

CHAPTER 4

PHOSPHORUS DIGESTIBILITY IN CONVENTIONAL CANOLA MEAL DETERMINED USING DIFFERENT BALANCE ASSAYS

ABSTRACT

Four experiments were conducted to determine the P retention (excreta) and digestibility (ileal) values for conventional canola meal (CCM) fed to roosters and chicks. The first experiment was an ad libitum-fed chick experiment which evaluated the effect of phytase on P digestibility and retention values for CCM based on ileal and excreta contents, respectively. The chicks were fed a P - deficient cornstarch - dextrose CM basal diet (.13% available P) as Diet 1. Diets 2 and 3 were the basal diet plus 125 or 250 FTU/kg of phytase, respectively. Diets were fed from Days 8 - 21 posthatch. On Day 22, the ileal digesta and excreta were collected and analyzed for P. There were no significant differences in ileal P digestibility among the dietary treatments ($P < 0.05$). The P retention values were determined to be 38.7%, 47.3%, and 51.0% for the diets containing 0, 125, or 250 FTU/kg phytase, and values for the diets containing added phytase were significantly higher ($P < 0.05$) than the zero phytase diet. The second experiment was a precision - fed chick assay conducted to determine the ileal P digestibility of CCM. The chicks were fed a nutritionally complete SBM starter diet from Days 1 - 20. The chicks were fasted overnight and then on Day 21, the chicks were tube fed 3, 6, or 9 g of CCM. Chicks were euthanized by CO₂ inhalation beginning 5 hours after feeding and ileal digesta were collected. There was not enough ileal digesta from the birds fed 3 g of CCM to be analyzed for P digestibility. Mean ileal P digestibility was determined to be 47.5% in chicks fed 6 g and 40.0% in chicks fed 9 g of CCM and the values were not significantly different. In Experiment 3, 15 cecectomized Single Comb White Leghorn roosters were used to determine P retention values for CCM using a precision-fed

rooster assay. After withdrawal of feed for 24 hours, the roosters were tube-fed 8, 16, or 24 g of CCM. All excreta (feces + urine) were collected 48 hours after feeding and analyzed for P. Phosphorus retention values were 34.6%, 28.5%, and 23.1% for birds tube-fed 8, 16, and 24 g of CCM, respectively, with the 34.6% value being significantly higher ($P < 0.05$) than the 23.1% value. Experiment 4 was an ad libitum-fed chick assay to determine the ileal P digestibility and retention (excreta) for CCM with and without increasing levels of dietary supplemental Ca. The chicks were fed a P deficient - dextrose - CCM basal diet (0.039% available P, 13.50% CCM) as diet 1. Diets 2-4 contained increasing levels of 27%, 40.50%, or 54% CCM, respectively. The Ca: available P ratio was maintained at a 2:1 ratio in Diets 1-4. Diets 5-8 were the same as Diets 1-4 but supplemental Ca was added so that the calcium: available P ratio was maintained at a 6:1 ratio. Diets were fed from Days 15-21 posthatch and excreta were collected on Days 21 and 22 and ileal digesta on Day 22. Phosphorus digestibility decreased with each increase in dietary CCM in Diets 1-4. There was also a reduction in P digestibility in Diets 5-8 compared to Diets 1-4 and also with increased Ca: P ratio. Phosphorus retention values also generally decreased with increased Ca: P ratio. The results of this study indicated the P digestibility and retention values for CCM varied among balance methods and sometimes among levels of CCM fed.

Keywords: canola meal, chick, rooster, digestibility, retention, phosphorus

INTRODUCTION

There has been little research conducted concerning P digestibility in CM for poultry. This is likely because conventional canola meal (CCM) inclusion in poultry diets has traditionally been limited due to high fiber and glucosinolate content. However, with new CM varieties that possess higher protein and lower fiber concentrations, it may be possible, in the future, to feed CM to

poultry at greater amounts than has been done in the past. The small amount of research that has been conducted on the P digestibility in CM has produced varying results. The poultry NRC (1994) states that the total P content in CM is 1.17% and the phytate bound P content is 0.30%. By dividing the phytate fraction by the total P content, it can be calculated that approximately 25% of the total P in CCM is digestible. In a chick bioassay conducted by Mutucumarana et al. (2014), ileal digesta and excreta were collected to determine P digestibility and retainability in CCM. The results indicated that P in CCM was 47% digestible (ileal) and 49% retainable (excreta), almost double the value calculated from the NRC (1994). The results obtained by Mutucumarana et al. (2014) also suggested that ileal digestibility decreased with increased dietary levels of P and CCM but excreta retention was not affected by dietary P and CM level.

Precision-fed rooster assays have been a common method used in determining TMEn and amino acid digestibility (Ravindran and Bryden, 1999; Parsons, 2002). However, little or no research has been conducted or published using precision-fed rooster assays to determine P retention values for feed ingredients. Precision-fed rooster assays are advantageous because they require only a small amount of feed sample and feed ingredients can be tested in a relatively short amount of time with only a few birds (Parsons, 2002).

Kim et al. (2011) developed a precision-fed ileal chick assay for determining amino acid digestibility. Three week old broiler chicks were used in the procedure and fasted for at least 8 hours prior to tube feeding. Approximately 10 g of feed ingredient was tube-fed and ileal digesta were collected 4 hours after feeding. This assay was successful in determining digestibility values for amino acids; however, no research has been conducted using this procedure to determine mineral digestibility.

Therefore, the purpose of the experiments conducted in this chapter was to evaluate and

compare an ad libitum-fed chick assay, a precision-fed chick assay and a precision-fed rooster assay for determining ileal P digestibility values or P retention values for CCM.

MATERIALS AND METHODS

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

Nutrient Analysis of Conventional Canola Meal

A sample of commercial solvent-extracted CCM was obtained from a commercial company and the same sample was used in all animal assays. Bomb calorimetry (Model 6300; Parr Instruments, Moline, IL) was used to analyze CCM for GE. Crude protein was analyzed by combustion (Method 990.03; AOAC International, 2007) using a Rapid N Cube (Elementar Americas Inc, Mt. Laurel, NJ) with Asp as the standard. Ash was analyzed using Method 942.05 (AOAC International, 2007). The CCM was also analyzed for amino acids (Method 982.30 E [a, b, and c]; AOAC International, 2007), ADF (Method 973.18, AOAC International, 2007), and NDF (Holst, 1973). The CCM was also analyzed for DM by forced air oven drying for 2 hours at 135°C (Method 930.15; AOAC International, 2007). Calcium and P were determined by using inductively coupled plasma spectroscopy (Method 985.01 A, B, and C; AOAC International, 2007) after wet ashing (Method 975.03 B[b]; AOAC International, 2007). Titanium was analyzed by UV spectroscopy (Meyers, 2004). All analyses except GE and ash were determined at the University of Missouri Experiment Station Field Laboratory.

Diets and Experimental Design

In Experiment 1, P apparent retention (excreta) and digestibility (ileal) values for CCM were determined in ad libitum-fed chicks. A total of 75 New Hampshire x Columbian male chicks were fed a nutritionally complete corn and SBM starter diet for 7 days. On Day 7, the chicks were fasted overnight prior to being placed on the experimental diets. On Day 8, the chicks were weighed, wing banded, and allotted to the 3 dietary treatments via a complete randomized design with a similar mean body weight across treatments. There were 5 chicks per pen with 5 replicate pens per treatment. Diet 1 was a P - deficient cornstarch-dextrose-CCM basal diet (Table 4.1) calculated to contain 0.13% available P (CCM was the only source of P in the diet). Diets 2 and 3 were the basal diet plus 125 or 250 FTU/kg of phytase, respectively (Table 4.1). Titanium dioxide was added to each diet at a level of 0.4% as an indigestible marker. The chicks were housed in an environmentally controlled room and in Petersime thermostatically controlled starter batteries with raised wire flooring. From Days 8-21 of age, the experimental diets and water were available ad libitum. On Day 22 of age, chicks were euthanized by CO₂ inhalation. Ileal digesta from Meckel's diverticulum to the ileal-cecal junction were collected. Excreta were also collected from pans under the cages. Ileal digesta and excreta were both frozen and stored at -20°C, freeze-dried, ground with mortar and pestle, and analyzed for P and Ti concentration.

The second experiment was a precision-fed chick assay (Kim et al., 2011) which was conducted to determine the apparent ileal digestibility of P in CCM. Ross 308 broiler chicks were fed a nutritionally complete SBM starter diet from Days 1 - 20 of age and were housed in thermostatically controlled Petersime starter batteries with 4 chicks per pen. After feed withdrawal for 10 hours on Day 21, they were weighed and wing-banded so that each dietary

treatment had a similar mean body weight. The chicks were then tube-fed 3, 6, or 9 g of CCM with 4 pens of 4 chicks assigned to each CCM level. Titanium dioxide was added to the CCM at a level of 0.4% as an indigestible marker. Chicks were euthanized by CO₂ inhalation beginning 4 hours after feeding and ileal digesta from Meckel's diverticulum to the ileo-cecal junction were collected. Ileal digesta were freeze-dried, ground, and analyzed for P and Ti.

In Experiment 3, a precision-fed rooster assay was utilized to determine standardized P retention values for CCM. Fifteen roosters were housed in individual cages in an environmentally controlled room. They were withdrawn from feed for 24 hours and then 5 roosters were tube-fed 8, 16, or 24 g of CCM. Excreta were quantitatively collected 48 hours after feeding, freeze-dried, ground with a mortar and pestle, and analyzed for P. Standardized P retention values were calculated by correcting the values for basal endogenous P losses, which were determined from roosters that had been fasted for 48 hours.

Experiment 4 was an ad libitum-fed chick assay conducted to determine the ileal P digestibility and retention (excreta) for CCM and to evaluate the effect of the Ca: available P ratio on P digestibility and retention. Two hundred chicks were fed a nutritionally complete SBM starter diet from Days 1-14. On Day 15, the chicks were weighed, wing banded, and allotted to the 3 dietary treatments via a complete randomized design with a similar mean body weight across treatments. There were 5 chicks per pen with 5 replicate pens per treatment. Diet 1 was a P-deficient dextrose-CCM basal diet which contained 0.039% available P (Table 4.2). Diets 1-4 contained graded concentrations of CCM which were 13.5%, 27%, 40.50%, and 54% and the Ca: available P ratio was 2:1. Diets 5-8 were the same as Diets 1-4 but had additional Ca added at levels of .4%, .8%, 1.2%, or 1.6% from limestone, respectively, to maintain a 6:1 ratio of Ca: available P. On Day 22 of age, chicks were euthanized by CO₂ inhalation. Ileal digesta

from Meckel's diverticulum to the ileal-cecal junction were collected. Excreta were also collected from pans under the cages on Day 21 and 22. Ileal digesta and excreta were both frozen and stored at -20°C, freeze-dried, ground with mortar and pestle, and analyzed for P and Ti concentration.

Statistical Analysis

Data from all three experiments were analyzed using the ANOVA procedure of SAS (SAS Institute, Inc., Cary, NC) with pen (Experiments 1 and 2) or individual rooster (Experiment 3) as the experimental unit. Differences among treatment means were assessed using the least significant difference test. Significance was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

The analyzed nutrient composition of CCM is shown in Table 4.2. The CP and amino acid levels were slightly higher than those reported in the poultry NRC (1994). Total calcium and phosphorus concentrations were similar to values in the poultry NRC (1994) table.

Experiment 1

Ileal digestibility and retention values for P in CCM from the basal diet (no supplemental phytase) were similar (Table 4.4). There were no significant differences in ileal P digestibility with inclusion of 125 or 250 FTU/kg of phytase. The ileal P digestibility was 38.0% in the basal diet, and 44.8% and 47.6% for the diets with 125 or 250 FTU/kg of phytase, respectively. Although there were no significant differences among the letter values, there was a linear effect of

phytase on ileal P digestibility that approached significance ($P < 0.056$). The inclusion of phytase did have a significant effect on P retention values. The retention value for the basal diet was 38.7% which was significantly lower ($P < 0.05$) than the values obtained from birds fed diets supplemented with phytase. The phosphorus retention value for Diet 2, which was supplemented with 125 FTU/kg of phytase was 47.3%, and the retention value for Diet 3, which was supplemented with 250 FTU/kg of phytase was 51.0%. The P retention values from Diets 2 and 3 did not differ significantly from each other. There was a significant linear effect ($P < 0.004$) of phytase on P retention values and ileal P digestibility approached significance ($P < 0.056$). The average ileal P digestibility for all birds in Experiment 1 was 43.5% and 45.7% for P retention. The results obtained herein are similar to those published by Mutucumarana et al. (2014) who found ileal P digestibility to be 46.9% and P retention to be 48.6% when using regression analysis for birds fed increasing dietary levels of CCM.

Experiment 2

The ileal P digestibility values for the chicks tube-fed 6 or 9 g of CCM are presented in Table 4.5. There was not enough ileal digesta present in any of the repetitions of birds fed 3 g of CM to do both P and Ti analysis; thus this level of feed intake is probably too low for the tube-feeding ileal digestibility chick assay. The ileal P digestibility values for chicks tube-fed 6 or 9 g of CCM did not differ significantly from each other. The ileal P digestibility value for chicks fed 6 g of CCM was 47.5% and 40.0% for chicks fed 9 g. These ileal P digestibility values are similar to those obtained in Experiment 1 are also comparable to the 46.9% ileal P digestibility reported by Mutucumarana et al. (2014) for CCM.

Experiment 3

The P retention values for roosters tube-fed 8, 16, or 24 g CCM are presented in Table 4.6. The retention values are standardized, meaning predetermined endogenous losses were subtracted from the output in the digestibility equation. Basal endogenous P losses were estimated from fasted roosters. There was a significant difference among treatments. The retention values were 35.0%, 28.0%, and 23.0% from roosters fed 8, 16, and 24 g of CM, respectively. These values are similar to the P availability value of 25.6% that can be calculated for CM from the Poultry NRC (1994) by dividing the nonphytate P by the total P. The P digestibility values in this experiment for the two highest CM levels are also similar to the relative bioavailability values found in Chapter 3 of this thesis (Table 3.6). For example, using the slope-ratio procedure, the P in TCM was calculated to be 25.2% bioavailable and CCM 21.4% bioavailable, using tibia ash (%) in Chapter 3. In a study conducted by Mutucumarana et al. (2014), the authors found P retention to be 48.6%, on average, which is double the lowest value reported herein of 23.0% retainable for birds fed 24 g of CM. The P retention value of the roosters fed 8 g differed significantly from those fed 24 g, but not from the birds fed 16 g. Thus, P retention decreased as increasing amounts of CM were fed to the roosters and there was a significant linear ($P < 0.05$) effect of CM intake on P retention values. A similar result was observed in the previously mentioned study by Mutucumarana et al. (2014) in which P retention values for CM decreased with increased CM inclusion in the diet. A possible explanation for the latter effect in the current study is that the P requirement of the roosters was approached or exceeded when higher levels of dietary CM were fed, which could lead to absorbed P being excreted in the urine. Since urine is collected with excreta, this could make the P in the CM

appear less retainable than it actually is at higher CM and P intakes. The precision-fed rooster assay has potential in determining P retainability in CM but further research is needed to refine this method, particularly with respect to effects of feed ingredient and P intake on retention values. The precision-fed rooster assay is easier, faster, less expensive and requires far fewer animals than the other assays. If valid methodology can be developed for the rooster assay, it would facilitate determining P availability in feed ingredients for poultry.

Experiment 4

The ileal P digestibility and retention values for P in CCM are shown in Table 4.7. The ileal P digestibility value for birds fed the basal diet was 58.9% and digestibility decreased significantly with increasing CM and Ca: P ratio in Diets 2-8. A similar study as Experiment 4 herein was conducted by Mutucumarana et al. (2014) where on average P digestibility was reported as 46.9%, which is similar to many of the values in Experiment 4. Increasing CM or Ca: P ratio did not have as great of an effect on P retention values or P digestibility values, and there was not a consistent decrease in P retention in Diets 1-4 which contained the 2:1 Ca: P ratio. The P retention values were decreased in Diets 7 and 8 which retained the highest dietary Ca levels. The latter P retention values are similar to the bioavailability value of 25.6% that can be calculated from the NRC (1994) and from Experiment 1 (Chapter 3) of this thesis (Table 3.6). Retention values from the study conducted by Mutucumarana (2014) were reported to be 48.6% on average. That value is higher than all of the retention values from Experiment 4 which ranged from 20.4%-42.9%.

In summary, the four balance assays evaluated herein yielded similar P digestibility or retention values in some cases but different values in others. The level of CM and P intake also

affected values in some balance assays. The digestibility and retention values obtained in the balance assays were often higher than the relative bioavailability values obtained earlier using the chick-growth tibia ash assay. Thus, P bioavailability values may differ among assays and level of P intake.

LITERATURE CITED

- AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.
- Kim, E. J., P. L. Utterback, and C. M. Parsons. 2011. Development of a precision-fed ileal amino acid digestibility assay using 3-week-old broiler chicks. *Poult. Sci.* 90:396-401.
- NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. *Poult. Sci.* 93:412-419.
- Myers, W. D., P. A. Ludden, V. Nayigihugu and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82: 1: 179-183
- Parsons, C. M. 2002. Digestibility and bioavailability of protein and amino acids. pp 115-135 In: 15 Poultry feedstuffs: Supply, composition, and nutritive value J.M. McNab and K.N. Boorman (ed.). CABI, Oxon, UK.
- Ravindran, V., and W. L. Bryden. 1999. Amino acid availability in poultry – in vitro and in vivo measurements. *Aust. J. Agric. Res.* 50:889-908.

TABLES

Table 4.1. Ingredient composition of Diets 1-3 in Experiment 1 (as-fed basis).

Ingredient, %	Dietary treatments		
	CCM control ¹	CCM control plus phytase	
	1	2	3
Dextrose	15.40	15.40	15.40
Cornstarch	30.81	30.80	30.80
CCM	45.00	45.00	45.00
Soybean oil	5.00	5.00	5.00
Limestone	1.34	1.34	1.34
Dicalcium phosphate	-	-	-
Salt	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15
DL- Met	0.10	0.10	0.10
L-Lys HCL	.013	0.13	0.13
L-Arg	0.15	0.15	
L-Ile	0.15	0.15	0.15
TiO ₂	0.40	0.40	0.40
Choline chloride (60%)	0.10	0.10	0.10
KH ₂ PO ₄	-	-	-
phytase	-	0.006	0.013
Bacitracin-BMD premix	0.040	0.040	0.040

¹The P-deficient conventional canola meal (CCM) control diet was calculated to contain 3,042 kcal of ME/kg, 19% CP, 0.131% available P and 0.78% Ca.

²Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg;
DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic.

Table 4.2. Ingredient composition of experimental diets in Experiment 4 (as-fed basis).

Ingredient, %	Dietary treatments							
	1	2	3	4	5	6	7	8
Dextrose	81.25	67.75	54.25	40.75	80.85	66.95	53.05	39.14
Conventional canola meal	13.50	27.00	40.50	54.00	13.50	27.00	40.50	54.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	-	-	-	-	0.40	0.80	1.20	1.61
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ¹	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ²	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
NaHCO ₃	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
TiO ₂	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analysis ³								
Calcium	0.08	0.17	0.25	0.33	0.23	0.47	0.70	0.94
Nonphytate P	0.04	0.08	0.12	0.16	0.04	0.08	0.12	0.16

Table 4.2. cont.

¹Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL-α-tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

²Provided as milligrams per kilogram of diet: manganese, 75 from MnSO₄·H₂O; iron, 75 from FeSO₄·H₂O; zinc, 75 mg from ZnO; copper, 5 mg from CuO₄·5H₂O; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from Na₂SeO₃.

³The conventional canola meal was analyzed to contain .61% Ca and .29% nonphytate P.

TABLE 4.3. Analyzed nutrient composition of conventional canola meal.

Item	Amount
ME _n ¹ , kcal/kg	2,010
DM, %	88.90
CP, %	40.25
NDF ² , %	18.88
ADF ² , %	14.32
Ca, %	0.60
Total P ² , %	1.20
Available P ³ , %	0.40
Total AA ² , %	
Arg	1.66
His	1.01
Ile	1.57
Leu	2.67
Lys	2.17
Met	0.79
Phe	1.56
Thr	1.66
Trp	0.52
Val	2.02
Cys	1.04
Ileal digestible AA ¹ , %	
Arg	2.34
His	0.80
Ile	1.34
Leu	2.14
Lys	1.75
Met	0.71
Phe	1.17
Thr	1.36
Trp	0.51
Val	1.65
Cys	0.89

¹ME_n value calculated from TME_n determined in conventional roosters in a precision-fed rooster assay. Ileal digestible AA values determined using cecectomized roosters in a precision-fed rooster assay.

²NDF = neutral detergent fiber; ADF= acid detergent fiber; P = phosphorus; AA= amino acids.

³Calculated assuming that 30% of the total P was available.

Table 4.4. Mean ileal P apparent digestibility and retention values for conventional canola meal in Experiment 1.

Dietary treatment	Ileal digestibility (%) ¹	Retention (%) ¹
Basal Diet (B)	38.0 ^a	38.7 ^b
B + 125 FTU/kg ²	44.8 ^a	47.3 ^a
B + 250 FTU/kg ²	47.6 ^a	51.0 ^a
Pooled SEM	3.33	1.82

^{a-b}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹Values are means of 5 pens per dietary treatment with 5 chicks per pen at 21 days of age.

²Level of phytase provided from Optiphos®, Huvepharma, Sofia, Bulgaria.

Table 4.5. Mean ileal apparent P digestibility values determined in chicks tube-fed 6 or 9 g of canola meal in Experiment 2.

Canola meal (g)	Digestibility (%) ¹
6	47.5 ^a
9	40.0 ^a
Pooled SEM	4.25

^aMeans within a column with no common superscript differ significantly ($P < 0.05$).

¹Values are means of 4 pens of 4 chicks at 21 days of age.

Table 4.6. Mean standardized P retention values for roosters tube-fed 8, 16, or 24 g of canola meal in Experiment 3.

Canola meal (g)	Retention (%) ¹
8	35 ^a
16	28 ^{ab}
24	23 ^b
Pooled SEM	3.59

^{a-b}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹Values are means of 5 individually-caged roosters.

Table 4.7 Mean ileal P digestibility and retention values for conventional canola meal in Experiment 4.

Dietary treatment	Ileal digestibility (%) ¹	Retention (%) ¹
1. 13.5% canola meal	58.9 ^a	36.5 ^{ab}
2. 27.0% canola meal	54.9 ^a	39.3 ^a
3. 40.0% canola meal	46.5 ^{ab}	41.1 ^a
4. 54.0% canola meal	37.8 ^{bc}	29.1 ^{bc}
5. As 1 + Ca	30.5 ^c	39.1 ^a
6. As 2 + Ca	30.7 ^{bc}	42.9 ^a
7. As 3 + Ca	7.80 ^d	26.7 ^{cd}
8. As 4 + Ca	11.2 ^d	20.4 ^d
Pooled SEM	5.50	2.98

^{a-b}Means within a column with no common superscript differ significantly ($P < 0.05$).

¹Values are means of 5 pens per dietary treatment with 5 chicks per pen at 21 days of age.

²Diets 1-4 have a Ca: nonphytate P ratio of 2:1 and Diets 5-8 have a Ca: nonphytate P level of 6:1

CHAPTER 5

CONCLUSIONS

The focus of the research in this thesis was to evaluate the bioavailability of P in CM using several different methods. A TCM was found to contain greater CP and less fiber than the CCM, indicating that it may be of higher nutritional value for poultry. The experiments in Chapter 3 of this thesis determined relative bioavailability of P in TCM, CCM, and SBM and the effect of phytase on P bioavailability using a chick-growth tibia ash assay. Little information exists concerning the P digestibility for CM when fed to poultry and the objective of the experiments in Chapter 4 was to evaluate ileal P digestibility and P retention in CCM using 3 different balance assay methods.

From the experiments conducted in Chapter 3, it was concluded that the bioavailability of P in the TCM was statistically equal to or greater than that of the CCM. It was also determined that microbial phytase increased the P bioavailability in both CMs. The effect of phytase on P bioavailability was equal to or greater for TCM than CCM, indicating that the efficacy of phytase for reduced P excretion was not reduced by genetic selection of the TCM for increased CP and reduced fiber.

The experiments conducted in Chapter 4 yielded ileal P digestibility and P retention values that sometimes varied among the 4 methods and also among different dietary levels of CM and P. The 4 balance methods often produced ileal P digestibility and P retention values that were higher than the relative P bioavailability values obtained with the chick-growth tibia ash assay in Chapter 3. The use of a precision-fed rooster assay as a method of determining P retention shows potential but needs to be further studied to determine its accuracy.