

EVALUATION OF HIGH-PROTEIN CANOLA MEALS FED TO GROWING CHICKS AND
WEANLING PIGS

BY

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THESIS

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ABSTRACT

Three experiments were conducted to determine and compare the nutritional value among 2 high-protein canola meals (CMA and CMB), conventional canola meal (CM-CV), and soybean meal (SBM). The objective of Experiment 1 was to determine P bioavailability in the canola meals and SBM relative to KH_2PO_4 when fed to growing chicks and to determine if P bioavailability was increased by addition of microbial phytase to a P-deficient CMA diet. Results indicated that as P level was increased by addition of KH_2PO_4 , CMA, CMB, or SBM, weight gain and tibia ash (mg/tibia and %) were increased linearly ($P < 0.05$). Based on tibia ash %, bioavailabilities of P in CMA, CMB, conventional CM, and SBM relative to KH_2PO_4 were 15, 20, 13, and 42%, respectively. A linear increase ($P < 0.05$) in weight gain and tibia ash was observed with addition of KH_2PO_4 or phytase to the P-deficient CMA diet. The addition of 250 or 500 units/kg microbial phytase to P-deficient CMA diets resulted in approximately 0.13 and 0.18% P being released, respectively, as estimated using the standard curve method. In Experiment 2, the objective was to determine the digestibility of Ca and P in CMA and CMB fed to growing pigs without or with the addition of microbial phytase, and to compare values obtained in high-protein canola meal with digestibility of Ca and P in CM-CV and SBM. Results indicated that apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of P were not different among treatments. Apparent total tract digestibility of Ca was 62, 66, 69, and 73% for CMA, CMB, CM-CV, and SBM, respectively. Standardized total tract digestibility of P was 55, 60, 49, and 66% for CMA, CMB, CM-CV, and SBM, respectively. Inclusion of phytase to the diets reduced both Ca and P outputs ($P < 0.05$). Inclusion of phytase also improved ($P < 0.05$) ATTD of Ca and P and STTD of P, regardless of the ingredient in the diet, and there was no interaction between diet and phytase supplementation.

In Experiment 3, the objective was to evaluate effects of graded inclusion levels of CMA, CMB, and CM-CV on growth performance, organ weights, bone ash, and blood characteristics of weanling pigs. Results indicated that ADFI was linearly ($P < 0.05$) decreased if inclusion of CMA, CMB, or CM-CV increased. Average daily gain of pigs fed CMA tended to increase quadratically, with the maximum response observed if 10 or 20% canola meal was included in the diet ($P = 0.06$). However, G:F was linearly ($P < 0.05$) increased by adding CMA or CM-CV to the diets. Liver weights were also linearly ($P < 0.05$) increased if pigs were fed diets containing CMB, but kidney weights were linearly ($P < 0.05$) decreased by adding CM-CV to the diets. Thyroid gland weights increased linearly ($P < 0.05$) for pigs fed diets containing CMA. Addition of any of the 3 canola meals linearly ($P < 0.05$) increased bone ash percentage in the metacarpals. Inclusion of CMA or CM-CV linearly ($P < 0.05$) decreased serum triiodothyronine, and the inclusion of CMA also linearly ($P < 0.05$) decreased serum thyroxine in weanling pigs. In conclusion, CMA and CMB contained a numerically higher concentration of bioavailable P when fed to chicks than the CM-CV, and bioavailability was numerically increased with addition of microbial phytase. In contrast, there were no differences observed for ATTD of Ca or P or for STTD of P when the canola meals or SBM were fed to growing pigs. Furthermore, inclusion of any of the 3 canola meals up to 20% in diets for weanling pigs did not reduce growth performance or negatively affect organ, bone, or blood characteristics.

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TABLE OF CONTENTS

LIST OF TABLES	vii
CHAPTER 1	1
INTRODUCTION	1
LITERATURE CITED	3
CHAPTER 2 THE NUTRITIONAL VALUE OF CONVENTIONAL AND HIGH-PROTEIN CANOLA MEAL FED TO PIGS OR POULTRY: A LITERATURE REVIEW	4
INTRODUCTION	4
HIGH-PROTEIN CANOLA MEAL FED TO BROILER CHICKENS	7
HIGH-PROTEIN CANOLA MEAL FED TO PIGS	8
PHOSPHORUS IN CANOLA MEAL	9
CONCLUSIONS	12
LITERATURE CITED	13
TABLES	17
CHAPTER 3 PHOSPHORUS BIOAVAILABILITY IN HIGH-PROTEIN CANOLA MEALS, CONVENTIONAL CANOLA MEAL, AND SOYBEAN MEAL FED TO CHICKS	19
ABSTRACT	19
INTRODUCTION	20
MATERIALS AND METHODS	21
RESULTS AND DISCUSSION	24
LITERATURE CITED	29
TABLES	32

CHAPTER 4	PHOSPHORUS DIGESTIBILITY IN HIGH-PROTEIN CANOLA MEALS, CONVENTIONAL CANOLA MEAL, AND SOYBEAN MEAL FED TO GROWING PIGS .	42
	ABSTRACT.....	42
	INTRODUCTION	43
	MATERIALS AND METHODS.....	44
	RESULTS	47
	DISCUSSION.....	47
	LITERATURE CITED.....	51
	TABLES	54
CHAPTER 5	EFFECTS OF HIGH-PROTEIN OR CONVENTIONAL CANOLA MEAL ON GROWTH PERFORMANCE, ORGAN WEIGHTS, BONE ASH, AND BLOOD CHARACTERISTICS OF WEANLING PIGS.....	60
	ABSTRACT.....	60
	INTRODUCTION	61
	MATERIALS AND METHODS.....	62
	RESULTS	65
	DISCUSSION.....	67
	LITERATURE CITED.....	72
	TABLES	76
CHAPTER 6	89
	CONCLUSIONS	89

LIST OF TABLES

Table 2.1. Chemical composition of meals derived from black- and yellow- seeded canola.....	17
Table 2.2. Amino acid composition of black- and yellow- seeded canola meals	18
Table 3.1. Ingredient composition of experimental diets.....	32
Table 3.2. Composition of P-deficient-canola meal A control diet	34
Table 3.3. Analyzed nutrient composition of ingredients	36
Table 3.4. Growth performance from d 8-21 of age and tibia ash content of chicks.....	38
Table 3.5. Relative bioavailability of the P in canola meals and soybean meal	40
Table 3.6. Amount of phosphorus released from Canola meal A by phytase enzyme	41
Table 4.1. Analyzed nutrient composition of ingredients	54
Table 4.2. Ingredient composition of experimental diets.....	56
Table 4.3. Analyzed nutrient composition of experimental diets	58
Table 4.4. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of P in 2 sources of high-protein canola meal (CMA and CMB), in conventional canola meal (CM-CV), and in soybean meal (SBM) without or with microbial phytase	59
Table 5.1. Analyzed nutrient composition of ingredients	76
Table 5.2. Analyzed glucosinolate composition of ingredients	78
Table 5.3. Ingredient composition of experimental diets.....	79
Table 5.4. Analyzed nutrient composition of experimental diets	81
Table 5.5. Growth performance of weanling pigs fed diets containing graded inclusion levels of 3 different canola meals	84
Table 5.6. Organ, bone, and blood characteristics of weanling pigs fed diets containing graded inclusion levels of 3 different canola meals.....	85
Table 5.7. Complete blood count for weanling pigs fed diets containing graded inclusion levels of 3 different canola meals.....	87

CHAPTER 1

INTRODUCTION

The term “canola” is used to define a variety of rapeseed that is low in glucosinolates and erucic acid (Khajali and Slominski, 2012). Canola seeds are small and round and can vary in size and shape. They are produced by a yellow-flowering plant that grows in northern areas where soybeans do not mature very rapidly. In terms of production, canola is the second largest oilseed crop after soybeans (USDA, 2012), but has a greater and healthier oil content, making it more desirable for human consumption (Daun, 2011). After extraction of the oil for the food industry, the leftover meal is used as a feed ingredient for livestock. Canola meal has traditionally been fed to ruminants due to the high fiber content, which is poorly digested by pigs and poultry. Additionally, although canola meal typically contains more total P than soybean meal, a higher proportion of the total P in canola meal is bound to phytate (NRC, 2012), resulting in decreased availability and increased excretion. In addition to fiber and phytate, canola contains several other anti-nutritional factors that make it less desirable than soybean meal for pigs and poultry. These anti-nutrients include glucosinolates, erucic acid, tannins, and sinapine which may reduce feed intake as well as digestibility of nutrients (Bell, 1993).

In response to the limitations of feeding canola meal, canola breeding programs have aimed at reducing these anti-nutritional factors as well as increasing the protein content to better rival that of soybean meal (**SBM**) (Khajali and Slominski, 2012). Canola meal is already required to have less than 2% erucic acid and less than 30 $\mu\text{mol/g}$ glucosinolates to use the name “canola” (Bell, 1993). However, further reductions in glucosinolates as well as fiber are beneficial for livestock, especially pigs and poultry. Supplementation of phytase to diets

containing canola meal has resulted in improved P digestibility as well as decreased P output (Simons et al., 1990; Selle and Ravindran, 2007; Akinmusire and Adeola, 2009). Additionally, an increase in the protein content of canola meal (38% CP) would make it more competitive with dehulled SBM (48% CP) in the feeding of pigs and poultry.

New, yellow-seeded varieties of canola have a larger seed with a thinner hull when compared to black-seeded varieties (Thacker, 1990; Slominski et al., 1994; Khajali and Slominski, 2012). This thinner hull contributes less fiber to the total seed than the traditional black-seeded varieties, resulting in a meal with lower fiber content. In addition, the embryo makes up a larger proportion of the total seed, providing greater protein content. Because of these changes in composition, it is believed that meal produced from yellow-seeded canola has a greater nutritional value than meal produced from the black-seeded varieties. However, research is needed to determine if yellow-seeded varieties have improved nutritional value if fed to pigs or poultry compared with the black-seeded varieties.

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CHAPTER 2

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

INTRODUCTION

Rapeseed is an oilseed of the *Brassica* species that has been grown for centuries in many different parts of the world. Through the years, plant breeders have been able to improve the nutritional quality of rapeseed so that it is more valuable as a feed ingredient to livestock, in part by selecting against anti-nutritional factors. Rapeseed (*Brassica rapa*) traditionally contained 25 to 45% erucic acid and 50 to 100 $\mu\text{mol/g}$ glucosinolates (Bell, 1993). Erucic acid causes fatty deposits on the heart and skeletal muscle as well as growth retardation (Przybylski and Eskin, 2011). Glucosinolates are not a problem themselves, but myrosinase, an enzyme present in rapeseed, degrades the glucosinolates into toxic metabolites (Fenwick and Curtis, 1980). In pigs and poultry, these metabolites may cause reduced feed intake, thyroid enlargement, decreased thyroid hormone production, liver enlargement, liver hemorrhages, kidney enlargement, taint in brown eggs, and perosis in chicks (Fenwick and Curtis, 1980; Bell, 1993). Therefore, rapeseed meal has traditionally only been fed to ruminant animals, as they are less sensitive to glucosinolates (Burel et al., 2000). Through plant breeding, erucic acid levels have been brought below 2% and glucosinolate levels below 30 $\mu\text{mol/g}$, resulting in “double-low” rapeseed. These “double-low” varieties are called “canola” in North America (Bell, 1993).

In addition to erucic acid and glucosinolates, canola contains other anti-nutritional factors such as tannins, sinapine, phytic acid, and fiber (Bell, 1993; Khajali and Slominski, 2012). Tannins, which are present in the hull portion of canola, decrease energy digestibility and may

form complexes with protein, decreasing the digestibility of protein in the diet (Thacker, 1990; Khajali and Slominski, 2012). Making up 1.5 to 3% of canola meal (Bell, 1993), tannins give the meal its dark color and 70 to 96% of the tannins in canola meal are insoluble (Khajali and Slominski, 2012). Mansoori and Acamovic (2007) observed increased endogenous losses of Met, Lys, and His following dietary addition of water-soluble tannins, or tannic acid. Because Met and Lys are the first limiting AA for pigs and poultry, the presence of tannins in canola meal prevents use of high inclusions of canola meal in diets for these animals. Sinapine resides in the embryo of the canola seed and causes a bitter taste which decreases feed intake when high levels of canola meal are fed (Thacker, 1990). Canola meal can contain 0.6 to 1.8% sinapine (Bell, 1993). Research has also shown that the presence of sinapine at high levels causes a fishy taint in the eggs of brown egg layers. This is a result of the hens lacking the enzyme trimethylamine oxidase, rendering them unable to handle the high amounts of choline produced from hydrolysis of sinapine. The enzyme deficiency causes a buildup of trimethylamine, which is then transferred to the eggs (Bell, 1993; Khajali and Slominski, 2012).

Phytic acid, or phytate, is the primary storage form of P, binding it and reducing P absorption and utilization by pigs and poultry (Nwokolo and Bragg, 1977; Khajali and Slominski, 2012). Phytate also binds Ca, Fe, K, Mn, Mg, and Zn and reduces the availability of these minerals (Nwokolo and Bragg, 1977). Phytate may also bind free Lys as well as protein, forming phytate-protein complexes that reduce protein digestion (Kies et al., 2006). Addition of microbial phytase to diets hydrolyzes some of these complexes, resulting in more available P and Ca for the animal (Simons et al., 1990; Selle et al., 2009; González-Vega et al., 2013). Protein and minerals other than Ca and P that are bound by phytate may also be made more available by the addition of exogenous phytase. Canola meal, unlike soybean meal (**SBM**), contains all the

hulls from the seeds, resulting in greater fiber content than SBM. Fiber, in addition to being poorly digested by pigs and poultry, binds minerals such as P, Ca, Mg, Mn, Zn, and Cu and also decreases the energy value of the meal (Nwokolo and Bragg, 1977; Bell, 1993).

Canola contains 44 to 50% oil, of which 97 to 99% is removed by prepress solvent extraction (Barthet and Daun, 2011) to make the meal. This oil is marketed in the food industry as the vegetable oil with the lowest saturated fatty acid content, containing only approximately 50% of the amount of saturated fatty acids in corn and soybean oil (Aukema and Campbell, 2011). Canola meal contains 36 to 44% CP (Akinmusire and Adeola, 2009; Barthet and Daun, 2011; Khajali and Slominski, 2012; Slominski et al., 2012; González-Vega et al., 2013; Rodríguez et al., 2013). The concentration of NDF in canola meal is 22 to 26% compared with 8 to 12% in SBM (Slominski et al., 1994; Khajali and Slominski, 2012; NRC, 2012).

Canola breeding programs have produced yellow-seeded varieties of canola that are nutritionally superior to conventional black-seeded varieties. These yellow-seeded varieties have increased CP and decreased fiber because yellow seeds are larger and have a thinner hull than brown seeds (Downey and Bell, 1990; Thacker, 1990; Khajali and Slominski, 2012; Table 2.1). The larger seed contributes proportionally more embryo to the whole seed, which is where most of the protein resides. As a consequence, canola meal derived from yellow-seeded canola contains 46 to 50% CP (Simbaya et al., 1995; Slominski et al., 2012) and also contains greater AA concentrations than black-seeded canola meal (Slominski et al., 2012; Table 2.2). The thinner hull also contributes proportionally less of the whole seed, resulting in less dietary fiber in the meal from yellow-seeded varieties (Khajali and Slominski, 2012). Therefore, dietary fiber concentration of canola meal from yellow-seeded canola is 24 to 27% compared with 30 to 34% dietary fiber in canola meal from black-seeded varieties of canola (Slominski et al., 1994;

Simbaya et al., 1995; Slominski et al., 1999; Slominski et al., 2012). The yellow-seeded canola meal contains less oil than conventional canola meal (Simbaya et al., 1995; Slominski et al., 1999; Slominski et al., 2012). Yellow-seeded canola meal also contains approximately 10 $\mu\text{mol/g}$ less glucosinolates than black-seeded canola meal (Daun and DeClercq, 1988; Slominski et al., 2012). The changed nutrient composition of canola meal from yellow-seeded canola compared with meal from black-seeded canola makes it necessary to conduct research on the value of yellow-seeded canola meal as a feed ingredient in diets fed to pigs or poultry.

HIGH-PROTEIN CANOLA MEAL FED TO BROILER CHICKENS

Slominski et al. (1999) compared brown- and yellow-seeded varieties of *Brassica napus* in a growth trial from 4 to 18 d of age. The CP content of the yellow-seeded canola meal was 0.8% greater than in the brown-seeded meal and the yellow-seeded meal contained 5.9% less dietary fiber and 13% less fat than the brown-seeded meal. Glucosinolate concentration in the 2 meals was not different. The brown and yellow-seeded canola meals were included in experimental diets at 29.7 and 29.5%, respectively. Weight gain of broilers fed the 2 canola meal varieties was not different, however, the feed to gain ratio was improved for birds fed diets containing the yellow-seeded canola meal. Data for feed intake were not reported, but presumably, feed intake was less for birds fed diets containing the yellow-seeded canola meal. Slominski et al. (1999) concluded that the yellow-seeded *Brassica napus* was superior to the black-seeded canola meal of the same species.

Jia et al. (2012) conducted a similar experiment to compare the effects of yellow- or black-seeded *B. napus* on the growth performance of broilers from 3 to 17 d of age. The yellow-seeded canola meal contained 6% more CP and 6% less fiber, 0.06% less P, and 10% less

glucosinolates than the black-seeded canola meal (Slominski et al., 2012). No differences were observed for BW gain, feed intake, or feed:gain. The greatest digestibility values for total and indispensable AA were for diets containing the yellow-seeded canola meal, indicating that protein from the yellow-seeded canola meal is more digestible than protein from the black-seeded meal. Additionally, the yellow-seeded canola meal contained the greatest amount of ME. It was concluded that the yellow-seeded *B. napus* had superior quality characteristics in terms of available energy and AA content, but it is not clear why broilers fed the yellow-seeded canola meal did not out-perform broilers fed black-seeded canola meal.

HIGH-PROTEIN CANOLA MEAL FED TO PIGS

Montoya and Leterme (2009) conducted an experiment to compare the DE and NE in yellow- and black-seeded canola meals fed to growing pigs. The yellow-seeded *B. napus* contained 5.2% more CP and 4.9% less NDF, 6.4% less ADF, and 3.3% less lignin than black-seeded canola meal. Pigs fed diets containing the yellow-seeded canola meal had 2-4% greater digestibility of DM and 2-5% greater digestibility of GE and the yellow-seeded canola meal contained more DE and NE than the black-seeded canola meal.

Trindade Neto et al. (2012) conducted an experiment to determine the apparent and standardized ileal digestibility of protein and AA in yellow- and black-seeded canola meals fed to pigs. The yellow-seeded *B. napus* contained 5% more CP and 5.5% less total dietary fiber than the black seeded *B. napus*. However, there were no differences for apparent or standardized ileal digestibility of CP or AA between the 2 meals. Sanjayan (2013) conducted a similar experiment; however, the yellow-seeded *B. napus* contained 1.7% less CP, 0.6% less fat, and 3.8% less glucosinolates, but 3.7% more fiber than black-seeded canola meal, which is contradictory to the

composition of meals used in the previous experiment. The author attributed these differences to cultivar and processing condition. Therefore, growing pigs fed diets containing the yellow-seeded canola meal had 7.9% lower apparent and 6.5% lower standardized ileal digestibility values of protein as well as lower apparent and standardized ileal digestibility values of Lys, Met, Thr, Val, Ala, Asp, Cys, Gly, and Ser than pigs fed diets containing the black-seeded canola meal.

PHOSPHORUS IN CANOLA MEAL

Conventional black-seeded canola meal contains 1.00 to 1.08% P (Bell, 1993; Khajali and Slominski, 2012; NRC, 2012; González-Vega et al., 2013; Rodríguez et al., 2013). Of this, about two-thirds is bound to phytate (Simons et al., 1990), making it largely unavailable to pigs and poultry, which lack sufficient endogenous phytase to completely hydrolyze the phytate-P complexes (Selle and Ravindran, 2007; Akinmusire and Adeola, 2009). There are few published values for the P content of high-protein canola meal from yellow-seeded varieties of canola. Slominski et al. (2012) reported a P content of 1.30% for black-seeded canola meal, a much greater value than reported by others, and 1.24% for yellow-seeded canola meal. Whereas the phytate-bound P concentration was similar between the 2 canola meals, the concentration of non-phytate-bound P was less in the yellow-seeded canola meal (Slominski et al., 2012). This indicates that P digestibility may be less in the yellow-seeded canola meal than in the black-seeded canola meal.

Phosphorus Digestibility by Broilers

Mutucumarana et al. (2014) conducted an experiment to determine P availability of canola meal when fed to broiler chickens. Diets were formulated to contain graded

concentrations of total P, where canola meal was the only source of P in the diet. Total tract digestibility of P and true ileal digestibility of P were calculated. The apparent ileal P digestibility of canola meal was not different among different dietary P concentrations and the coefficient for P retention decreased from 0.697 to 0.539 with increasing P concentrations. There was a difference in P output between the excreta and ileal contents, indicating that there was post-ileal absorption of P. Phytate-P hydrolysis in canola meal was 25.2%, but the authors noted that utilization of phytate-P in chickens can vary widely and can be influenced by dietary factors such as low Ca concentrations which were present in these diets.

There are few other articles discussing P digestibility of canola meal in broiler chickens. However, dietary addition of phytase increases bioavailable P and reduces P excretion (Selle and Ravindran, 2007). Simons et al. (1990) conducted an experiment to evaluate the effect of phytase on phosphorus availability to broilers. Addition of phytase to maize-sorghum-SBM diets increased the availability of P to over 60% and decreased the amount of P in the excreta by 50%. Similarly, Paik (2003) conducted multiple broiler trials to determine the effect of phytase supplementation on P excretion. By supplementing phytase to corn-SBM diets, levels of non-phytate-P in the diets were decreased 0.2% without affecting growth performance. Phytase also increased availability of P by up to 14.8% and availability of some other minerals by up to 30% and decreased P excretion by up to 60%. Zyla and Koreleski (1993) also observed the positive effects of dietary phytase addition, allowing for elimination of inorganic phosphate from formulation of diets containing rapeseed meal with high levels of phytate P. Sebastian et al. (1996) observed increased retention of P, Ca, Cu, and Zn (12.4, 12.2, 19.3, and 62.3%, respectively) when phytase was added to a low-P corn-SBM diet as well as 13.2% increased BW

gain. More research is needed to determine the effect of microbial phytase on the P availability of canola meal when fed to broiler chickens.

Phosphorus Digestibility by Pigs

Akinmusire and Adeola (2009) conducted an experiment with growing pigs to estimate the true P digestibility of canola meal or SBM, where the canola meal or SBM was the only source of dietary P. Apparent total tract digestibility (ATTD) of P from canola meal ranged from 26 to 33%, depending on the dietary inclusion level of canola meal. The ATTD of P from soybean meal was 34 to 38%, despite the SBM having much less total P than the canola meal. There was also greater P output from pigs fed diets containing canola meal compared with those fed diets containing SBM. The addition of microbial phytase to diets containing phytate-bound P usually improves P digestibility and decreases P output (Simons et al., 1990; Kies et al., 2006), and inclusion of phytase to diets containing canola meal and fed to pigs, increases true P digestibility and decreases total P output (Akinmusire and Adeola, 2009; González-Vega et al., 2013; Rodríguez et al., 2013).

Addition of phytase to diets containing canola meal can increase ATTD of Ca by hydrolyzing phytate-Ca bonds. González-Vega et al. (2013) conducted an experiment feeding 4 different levels of canola meal without or with microbial phytase to weaned barrows. Diets were formulated to contain 0.32% standardized total tract digestible P and increasing levels of Ca. Inclusion of microbial phytase to the diets increased ATTD of P as well as ATTD of Ca. Likewise, Rodríguez et al. (2013) conducted an experiment feeding diets containing 1 of 7 different ingredients, including canola meal, without or with microbial phytase to growing barrows. Inclusion of phytase to diets containing any of the ingredients except de-hulled sunflower meal increased ATTD of P by anywhere from 15 to 22 percentage units and increased

standardized total tract digestibility (STTD) of P by 15 to 25 percentage units. Phytase inclusion decreased P output from the canola meal diet by 32%. Addition of phytase to diets containing canola seeds, canola meal, sunflower seeds, or sunflower meal increased Ca digestibility by 11 to 19 percentage units.

CONCLUSIONS

High-protein, yellow-seeded canola meal has a higher concentration of CP and decreased concentration of fiber and glucosinolates compared with conventional canola meal. This indicates improved feeding value for pigs and poultry. Canola meal contains more P than SBM, but a greater proportion of the P is bound to phytate, limiting P digestibility and absorption. Yellow-seeded canola meal also contains more phytate-bound P than the black-seeded varieties, indicating reduced P digestibility. However, the inclusion of microbial phytase to corn-SBM diets and diets containing canola meal for pigs and poultry results in increased hydrolysis of phytate-P complexes and improves P absorption. Improved P digestibility and decreased P excretion in canola meal diets supplemented with microbial phytase for pigs have been documented. Whereas no research has been conducted to determine P availability in high-protein canola meal for poultry, it has been documented that microbial phytase inclusion in both corn-SBM and rapeseed meal diets for broiler chickens results in increased digestibility of P and other minerals. More research is needed to determine the effect of microbial phytase on P availability in canola meal when fed to broilers and to further evaluate its effect on P availability of canola meal when fed to pigs. Research is also needed to evaluate the effect of feeding high-protein canola meal compared with conventional canola meal on growth performance, organ, bone, and blood characteristics of pigs.

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TABLES

Table 2.1. Chemical composition of meals derived from black- and yellow- seeded canola¹

Item (%)	Black	Yellow
DM	91.28	90.90
CP	42.73	47.82
Fat	3.55	2.80
Ash	7.02	7.07
Phosphorus	1.19	1.24
Dietary Fiber	30.32	25.25
NDF	22.95	17.20
Glucosinolates ($\mu\text{mol/g}$)	19.45	14.25

¹Average values calculated from Slominski et al. (1994); Simbaya et al. (1995);

Slominski et al. (1999); NRC (2012); Slominski et al. (2012); and Trindade Neto et al. (2012).

Table 2.2. Indispensable AA composition of black- and yellow- seeded canola meals¹

Item (%)	Black	Yellow
Arginine	5.7 ^b	6.3 ^a
Isoleucine	3.9	4.1
Leucine	6.9	7.2
Lysine	6.1 ^a	5.7 ^b
Methionine	2.1 ^a	1.9 ^b
Methionine + Cysteine	4.5 ^a	4.2 ^b
Phenylalanine	4.0 ^b	4.2 ^a
Threonine	4.3	4.3
Tryptophan	0.9	0.8
Valine	4.5	4.8
Total AA	92.4 ^b	95.0 ^a

¹Adapted from Slominski et al. (2012).

CHAPTER 3

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

ABSTRACT

An experiment was conducted to evaluate two high protein canola meals (CMA and CMB), a conventional canola meal (CM), and a control soybean meal (SBM). For determination of P bioavailability in the CM and SBM, a phosphorus-deficient cornstarch-dextrose-SBM basal diet was fed as Diet 1. Diets 2 and 3 had 0.05 and 0.10% P added from KH_2PO_4 , respectively. Diets 4-11 had 12.5 and 25% added CM from each of the 3 different sources or the SBM, added in place of cornstarch and dextrose. Diets 12-16 were used to evaluate the effect of phytase enzyme on bioavailability of the P in CMA. Diet 12 was a P-deficient CMA-cornstarch-dextrose diet, with CMA as the only source of dietary P. Diets 13 and 14 had 0.05 and 0.10% P added from KH_2PO_4 , respectively. Diets 15 and 16 were the same as Diet 12 with 250 and 500 units phytase added per kg of diet, respectively. A total of 320 New Hampshire \times Columbian male chicks were weighed, wing banded, and allotted to the 16 dietary treatments via a completely randomized design, so that each pen had a similar mean initial body weight. There were 5 chicks per pen and 4 replicate pens per treatment. Chicks were fed the experimental diets from 8 to 21 d posthatch and bioavailability of P was estimated using the slope ratio method where tibia ash was regressed on P intake. The total P content of CMA, CMB, conventional CM, and SBM was 1.26, 1.16, 1.16, and 0.57%, respectively. A linear increase in weight gain and tibia ash (mg/tibia and %) was observed as the P level was increased by addition of KH_2PO_4 , CMA, CMB, or SBM. Based on tibia ash %, bioavailabilities of P in CMA, CMB, conventional CM, and SBM relative to

KH_2PO_4 were 15, 20, 13, and 42%, respectively. A linear increase in weight gain and tibia ash was observed with addition of KH_2PO_4 or phytase to the P-deficient CMA diet (Diets 12-14). The addition of 250 or 500 units/kg microbial phytase to P-deficient CMA diets resulted in approximately 0.13 and 0.18% P being released, respectively, as estimated using the standard curve method. In conclusion, the high-protein CMA and CMB contained a numerically higher concentration of bioavailable P than the conventional CM. Furthermore, microbial phytase can greatly increase the bioavailability of P in the new high protein CM.

Key words: bone ash, canola meal, chick, digestibility, phosphorus

INTRODUCTION

Canola meal (**CM**) inclusion has usually been limited in the past in diets for poultry. The major causes for this are the high levels of anti-nutritional factors present, mainly glucosinolates and fiber, which reduce the value of the meal for monogastric animals. The presence of glucosinolates has been shown to result in reduced feed intake, reduced growth, thyroid enlargement, perosis, and mortality in broilers (Fenwick and Curtis, 1980; Khajali and Slominski, 2012). High fiber content is known to decrease the energy value of the feedstuff and also decrease the digestibility of some minerals (Nwokolo and Bragg, 1977; Bell, 1993). Breeding programs to reduce these factors have resulted in yellow-seeded varieties of canola (*Brassica napus*). These yellow-seeded varieties contain lower levels of glucosinolates and fiber as well as having greater protein content (Simbaya et al., 1995; Slominski et al., 1999; Slominski et al., 2012). The larger seed of the yellow-seeded varieties has a thinner hull that is proportionally less of the whole seed than in the traditional dark-seeded varieties (Khajali and Slominski, 2012). Although the yellow-seeded varieties of high-protein canola have been

reported to contain less P than conventional varieties (Slominski et al., 2012), the reduction in fiber content may result in improved digestibility of P for chicks.

About two-thirds of the P in plant products is bound to phytate (Simons et al., 1990), rendering it largely unavailable to pigs and chickens. Poultry do not possess enough natural phytase to be able to degrade the phytate-P complexes (Selle and Ravindran, 2007), so exogenous phytase may be added to diets to increase digestibility of P as well as other minerals (Simons et al., 1990; Selle and Ravindran, 2007). Therefore, the objective of this study was to determine the bioavailable P content of 2 new high-protein canola meals compared to conventional canola meal (**CM-CV**) and soybean meal (**SBM**) and to evaluate the effect of phytase enzyme on bioavailability of P in one of the high-protein CM.

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

Ingredients were analyzed for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL), 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 acid hydrolyzed ether extract (Method 2003.06, AOAC International, 2007) on an automated analyzer (Soxtec 2050; FOSS North America, Eden Prairie, MN), ADF (Method 973.18, AOAC International, 2007), NDF (Holst, 1973), and amino acids (Method 982.30 E [a, b, and c]; AOAC International, 2007). Ingredient samples were also analyzed for DM by forced air oven drying for 2 h at 135°C

(Method 930.15; AOAC International, 2007), Ca and P 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) and phytate concentration (Ellis et al., 1977). The concentration of phytate-bound P in each ingredient was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004). Non-phytate-bound P was calculated as the difference between total P and phytate-bound P.

Diets and Experimental Design

Two sources of CM produced from new varieties of high-protein canola seeds (**CMA** and **CMB**), a CM-CV, and a control SBM were evaluated (Table 3.3). For determination of P bioavailability in the CM and SBM, a phosphorus-deficient cornstarch-dextrose-SBM basal diet was fed as Diet 1 (Table 3.1). Diets 2 and 3 had 0.05 and 0.10% P added from KH_2PO_4 , respectively. Diets 4-11 had 12.5 and 25% added CM from each of the 3 different sources, or the SBM, added in place of cornstarch and dextrose. Diets 12-16 were used to evaluate the effect of phytase enzyme on bioavailability of the P in CMA. Diet 12 was a P-deficient CMA-cornstarch-dextrose diet in which the only source of dietary P was CMA (Table 3.2). Diets 13 and 14 had 0.05 and 0.10% P added from KH_2PO_4 , respectively. Diets 15 and 16 were the same as Diet 12 with 250 and 500 units phytase added per kg of diets, respectively.

A total of 320 New Hampshire \times Columbian male chicks were fed a nutritionally complete corn and SBM starter diet for 7 d. On d 7 of age, chicks were fasted overnight prior to being placed on experiment. At the initiation of the experiment, chicks were weighed, wing banded, and allotted to the 16 dietary treatments via a complete randomized design so that each pen had a similar mean initial body weight. There were 5 chicks per pen and 4 replicate pens per treatment. During the experiment, chicks were housed in thermostatically-controlled Petersime

starter batteries with raised-wire flooring in an environmentally controlled room with continuous lighting. Experimental diets and water were available free-access to chicks from 8 to 21 d of age. Feed intake per pen was recorded and final BW of each chick was recorded at the conclusion of the experiment.

At the conclusion of the experiment, data were summarized to calculate weight gain, feed intake, and gain:feed ratio. Chicks were euthanized via CO₂ inhalation and right tibia bones were collected and pooled within pen. Bones were autoclaved and cheesecloth was used to aid in removal of adhering tissue. Bones were dried for 24 h at 100°C, weighed, and then dry-ashed in a muffle furnace for 24 h at 600°C. Ash weight was expressed as a percentage of dry bone weight (Chung and Baker, 1990) and as milligrams per tibia.

Statistical Analysis

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. Data for Dietary Treatments 1-11 were analyzed by multiple linear regression (GLM procedure of SAS) 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. Bioavailability of P in CM or SBM relative to KH₂PO₄ was then estimated using the slope-ratio method (Finney, 1978). For dietary treatments 12-14, a standard curve was calculated using simple linear regression to estimate the amount of P released from CMA by phytase by regressing tibia ash (mg/tibia) and tibia ash percent on supplemental P intake (mg/chick) from KH₂PO₄. The tibia ash values (mg/tibia and percent) for the two supplemental phytase treatments (dietary treatments 15 and 16) were then substituted into the

respective regression equation for Y. Solving for X gave an estimate of the amount of P released from the CMA by the phytase enzyme.

RESULTS AND DISCUSSION

Nutrient Composition

Nutrient compositions of the CM and SBM are presented in Tables 3.3. As expected, the CP content of CMA and CMB was higher than for the CM-CV, with a difference of over 10 percentage units (as-fed basis). The CP and AA values for the CM-CV were lower than previously published values (NRC, 2012). The CP content of CMA and CMB was higher than values published in NRC (2012), but in agreement with previous research using yellow-seeded CM (Simbaya et al., 1995; Slominski et al., 2012). Due to the increase in CP, CMA and CMB also had higher levels of all AA than the CM-CV. Concentrations of all AA were greater in CMA and CMB than values for CM in NRC (2012). The concentration of CP in CMA and CMB was similar to the SBM sample, which was close to published values (Khajali and Slominski, 2012; NRC, 2012). Though the SBM had higher levels of several AA than the high-protein CM, similar levels of His, Thr, and Trp were seen among the samples, while CMA and CMB had higher levels of Gly, Pro, Cys, and Met when compared to SBM. The gross energy content of the high-protein CM was 230 kcal/kg greater than the conventional CM and 200 kcal/kg greater than the SBM sample (as-fed basis). The concentrations of NDF and ADF were 18.32 and 12.66% in CMA and 17.90 and 10.95% in CMB. These levels were substantially less than 25.04% NDF and 17.53% ADF in the CM-CV, but all CM samples had higher levels of NDF and ADF than SBM. Values for NDF of all 3 CM were in agreement with those published by Slominski et al. (1994);

however, values for ADF and NDF of the CM-CV were slightly higher than those reported in NRC (2012).

Canola meal A had a higher concentration of P than the CM-CV, but CMB and the CM-CV had similar levels of P (Table 3.3). All three CM samples had more P than previously reported (NRC, 2012) for brown-seeded CM and more than twice the amount present in SBM. The P content in the CM-CV was lower than reported by Nwokolo and Bragg (1977) and Slominski et al. (2012), but higher than reported by Khajali and Slominski (2012). In accordance with Raboy (1997) who reported that 65 to 85% of the total P of plant origin is bound to phytate, CMA and CMB had 80 and 78% of the total P as phytate-bound P, respectively. The CM-CV had 66% and the SBM had 70% of the total P bound to phytate, which is in agreement with previous research by Selle and Ravindran (2007) and Slominski et al. (2012). The lower concentration of phytate P in the CM-CV compared to CMA and CMB suggests that P digestibility may be higher in CM-CV. The high-protein CM were obtained from a different source than the CM-CV so differences in soil composition as well as growing conditions could account for the increased phytate P concentration in the high-protein meals. The CM-CV also had a much greater Ca concentration than either CMA or CMB, with SBM having the lowest Ca concentration overall. Values for Ca concentration for CMA and SBM were similar to published values (Bell, 1993; Khajali and Slominski, 2012), but the Ca concentration in CMB was lower and in CM-CV was higher than the previously published values.

Phosphorus Bioavailability in CM and SBM

Feed intake generally only increased with the upper inclusions of KH_2PO_4 or CM ($P < 0.05$) (Table 3.4). There were no consistent effects of dietary treatment on feed conversion. A linear increase in weight gain and tibia ash (mg/tibia and %) was observed as the P level was

increased by addition of KH_2PO_4 . This agrees with previous research by Kim et al. (2008). There was also a linear increase in weight gain and tibia ash (mg/tibia and %) observed as the P level was increased by addition of CMA, CMB, or SBM. Multiple regressions of tibia ash (mg/tibia and %) on supplemental P intake were highly significant (R^2 values were 0.80 and 0.84, respectively; $P < 0.001$). Total P content and estimated P bioavailability values (relative to KH_2PO_4) are shown in Table 3.5. The bioavailability values estimated were greater for CMA and CMB than for the CM-CV, but were lower than for SBM. However, when the bioavailability values are multiplied by the total P content, the concentration of bioavailable P in CMA and CMB was comparable to SBM. The latter is due to the lower total P content of SBM. There was numerically greater bioavailable P content for CMA and CMB than for the CM-CV due to the lower P bioavailability value for the CM-CV. Although CMA and CMB had less nonphytate-bound P than the CM-CV (Table 3.3), their higher bioavailability values led to numerically increased bioavailable P content when compared to the CM-CV.

Dividing the non-phytate-bound P content by the total P content (analytical values provided) resulted in expected values for bioavailability of P in each of the P sources. The expected bioavailability of P based on these calculations in CM from the NRC (2012) is 40%. The expected P bioavailability value calculated for CMA and CMB was about 20%, whereas for CM-CV, the expected value was about 34%. The P bioavailability values for CMA and CMB were close to the expected values, particularly for bone ash %, but the estimated values of 10 to 13% for the CM-CV were substantially lower than expected from the analytical values obtained herein and the NRC (2012) values. The reason for these discrepancies is unknown. Perhaps, part of the difference is due to errors in phytate analyses or to the higher fiber content of the CM-CV (Slominski et al., 2012).

Phytase Effect

A significant increase in weight gain and tibia ash was observed with addition of KH_2PO_4 or phytase to the Basal 2 diet containing CMA as the only source of dietary P (Table 3.4). This is in agreement with previous findings (Nelson et al., 1971; Mitchell and Edwards, 1996; Green, 2011). Simple linear regressions of tibia ash (mg/tibia and %) on supplemental P intake from KH_2PO_4 were highly significant (R^2 values were 0.94 and 0.88, respectively; $P < 0.001$). Unexpectedly, weight gain and tibia ash responses to phytase addition exceeded the response to KH_2PO_4 (Tables 3.4 and 3.6). Because the tibia ash responses were greater between the Basal 2 diet and the 250 U/kg phytase treatments than between the 250 and 500 U/kg treatments (not linear), the standard curve method was used to estimate P release for each of the individual phytase additions. The standard curve method to estimate P release by phytase yielded values of 0.13 and 0.19% P released for tibia ash (mg/tibia) and 0.12 and 0.17% P released for tibia ash (%) for 250 and 500 U/kg of phytase, respectively (Table 3.6).

Overall, results indicated that the phytate P of CMA is highly susceptible to release by microbial phytase. The addition of 250 units phytase/kg diet released well in excess of the expected 0.1% P from CMA, and addition of 500 units phytase/kg diet released almost 0.2% P. The actual release values may not be completely accurate since the tibia ash responses exceeded the range of the standard curve for the added levels of KH_2PO_4 . This occurred because it was expected that the phytase would only be able to liberate approximately 0.1% P from CMA, whereas the actual estimated release was well in excess of that. The estimated P release value of approximately 0.12% for the 250 units/kg phytase is likely accurate, since Augspurger et al. (2003) showed that, when using the same type of chicks and similar P-deficient diets used herein, tibia ash responses were linear up to 0.15% added P. The P release value for the 500

units/kg phytase may be slightly underestimated because tibia ash response may have been in excess of the linear response range.

In conclusion, even though the total P content of the high-protein CM did not differ consistently from the CM-CV, the bioavailability of the P was higher in the high-protein CM. Thus, the bioavailable P concentration was higher in the high-protein CM. Furthermore, the research with CMA indicates that microbial phytase can greatly increase the bioavailability of the P in the high-protein CM.

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TABLES

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

Ingredient, %	Diet										
	P- deficient control ²	KH ₂ PO ₄		Canola meal A		Canola meal B		Conventional canola meal		Soybean meal	
	-	0.23%	0.45%	12.5%	25%	12.5%	25%	12.5%	25%	12.5%	25%
Dextrose	16.68	16.61	16.54	12.52	8.35	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	25.04	16.71
Test canola or SBM	-	-	-	12.50	25.00	12.50	25.00	12.50	25.00	12.50	25.00
Soybean meal (SBM)	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Dical	0.10	0.10	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	0.40	0.40
Vitamin mix ³	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ⁴	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table 3.1 (cont.)

DL-Met	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride (60%)	0.10	0.10	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 ⁵	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²The P-deficient control diet was calculated to contain 3.328 kcal ME/kg, 20.5% CP, 0.11% available P, and 0.76% Ca.

³Provided 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.

⁴Provided 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.

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

Table 3.2. Composition of P-deficient-canola meal A control diet (Diet 12, as-fed basis)

Ingredient ¹	Amount (%)
Dextrose	15.68
Cornstarch	31.38
Test Canola meal A	45.00
Soybean oil	5.00
Limestone	1.34
Salt	0.40
Vitamin mix ²	0.20
Mineral mix ³	0.15
DL-Methionine	0.25
L-Lysine HCl	0.25
L-Arg	0.13
L-Ile	0.03
L-Thr	0.05
Choline chloride (99%)	0.10
Bacitracin ⁴	0.04
Calculated nutrients	
CP (%)	17.20
ME (kcal/kg)	3,073
Available phosphorus (%)	0.135
Calcium (%)	0.820

¹KH₂PO₄ or phytase added in place of starch.

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 ethylene diamine dihydroiodide; selenium, 9.1 from Na₂SeO₃.

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

Table 3.3. Analyzed nutrient composition of ingredients (as-fed basis)¹

Item	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal
GE, kcal/kg	4,450	4,403	4,172	4,222
DM, %	91.24	91.13	89.90	88.15
CP, %	45.69	46.97	35.10	46.67
Ash, %	6.88	6.10	7.98	5.57
Ether extract, acid hydrolyzed, %	3.48	3.28	3.77	2.48
NDF, %	18.32	17.90	25.04	8.23
ADF, %	12.66	10.95	17.53	4.81
Ca, %	0.64	0.51	1.25	0.29
P, %	1.26	1.16	1.16	0.57
Phytate, %	3.57	3.20	2.69	1.43
Phytate-bound P ² , %	1.01	0.90	0.76	0.40
Non-phytate-bound P ³ , %	0.25	0.26	0.40	0.17
Indispensable AA, %				
Arg	2.79	2.87	2.09	3.43
His	1.23	1.23	0.91	1.24
Ile	1.77	1.89	1.35	2.32
Leu	3.18	3.31	2.53	3.73
Lys	2.61	2.67	2.02	3.07
Met	0.91	0.91	0.68	0.69
Phe	1.80	1.90	1.38	2.45
Thr	1.85	1.84	1.49	1.82
Trp	0.67	0.71	0.46	0.68
Val	2.23	2.48	1.72	2.50
Dispensable AA, %				
Ala	1.88	1.92	1.50	2.02
Asp	3.00	3.35	2.44	5.34
Cys	1.21	1.19	0.82	0.68

Table 3.3 (cont.)

Glu	7.36	7.45	5.41	7.86
Gly	2.18	2.20	1.69	1.98
Pro	2.81	2.84	2.08	2.29
Ser	1.74	1.67	1.32	2.03
Tyr	1.20	1.24	0.96	1.75

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

³Non-phytate-bound P was calculated as the difference between total P and phytate-bound P.

Table 3.4. Growth performance from d 8-21 of age and tibia ash content of chicks^{1,2}

Dietary Treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:Feed (g/kg)	Bone Ash ³ (mg/tibia)	Bone Ash ⁴ (%)
1. Basal Diet (B) ⁵	268 ^{gh}	414 ^{ef}	649 ^{bcd}	300 ^{fg}	31.0 ^{ghi}
2. B + 0.05% P ⁶	286 ^f	438 ^{de}	654 ^{bcd}	348 ^{de}	33.9 ^{de}
3. B + 0.10% P ⁶	328 ^{bc}	493 ^{ab}	665 ^{bc}	477 ^b	39.0 ^b
4. B + 12.5% CMA ⁷	290 ^{ef}	430 ^{def}	676 ^{ab}	322 ^{ef}	31.3 ^{gh}
5. B + 25% CMA ⁷	297 ^{def}	452 ^{cd}	659 ^{bcd}	351 ^{de}	34.2 ^{de}
6. B + 12.5% CMB ⁷	288 ^{ef}	431 ^{def}	668 ^{bc}	312 ^{ef}	31.8 ^g
7. B + 25% CMB ⁷	296 ^{ef}	453 ^{cd}	653 ^{bcd}	369 ^d	35.0 ^d
8. B + 12.5% CM-CV ⁷	285 ^f	426 ^{def}	669 ^b	317 ^{ef}	30.4 ^{hi}
9. B + 25% CM-CV ⁷	284 ^{fg}	442 ^{de}	642 ^{bcd}	330 ^{def}	33.5 ^{ef}
10. B + 12.5% SBM ⁷	296 ^{ef}	439 ^{de}	674 ^{ab}	333 ^{def}	32.3 ^{fg}
11. B + 25% SBM ⁷	298 ^{def}	424 ^{def}	710 ^a	365 ^d	34.8 ^{de}
12. Basal Diet (B2) ⁸	255 ^h	406 ^f	629 ^{cd}	264 ^g	29.6 ⁱ
13. B2 + 0.05% P ⁶	303 ^{de}	473 ^{bc}	641 ^{bcd}	366 ^d	35.2 ^d
14. B2 + 0.10% P ⁶	313 ^{cd}	502 ^{ab}	625 ^d	430 ^c	37.1 ^c
15. B2 + 250 units/kg phytase ⁹	333 ^{ab}	503 ^{ab}	662 ^{bcd}	480 ^b	38.9 ^b
16. B2 + 500 units/kg phytase ⁹	346 ^a	511 ^a	678 ^{ab}	592 ^a	42.8 ^a
Pooled SEM	5.6	10.8	13.7	14.1	0.52

^{a-1}Means within a column with no common superscript letter differ ($P < 0.05$).

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Means represent 4 pens of 5 chicks per treatment; average initial BW was 93.4 g.

³Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH₂PO₄ (X₁), CMA (X₂), CMB (X₃), CM-CV (X₄), and SBM (X₅) yielded the equation: $Y = 287.7 + 0.369 \pm 0.031X_1 + 0.046 \pm 0.011X_2 + 0.058 \pm 0.016X_3 + 0.036 \pm 0.012X_4 + 0.128 \pm 0.024X_5$ ($R^2 = 0.80$). The (\pm) values are standard errors of the regression coefficients.

⁴Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH₂PO₄ (X₁), CMA (X₂), CMB (X₃), CM-CV (X₄), and SBM (X₅) yielded the equation: $Y = 29.89 + 0.0185 \pm 0.0014X_1 + 0.0028 \pm 0.00046X_2 + 0.0037 \pm 0.00051X_3 + 0.0024 \pm 0.00052X_4 + 0.0077 \pm 0.0010X_5$ ($R^2 = 0.84$). The (\pm) values are standard errors of the regression coefficients.

⁵P-deficient control diet in Table 3.1.

⁶From KH₂PO₄.

⁷CMA= Canola meal A, CMB= Canola meal B, CM-CV= Conventional canola meal, SBM= Soybean meal.

⁸P-deficient CMA diet in Table 3.2.

⁹*Optiphos 2000*, Enzyvia LLC, Sheridan, IN.

Table 3.5. Relative bioavailability of the P in canola meals and soybean meal¹

Sample	Total P (%)	Bioavailability values ² (%)		Bioavailable content ³ (%)	
		Tibia ash (mg)	Tibia ash (%)	Tibia ash (mg)	Tibia ash (%)
Canola meal A	1.26	12.5	15.1	0.16	0.19
Canola meal B	1.16	15.7	20.0	0.18	0.23
Conventional canola meal	1.16	9.8	13.0	0.11	0.15
Soybean meal	0.57	34.7	41.6	0.20	0.24

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Calculated by the slope-ratio method using the multiple regression equations in footnotes 3 and 4 of Table 3.4. These are bioavailability values relative to the P in KH_2PO_4 which was set at 100%.

³Bioavailable content = total P \times bioavailability value.

Table 3.6. Amount of phosphorus released from Canola meal A by phytase enzyme

Dietary treatments	Based on bone ash as mg/tibia ¹		Based on bone ash as % ²	
	Bone Ash	P Released ³	Bone Ash	P Released ³
12. Basal Diet (B2) ⁴	264 ^c		29.6 ^c	
13. B2 + 0.05% P ⁵	366 ^d		35.2 ^d	
14. B2 + 0.10% P ⁵	430 ^c		37.1 ^c	
15. B2 + 250 units/kg phytase ⁶	480 ^b	0.126%	38.9 ^b	0.115%
16. B2 + 500 units/kg phytase ⁶	592 ^a	0.194%	42.8 ^a	0.167%
Pooled SEM	14.1		0.52	

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

¹Linear regression of tibia ash (Y; mg) on supplemental P (% of diet) yielded the equation: $Y = 270.49 + 1660.2 \pm 8.76X$ ($R^2 = 0.94$). The (\pm) value is the standard error of the regression coefficient.

²Linear regression of tibia ash (Y; %) on supplemental P (% of diet) yielded the equation: $Y = 30.17 + 75.65 \pm 0.566X$ ($R^2 = 0.88$). The (\pm) value is the standard error of the regression coefficient.

³Estimated by the standard curve method using the regression equations in footnotes 1 and 2.

⁴P-deficient CMA control diet in Table 3.2.

⁵From KH_2PO_4 .

⁶*Optiphos 2000*, Enzyvia LLC, Sheridan, IN.

CHAPTER 4

PHOSPHORUS DIGESTIBILITY IN HIGH-PROTEIN CANOLA MEALS, CONVENTIONAL CANOLA MEAL, AND SOYBEAN MEAL FED TO GROWING PIGS

ABSTRACT

An experiment was conducted to determine the digestibility of Ca and P in 2 high-protein canola meals (CMA; 45.7% CP, and CMB; 47.0% CP) fed to growing pigs, and to compare values obtained in high-protein canola meal with digestibility of Ca and P in conventional canola meal (CM-CV; 35.1% CP) and soybean meal (SBM). The Ca and P contents of CMA, CMB, and CM-CV were 0.64 and 1.26%, 0.51 and 1.16%, and 1.25 and 1.16%, respectively. Four cornstarch-based diets were formulated using each source of canola meal or SBM as the sole source of P in the diet. Four additional diets, similar to the initial 4 diets with the exception that 500 FTU/kg of microbial phytase were added to each diet, were also formulated. Therefore, a total of 8 diets were formulated. Forty-eight barrows were divided into 2 periods and randomly allotted via a randomized complete block design using a 2×4 factorial arrangement to the 8 dietary treatments based on initial BW. There were 6 replicate pigs per dietary treatment. Experimental diets were provided for 12 d with the initial 5 d being the adaptation period. Indigo carmine was added as an indigestible marker to the morning meals on d 6 and 11, respectively. Fecal collections started when the first marker appeared in the feces and ceased when the second marker appeared. The endogenous loss of P was assumed to be $190 \text{ mg kg}^{-1} \text{ DMI}$. At the conclusion of the experiment, feed intake, Ca and P intake, apparent total tract digestibility (ATTD) of Ca and P, and standardized total tract digestibility (STTD) of P were calculated. Results indicated that ATTD

of Ca and P and STTD of P were not different among treatments. Apparent total tract digestibility of Ca was 62, 66, 69, and 73% for CMA, CMB, CM-CV, and SBM, respectively. Standardized total tract digestibility of P was 55, 60, 49, and 66% for CMA, CMB, CM-CV, and SBM, respectively. Inclusion of phytase to the diets reduced both Ca and P outputs ($P < 0.05$). Inclusion of phytase also improved ($P < 0.05$) ATTD of Ca and P (by 8 and 16%, respectively) and STTD of P (by 17%), regardless of the ingredient in the diet, and there was no interaction between diet and phytase supplementation.

Key words: apparent digestibility, canola meal, phosphorus, pig, standardized digestibility

INTRODUCTION

One limitation to using canola meal (**CM**) in swine diets is the high fiber content, which, in addition to high levels of phytic acid, reduces availability of P and results in poor apparent total tract digestibility (**ATTD**) of P and greater P excretion (Nwokolo and Bragg, 1977).

Yellow-seeded canola (*Brassica napus*) has a larger seed size and contains more protein (46 to 49%) and less dietary fiber (24 to 27%) than black-seeded *B. napus* (Slominski et al., 1994; Simbaya et al., 1995; Slominski et al., 2012), making it more desirable for use in swine diets.

Though this high-protein canola has similar P content as the conventional black-seeded varieties (Sanjayan, 2013), the reduction in fiber may result in greater ATTD of P and less P excretion.

About two-thirds of the P in plant products is bound to phytate (Simons et al., 1990). Pigs do not produce sufficient intestinal phytase to break the phytate-P bond, but addition of microbial phytase to the diet may result in increased ATTD of P and decreased P excretion (Simons et al., 1990; Akinmusire and Adeola, 2009; Rodriguez et al., 2013). However, ATTD does not account for endogenous P losses and is, therefore, not a correct value to use in diet

formulation. Values based on standardized total tract digestibility (**STTD**) of P are corrected for basal endogenous losses of P and are additive among feed ingredients (NRC, 2012). Therefore, diets are more accurately formulated using STTD values than ATTD values (NRC, 2012).

Concentration of Ca in the diet may also have a negative effect on ATTD of P (González-Vega et al., 2013). Stein et al. (2011) hypothesized that increased Ca in the diet may form Ca-P complexes in the intestinal tract and inhibit P from being absorbed. Therefore, the objectives of this experiment were to determine the digestibility of Ca and P in high-protein CM fed to growing pigs, and to compare values obtained in high-protein CM with digestibility of Ca and P in conventional canola meal (**CM-CV**) and soybean meal (**SBM**).

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were offspring of G-performer boars × Fertiliium 25 females (Genetiporc, Alexandria, MN).

Diets, Animals, and Experimental Design

Two sources of CM that were produced from new varieties of high-protein canola seeds (**CMA** and **CMB**) and a CM-CV were used (Table 4.1). Four cornstarch-based diets were formulated using each source of CM or SBM as the sole source of P in the diet. Four additional diets, similar to the initial 4 diets with the exception that 500 FTU/kg of microbial phytase (Optiphos; Enzyvia, Sheridan, IN) were added to each diet, were also formulated. Therefore, a total of 8 diets were formulated (Tables 4.2 and 4.3).

Forty-eight barrows (initial BW: 16.60 ± 3.96 kg) were divided into 2 periods and placed in metabolism cages equipped with a feeder and a nipple drinker. Pigs were randomly allotted

via a randomized complete block design using a 2×4 factorial arrangement to the 8 dietary treatments based on initial BW. There were 6 replicate pigs per dietary treatment.

Individual pig BW was recorded at the initiation and at the conclusion of the experiment. Feed was supplied at 2.5 times the daily maintenance energy requirement for the smallest pig in each replicate and divided into 2 equal meals per day. The amount of feed supplied each day was recorded and water was available at all times.

Sample Collection and Analysis

Experimental diets were provided for 12 d with the initial 5 d being the adaptation period. Indigo carmine was added as an indigestible marker to the morning meals on d 6 and 11, respectively. Fecal collections started when the first marker appeared in feces and ceased when the second marker appeared. Feces were collected twice daily and stored immediately after collection at -20°C . At the conclusion of the experiment, fecal samples were thawed and pooled within animal and dried in a forced-air drying oven. Samples were finely ground and a subsample was collected for analysis.

Diets and ingredients were analyzed for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL), 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 acid hydrolyzed ether extract (Method 2003.06, AOAC International, 2007) using an automated analyzer (Soxtec 2050; FOSS North America, Eden Prairie, MN), ADF (Method 973.18, AOAC International, 2007), NDF (Holst, 1973), AA (Method 982.30 E [a, b, and c]; AOAC International, 2007), and phytate concentration (Ellis et al., 1977). Diet, ingredient, and fecal samples were also analyzed for DM by forced air oven drying for 2 h at 135°C (Method 930.15; AOAC International, 2007),

and for Ca and P 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).

Calculations and Statistical Analysis

The concentration of phytate-bound P in each ingredient was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004). Non-phytate-bound P in each ingredient was calculated as the difference between total P and phytate-bound P. Feed consumption was summarized for each pig to calculate ADFI. The Ca and P intake for each treatment were calculated by multiplying ADFI by the analyzed Ca or P concentration in the diet. The Ca and P output for each treatment were calculated by multiplying the fecal output (DM) by the analyzed Ca or P concentration in feces. Values for ATTD of Ca and P were calculated as described previously (NRC, 2012). Standardized total tract digestibility of P was calculated by correcting ATTD values for the endogenous loss of P, which was assumed to be 190 mg kg⁻¹ DMI (NRC, 2012). Normality was confirmed and outliers were tested using the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC). Outliers were identified as values that deviated from the treatment mean by more than 3 times the interquartile range. Data for ATTD of Ca and P, STTD of P, Ca and P intake, and fecal Ca and P output were analyzed by ANOVA using the MIXED procedure of SAS with pig as the experimental unit. The model included dietary treatment, microbial phytase, and the interaction between dietary treatment and phytase as fixed effects and period as random effect. Least squares means were calculated for each independent variable. If diet was a significant source of variation, means were separated using the PDIF option of SAS. Significance was assessed at $P \leq 0.05$ and tendencies were determined at $0.05 < P \leq 0.10$.

RESULTS

There were no differences in feed intake among dietary treatments (Table 4.4). Pigs fed CM-CV had greater Ca intake compared with pigs on the other treatments ($P < 0.05$). Pigs fed CMA had the greatest ($P < 0.05$) P intake, whereas pigs fed SBM had the least P intake ($P < 0.05$). Pigs fed SBM also had the least fecal output, whereas pigs fed CM-CV and CMA had the greatest ($P < 0.05$) fecal output. The greatest ($P < 0.05$) Ca output was observed for pigs fed the CM-CV and the greatest P output was for pigs fed CMA ($P < 0.05$). However, pigs fed SBM had the least ($P < 0.05$) Ca and P outputs ($P < 0.05$). Inclusion of phytase in the diets reduced both Ca and P outputs ($P < 0.05$). There was no difference among the 3 CM for ATTD of Ca or P or for STTD of P. Likewise, no differences in ATTD of Ca or P or STTD of P were observed between the CM and the SBM. Inclusion of phytase improved ($P < 0.05$) ATTD of Ca and P and STTD of P regardless of the ingredient in the diet and there was no interaction between diet and phytase supplementation.

DISCUSSION

The analyzed nutrient composition of the CM and SBM used in this experiment are presented in Table 4.1. The CP of the high-protein CM was 45.7% for CMA and 47.0% for CMB. The SBM had a CP value of 46.7%. The CM-CV had the least CP value at 35.1%. The content of most AA was greatest in SBM, but the high-protein CM had the greatest levels of Met, Cys, Gly, and Pro. Levels of His, Thr, Trp, and Val were similar among the high-protein CM and SBM. The CM-CV had the least amounts of all AA. The CP and AA values for the high-protein CM were less than the values reported by Slominski et al. (2012) for yellow-seeded CM. However, CP in these meals was greater than values reported in NRC (2012) for CM-CV

and greater than those reported by Simbaya et al. (1995) and Trindade Neto et al. (2012) for yellow-seeded CM. Values of several AA for the high-protein CM were close to those reported by Trindade Neto et al. (2012) for yellow-seeded CM, but levels of Lys, Met, Cys, Trp, Val, and Pro were greater in the meals used in the current study. Values for all AA in the high-protein CM were higher than those published in NRC (2012), and CP content and most AA values for CM-CV were less than the published values (NRC, 2012). Crude protein and AA values of the SBM were close to published values (Baidoo et al., 1987; NRC, 2012; Rojas and Stein, 2012).

Values for ADF and NDF of the high-protein CM were less than those reported by NRC (2012) and Rodríguez et al. (2013). Values for NDF of the high-protein CM and CM-CV agreed with values published by Slominski et al. (1994). Values for NDF and ADF of the CM-CV were slightly greater than published values (NRC, 2012). The NDF value for SBM agrees with NRC (2012), but the value for ADF was slightly lower than the NRC value, and both NDF and ADF values for SBM were less than values reported by Rodríguez et al. (2013). The greater CP content and lower fiber content of the high-protein CM compared with the CM-CV are likely a result of the increased seed size of the canola. Therefore, the hull makes up a reduced proportion of the seed, resulting in less fiber, and the embryo makes up a greater proportion, resulting in greater CP content.

Values for DM and ash of the high-protein CM agree with published values (Simbaya et al., 1995; NRC, 2012) and values for DM and ash of the CM-CV and SBM were close to values previously reported (NRC, 2012; Rodríguez et al., 2013). The GE content of the high-protein CM was greater than values for CM-CV (NRC, 2012; Rodríguez et al., 2013) but the GE in CM-CV was less than previous values. Values for GE of SBM were in agreement with published values (Akinmusire and Adeola, 2009; NRC, 2012; Rodríguez et al., 2013). All 3 CM had

greater P content than published in NRC (2012), but also contained more phytate P. The P content of the CM-CV was less than values previously reported (Nwokolo and Bragg, 1977; Slominski et al., 2012), but was in agreement with values reported by Akinmusire and Adeola (2009). The P content of CMA was close to values published by Nwokolo and Bragg (1977) and Slominski et al. (2012), but values for CMB were less. The concentration of P in CMB and the CM-CV was not different and both contained less P than CMA. The phytate P content of the CM-CV was in agreement with values previously reported (Slominski et al. 2012; Rodríguez et al., 2013); however, there was more phytate P in the high-protein CM. Phosphorus and phytate P content of the SBM are close to published values (Akinmusire and Adeola, 2009; NRC 2012; Rojas and Stein, 2012). The most likely reason for the increased phytate P concentration in the high-protein CM is that phytate is located in the embryo portion of the seed. With the increased seed size and thinner hull, the embryo makes up a greater proportion of the seed and, therefore, there are greater amounts of all constituents in the embryo, including phytate. Variations in soil composition where the canola was grown may also be partly responsible for differing levels of phytate P between the high-protein CM and the CM-CV, which were obtained from different sources.

The STTD of P calculated for all 3 CM is in agreement with published values (Rodríguez et al., 2013; Maison, 2013). The increased STTD of P for all treatments with the addition of phytase is in agreement with published results (Akinmusire and Adeola, 2009; Maison, 2013; Rodríguez et al., 2013). This observation is a result of the phytase dephosphorylating the phytate to release P from the phytate-P complexes for absorption in the gastrointestinal tract (Selle et al., 2009).

All CM diets were formulated to have 0.70% limestone, so that any variability in ATTD of Ca among these 3 diets would have been due to differences in Ca digestibility in the canola meals. Furthermore, the increase in ATTD of Ca with addition of phytase supports previous data (González-Vega et al., 2013; Maison, 2013; Rodríguez et al., 2013) and was expected because phytate may bind Ca and form Ca-phytate complexes in the gastrointestinal tract (Selle et al., 2009).

In conclusion, a large proportion of the P in CM is phytate bound, resulting in high P excretion and poor P digestibility. Phytate also binds Ca to some extent, resulting in increased excretion and decreased digestibility. The addition of microbial phytase to diets containing CM significantly improves STTD of P and ATTD of Ca and reduces P and Ca output.

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TABLES

Table 4.1. Analyzed nutrient composition of ingredients (as-fed basis)¹

Item	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal
GE, kcal/kg	4,450	4,403	4,172	4,222
DM, %	91.24	91.13	89.90	88.15
CP, %	45.69	46.97	35.10	46.67
Ash, %	6.88	6.10	7.98	5.57
Ether extract, acid hydrolyzed, %	3.48	3.28	3.77	2.48
NDF, %	18.32	17.90	25.04	8.23
ADF, %	12.66	10.95	17.53	4.81
Ca, %	0.64	0.51	1.25	0.29
P, %	1.26	1.16	1.16	0.57
Phytate, %	3.57	3.20	2.69	1.43
Phytate-bound P ² , %	1.01	0.90	0.76	0.40
Non-phytate-bound P ³ , %	0.25	0.26	0.40	0.17
Indispensable AA, %				
Arg	2.79	2.87	2.09	3.43
His	1.23	1.23	0.91	1.24
Ile	1.77	1.89	1.35	2.32
Leu	3.18	3.31	2.53	3.73
Lys	2.61	2.67	2.02	3.07
Met	0.91	0.91	0.68	0.69
Phe	1.80	1.90	1.38	2.45
Thr	1.85	1.84	1.49	1.82
Trp	0.67	0.71	0.46	0.68
Val	2.23	2.48	1.72	2.50
Dispensable AA, %				
Ala	1.88	1.92	1.50	2.02

Table 4.1 (cont.)

Asp	3.00	3.35	2.44	5.34
Cys	1.21	1.19	0.82	0.68
Glu	7.36	7.45	5.41	7.86
Gly	2.18	2.20	1.69	1.98
Pro	2.81	2.84	2.08	2.29
Ser	1.74	1.67	1.32	2.03
Tyr	1.20	1.24	0.96	1.75

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

³Non-phytate-bound P was calculated as the difference between total P and phytate-bound P.

Table 4.2. Ingredient composition of experimental diets (as-fed basis)¹

Ingredient, %	Diets ²			
	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal
Cornstarch	47.60	47.60	47.60	38.40
Canola meal A	38.00	-	-	-
Canola meal B	-	38.00	-	-
Conventional canola meal	-	-	38.00	-
Soybean meal	-	-	-	47.00
Soybean oil	3.00	3.00	3.00	3.00
Limestone	0.70	0.70	0.70	0.90
Sucrose	10.00	10.00	10.00	10.00
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix ³	0.30	0.30	0.30	0.30
Calculated energy and nutrients				
ME, kcal/kg	3,512	3,512	3,512	3,731
CP, %	17.37	17.86	13.34	21.95
Fat, %	4.30	4.30	4.30	3.50
Lys, %	0.68	0.68	0.68	1.42
Ca, %	0.51	0.51	0.51	0.50
P, %	0.38	0.38	0.38	0.32
Digestible P, %	0.10	0.10	0.10	0.13

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Four additional diets containing 500 FTU/kg microbial phytase (Optiphos 2000, Enzyvia LLC, Sheridan, IN) were formulated and phytase was added at the expense of starch.

³Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU;

vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 4.3. Analyzed nutrient composition of experimental diets (as-fed basis)¹

Item	Without Phytase				With Phytase			
	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal
GE, kcal/kg	4,039	4,090	4,017	4,065	4,085	4,098	3,991	4,055
DM, %	91.68	91.90	91.38	90.66	91.69	91.88	90.77	90.55
CP, %	18.76	19.70	13.59	22.02	19.12	18.64	13.70	24.26
Ash, %	3.91	4.24	4.51	4.46	4.36	3.75	4.49	4.29
Ca, %	0.589	0.573	0.771	0.568	0.587	0.557	0.786	0.577
P, %	0.501	0.468	0.395	0.309	0.495	0.452	0.405	0.310

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

Table 4.4. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of P in 2 sources of high protein canola meal (CMA and CMB), in conventional canola meal (CM-CV), and in soybean meal (SBM) without or with microbial phytase¹

Item	Without Phytase				With Phytase				SEM	<i>P</i> -value		
	CMA	CMB	CM-CV	SBM	CMA	CMB	CM-CV	SBM		Diet	Phytase	Interaction
Feed intake, DM, g/5d	3,046	3,034	3,018	2,880	3,053	3,035	3,075	2,918	267.1	0.49	0.75	0.99
Ca intake, g/5d	17.94 ^b	17.38 ^b	23.27 ^a	16.36 ^b	17.92 ^b	16.91 ^b	24.17 ^a	16.84 ^b	1.70	<0.01	0.68	0.81
P intake, g/5d	15.26 ^a	14.20 ^{ab}	11.92 ^d	8.90 ^e	15.11 ^{ab}	13.72 ^{bc}	12.45 ^{cd}	9.05 ^e	1.10	<0.01	0.97	0.76
Fecal output, DM, g/5d	262.4 ^{ab}	212.3 ^b	268.2 ^{ab}	130.2 ^{cd}	237.2 ^{ab}	201.0 ^{bc}	298.2 ^a	86.4 ^d	34.40	<0.01	0.54	0.63
Ca output, g/5d	6.78 ^{ab}	5.99 ^{abc}	7.40 ^a	4.23 ^{cd}	5.07 ^{bcd}	3.53 ^{cd}	5.84 ^{abc}	3.14 ^d	1.03	<0.01	<0.01	0.89
P output, g/5d	7.53 ^a	6.45 ^{ab}	6.75 ^{ab}	3.50 ^{cd}	5.21 ^{abc}	4.69 ^{bcd}	4.34 ^{bcd}	1.75 ^d	1.23	<0.01	<0.01	0.97
ATTD of Ca, %	62.34 ^c	66.42 ^{bc}	68.81 ^{abc}	73.27 ^{abc}	71.29 ^{abc}	75.62 ^{ab}	74.91 ^{ab}	81.64 ^a	5.34	0.17	<0.05	0.98
ATTD of P, %	51.14 ^{bc}	55.94 ^{bc}	44.49 ^c	59.84 ^{abc}	64.56 ^{ab}	64.99 ^{ab}	69.52 ^{ab}	79.40 ^a	8.22	0.31	<0.01	0.68
STTD of P, ² %	54.93 ^{bc}	60.00 ^{bc}	49.30 ^c	65.99 ^{abc}	68.40 ^{abc}	69.19 ^{ab}	74.21 ^{ab}	85.53 ^a	8.22	0.19	<0.01	0.69

^{a-d}Within a row, means without a common superscript letter are different ($P < 0.05$).

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Values for STTD of P were calculated by correcting values for ATTD for basal endogenous losses. The endogenous P loss value used for calculation was 190 mg/kg DMI (NRC, 2012).

CHAPTER 5

EFFECTS OF HIGH-PROTEIN OR CONVENTIONAL CANOLA MEAL ON GROWTH PERFORMANCE, ORGAN WEIGHTS, BONE ASH, AND BLOOD CHARACTERISTICS OF WEANLING PIGS

ABSTRACT

An experiment was conducted to evaluate effects of 2 high-protein canola meals (CMA; 45.7% CP and CMB; 47.0% CP, respectively) and a conventional canola meal (CM-CV; 35.1% CP) on growth performance, organ weights, bone ash, and blood characteristics of weanling pigs. Inclusion rates of canola meal in the diets were 10, 20, 30, or 40% from CMA and CM-CV, whereas inclusions were 10, 20, or 30% from CMB. No 40% inclusion of CMB was used due to lack of ingredient. A control diet containing no canola meal was also used. Therefore, a total of 12 diets were tested in this experiment. A total of 420 pigs (initial BW: 9.8 ± 1.1 kg) were divided into 3 blocks and randomly allotted to 1 of the 12 diets with 8 replicate pens per treatment and 4 or 5 pigs per pen. The ADG, ADFI, and G:F were calculated and, at the conclusion of the experiment, one pig in each pen was sacrificed to measure organ weights and blood characteristics and to collect the 3rd and 4th metacarpals from the left foot. Results indicate that ADFI was linearly ($P < 0.05$) decreased if inclusion of CMA, CMB, or CM-CV increased. Average daily gain for pigs fed CMA tended to increase quadratically, with the maximum response observed if 10 or 20% canola meal was included in the diet ($P = 0.06$). However, G:F was linearly ($P < 0.05$) increased by adding CMA or CM-CV to the diets. Liver weights were also linearly ($P < 0.05$) increased if pigs were fed diets containing CMB, but kidney weights were linearly ($P < 0.05$) decreased by adding CM-CV to the diets. Thyroid gland weights

increased linearly ($P < 0.05$) for pigs fed diets containing CMA. No differences were observed on heart and bone weights if canola meal was added to the diets. Addition of any of the 3 canola meals linearly ($P < 0.05$) increased bone ash percentage in the metacarpals. Inclusion of CMA or CM-CV linearly ($P < 0.05$) decreased serum triiodothyronine, and the inclusion of CMA also linearly ($P < 0.05$) decreased serum thyroxine in weanling pigs. No differences were observed for complete blood counts or blood urea nitrogen if canola meal was added to the diets. In conclusion, high-protein or conventional canola meals can be included in diets for weanling pigs from 2 wk post-weaning at 20% minimum without reducing growth performance or negatively affecting organ, bone, or blood characteristics, but greater inclusion levels may result in reduced performance due to reduced feed intake.

Key words: bone ash, canola meal, growth performance, high-protein canola meal, organs, weanling pigs

INTRODUCTION

Canola and rapeseed meal are the second most commonly used protein sources in the world in animal diets (Arntfield and Hickling, 2011). Rapeseed meal has been fed to livestock for decades, though inclusion in swine diets has been limited in the past due to anti-nutritional factors such as glucosinolates, erucic acid, and fiber. In response, Canadian plant breeders developed a low-glucosinolate and low erucic acid variety of rapeseed, known as canola. Past studies have not shown consistency in the percentage of protein from soybean meal (**SBM**) that can be replaced by canola meal (**CM**) in diets fed to weanling pigs, varying from <10% (Rundgren, 1983) to complete replacement (Bowland, 1975). Results of more recent studies

indicate that 15 to 20 percentage units of SBM in diets can be replaced with CM with no effect on growth performance (Seneviratne et al., 2011; Landero et al., 2011).

Yellow-seeded *Brassica napus* has a larger, thinner seed size than the conventional black-seeded *B. napus* (Rahman et al., 2001) and contains more CP and less dietary fiber (Slominski et al., 2012). The seed coat of yellow-seeded canola contains less fiber as a percentage of the seed and also has increased energy digestibility. Results of research in which yellow-seeded CM was fed to growing pigs indicate that inclusion levels of 25% can be used without negative effects on growth performance (Sanjayan, 2013). Trindade Neto et al. (2012) included 35% CM in diets for growing pigs and reported no effects on ileal digestibility of AA. There is, however, limited information about feeding high-protein CM to weanling pigs.

Therefore, the objectives of this experiment were to compare growth performance, organ weights, bone ash, and blood characteristics between weaned pigs fed diets containing either high-protein CM or conventional canola meal (**CM-CV**) at increasing inclusion levels and to determine the maximum inclusion level of high-protein CM in diets for weanling pigs.

MATERIALS AND METHODS

The protocol concerning this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were offspring of G-performer boars × Fertiliun 25 females (Genetiporc, Alexandria, MN).

Diets, Animals, and Experimental Design

Two sources of CM produced from new varieties of high-protein canola seeds (**CMA** and **CMB**) and a CM-CV were used (Table 5.1). Canola meal A and the CM-CV were used at inclusion rates of 10, 20, 30, or 40% of diets fed to weanling pigs, whereas CMB was used only

at 10, 20, or 30% inclusion. A control diet containing no CM was also included in the experiment so a total of 12 diets were formulated (Tables 5.3 and 5.4). Canola meal primarily replaced SBM in the diets, and all diets were formulated based on the digestibility values for energy, AA, and P that were previously determined in the same batches of the 3 CM and the SBM that were used in this experiment.

A total of 420 pigs that had been weaned for 2 wk (initial BW: 9.8 ± 1.1 kg) were divided into 3 blocks based on date of birth and blocks were started 2 weeks apart. Within each block, pigs were randomly allotted via a randomized complete block design to the 12 dietary treatments based on initial BW. There were 3, 3, and 2 replicate pens in each block for a total of 8 replicates per dietary treatment. There were 5 replicates with 4 pigs per pen, and 3 replicates with 5 pigs per pen with an equal number of barrows and gilts on each treatment. Pens were 1.2×1.4 m with either slatted or mesh floors and each pen was equipped with a feeder and a nipple drinker.

Individual pig BW was recorded at the initiation of the experiment and final BW was recorded at the conclusion. Feed was added as needed to assure free access at all times throughout the 3 wk experiment. Feed intake per pen was recorded. Water was available at all times.

Sample Collection and Analysis

At the conclusion of the experiment, one pig of average BW in each pen was sacrificed, organ weights were recorded, and tissue and blood samples were collected. An equal number of barrows and gilts were sacrificed. Three tubes of blood were collected from each pig that was designated to be sacrificed via jugular venipuncture in EDTA tubes. Tubes were stored on ice immediately after collection. One blood sample from each pig was analyzed for complete blood count (CBC). The other 2 samples were centrifuged (3,000 rpm at 4°C for 10 min). Plasma was

collected from the centrifuged tubes and plasma from 1 tube was analyzed for triiodothyronine (**T3**) and thyroxine (**T4**) and plasma from the other tube was analyzed for blood urea nitrogen (**BUN**). Thyroid hormones were analyzed using an ELISA kit (Abnova, Taipei, Taiwan) and BUN was analyzed on an Olympus AU680 Chemistry Analyzer (Olympus Life Science Research Europa GmbH, Sauerbruchstr., Munich, Germany).

Following blood collection, pigs were killed via captive bolt penetration and exsanguination. The liver, heart, kidneys, and thyroid gland were removed, and the weight of each organ was recorded.

The third and fourth metacarpals were collected from the front left foot of each sacrificed pig. Bones were autoclaved for 40 min and cheesecloth was used to aid in the removal of soft tissue. Bones were defatted in ether for 3 d and then ashed in a muffle furnace at 600°C for 16 h for determination of total bone ash.

Diets and ingredients were analyzed for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL), DM by forced air oven drying for 2 h at 135°C (Method 930.15; AOAC International, 2007), and 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. Diets and ingredients were also analyzed for acid hydrolyzed ether extract (Method 2003.06, AOAC International, 2007) on an automated analyzer (Soxtec 2050; FOSS North America, Eden Prairie, MN), ADF (Method 973.18, AOAC International, 2007), NDF (Holst, 1973), and AA (Method 982.30 E [a, b, and c]; AOAC International, 2007). Diet and ingredient samples were also analyzed for Ca and P 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, ingredients, and bones were analyzed for ash (Method 942.05; AOAC

International, 2007). Ingredients were also analyzed for phytate concentration (Ellis et al., 1977) and glucosinolates (method MGLUC-01, SunWest Food Laboratory Ltd, Saskatoon, SK).

Calculations and Statistical Analysis

At the conclusion of the experiment, pigs were weighed and data were summarized to calculate ADG, ADFI, and G:F. Percent bone ash was calculated by dividing ashed bone weight by defatted bone weight. The concentrations of non-phytate- and phytate-bound P in CM and SBM were calculated at previously described (Rojas and Stein, 2012). Glucosinolate content in the diets was calculated by multiplying the total analyzed glucosinolate concentration of the CM used by the inclusion level of that CM in the diet. Data for ADG, ADFI, G:F, organ weights, bone ash, CBC, T3 and T4 levels, and BUN were analyzed using the PROC MIXED procedure of SAS (SAS inst. Inc., Cary, NC) with pen as the experimental unit. The model included treatment as the fixed effect and block as the random effect. Least squares means were calculated for each independent variable. Linear and quadratic effects of inclusion level of canola meal were determined using orthogonal contrasts. Significance was assessed at $P \leq 0.05$ and tendencies were determined at $0.05 < P \leq 0.10$.

RESULTS

Growth Performance

All pigs readily consumed their assigned diets; however, a total of 10 pigs were removed from the experiment because they became sick or lame. One pig was on the control diet, 2 pigs were from 20% diets, 3 from 30% diets, and 4 from 40% diets. None of the pig removals appeared to be treatment related. There were no significant differences in initial BW among pigs assigned to the 12 dietary treatments (Table 5.5). Feed intake decreased linearly ($P < 0.05$) with

increased inclusion of CM, regardless of variety. Average daily gain of pigs fed CMA tended to increase if 10 or 20% CM was used (quadratic, $P = 0.06$), but for CMB and the CM-CV, no differences among treatments were observed for ADG. The G:F improved (linear, $P < 0.05$) with increasing inclusion of CMA or CM-CV in the diets, but inclusion of CMB in the diets had no impact on G:F. Final BW was not affected by dietary treatments.

Organ, Bone, and Blood Characteristics

Liver weight tended to increase (linear, $P = 0.06$) for pigs fed diets containing CMA, and increased (linear, $P < 0.05$) for pigs fed CMB, but inclusion of the CM-CV in the diets did not influence liver weight (Table 5.6). Thyroid gland weight increased (linear, $P < 0.05$) for pigs fed diets containing CMA, but inclusion of CMB or the CM-CV did not influence thyroid gland weight. There were no effects of feeding CM on heart weight, bone weight, CBC, or BUN or on kidney weight for CMA or CMB. However, kidney weights from pigs fed diets containing the CM-CV decreased (linear, $P < 0.05$) with increasing inclusion of CM in the diets. Ashed bone weight tended to increase (linear, $P = 0.10$) for pigs fed diets containing CMA, but no effects on ashed bone weight were observed for pigs fed diets containing CMB or the CM-CV. Bone ash percent increased (linear, $P < 0.05$) for pigs fed diets containing CM, regardless of the variety. Levels of both T3 and T4 decreased (linear, $P < 0.05$) for pigs fed diets containing CMA. Levels of T3 also decreased (linear, $P < 0.05$) for pigs fed diets containing CM-CV. Levels of T4 tended to decrease (linear, $P = 0.07$) for pigs fed diets containing CMB, but no effects on T4 levels were observed for pigs fed diets containing the CM-CV.

DISCUSSION

Composition of Ingredients

The CM-CV had lower concentrations of DM, CP, GE, and most AA compared with published values for solvent-extracted CM. Likewise, concentrations of NDF and ADF were less than values in the literature (NRC, 2012).

Canola meals A and B were produced from high-protein varieties of canola and, therefore, had greater CP and AA concentrations than CM-CV. These high-protein varieties are yellow-seeded *Brassica napus*. Compared with black-seeded *B. napus*, the yellow seeds are larger and have a thinner hull. Simbaya et al. (1995) reported that the protein content of the yellow seeds was 3.8 percentage units greater than in the black seeds, but Slominski et al. (2012) reported a 6 percentage unit difference between black-seeded and yellow-seeded canola. The reason the difference observed in this study was more than 10 percentage units may be that the CP in CM-CV was less than in the CM-CV used by others. Values analyzed for the high-protein CM were in agreement with or slightly less than previously published values (Simbaya et al., 1995; Slominski et al., 2012; Sanjayan, 2013).

In addition to greater protein content, yellow-seeded canola varieties contain less fiber. This is partly due to the thinner hull, which, in addition to the larger seed, results in the hull fraction accounting for less of the total seed mass. Analyzed values for NDF were in agreement with those of Slominski et al. (1994) and were approximately 7 percentage units less than those of CM-CV. This is in accordance with work by Simbaya et al. (1995), who reported that dietary fiber content in yellow-seeded CM was 6 percentage units less than in black-seeded CM.

One of the main reasons for limited use of CM in swine diets is the high level of glucosinolates. Although canola refers to varieties of rapeseed that are already “low” in

glucosinolates (<30 $\mu\text{mol/g}$), high inclusion rates can cause deleterious effects on growth performance. The high-protein CM used in this study contained almost twice the levels of glucosinolates compared with the CM-CV.

Growth Performance

The lack of a difference in ADG between low levels of CM and the control diet is not consistent with data by Castell (1977) who concluded that pigs fed rapeseed meal had poorer ADG compared with pigs fed SBM, but the present observations are in agreement with more recent reports in which it was indicated that feeding up to 25% CM does not affect growth performance (King et al., 2001). The decrease in ADFI observed in this experiment is consistent with Baidoo et al. (1987), who conducted 4 experiments feeding graded inclusion levels of CM to pigs and reported decreased ADFI with increased CM inclusion. The observation that feed efficiency was improved for all pigs fed CM compared with pigs fed the control diet is in agreement with results of previous research (Castell, 1977). The reason G:F increased with increasing inclusion of CM is that ADG values were not different regardless of inclusion levels, but ADFI decreased as CM concentration increased. A potential explanation for this observation is that while the pigs gained the same, body composition may have been changed. It is unlikely that ME in the CM was underestimated because values that had been determined in the same batches of CM and SBM were used in diet formulations. The decrease in ADFI for pigs fed diets containing CM may be explained by the increase in glucosinolates in the diets, which cause a bitter taste. Schöne et al. (1997) recommended that diets not exceed 2 $\mu\text{mol/g}$ glucosinolates. Only the diets with 10% inclusion of high-protein CM contained glucosinolate levels below that recommendation, whereas the CM-CV is within that limitation up to 20% inclusion.

Organs

The increase in liver weight observed for pigs fed diets containing CMA may be due to a reduction in protein quality in the diets containing high-protein CM. In addition, the diets were not balanced nitrogenously, so increasing inclusion of CM increased the CP concentration in the diet. This indicates that there may have been an increase in the frequency of the urea cycle, causing the liver to work harder and build muscle, resulting in increased liver weights. These observations are in contrast to reports from previous studies indicating no change in liver weight of pigs fed diets containing CM (Slinger, 1977; Busato et al., 1991; Thomke et al., 1983).

The increased weight of the thyroid gland with increasing inclusion of CM is in agreement with studies published by Mullan et al. (2000) and Thomke et al. (1983) for conventional type CM. On the contrary, Busato et al. (1991) and King et al. (2001) reported that CM had no effect on thyroid gland weight. All CM diets except the 10% CMA diet produced greater thyroid gland weights than the control diet, which is in agreement with Slinger (1977), who showed an increase in thyroid gland weights of pigs fed CM over those fed a corn-SBM diet. The increase for pigs fed CMA may be due to the higher level of glucosinolates compared to the CM-CV. The lack of difference seen for pigs fed CMB is likely because there was no 40% inclusion level of this meal. However, there was a numerical increase within the levels included, indicating significance would have been observed with the additional inclusion level ($P = 0.11$).

Increased thyroid gland weight may be due to a lack of iodine to the thyroid gland, which can be impaired by goitrogens in CM (Underwood, 1977). Sihombing et al. (1974) conducted 4 studies to investigate the effect of iodine supplementation on thyroid gland weight in pigs with induced hypothyroidism. Results of all 4 studies indicated that thyroid gland weights were reduced with increasing iodine supplementation. However, iodine was included in the vitamin-

micromineral premix that was used in this experiment, which may have ameliorated the effects of glucosinolates on weight of the thyroid glands.

The lack of difference observed in the kidneys of pigs fed CM diets supports previous research by Thomke et al. (1983) using conventional type CM. Additionally, the lack of effect of treatment on heart weight agrees with research conducted by Slinger (1977).

Bones

Though the thyroid gland data indicate a potential shortage of iodine, the lack of a difference in bone weights indicates that there was not enough of an iodine deficiency to affect bone synthesis. The increase of bone ash percent with increasing inclusion of all 3 CM is likely a result of the increasing dietary P concentration. It may also indicate that the Ca in the CM is better digested than inorganic Ca. However, this is unlikely because the ATTD of Ca in CM is less than the ATTD of Ca in calcium carbonate (Stein et al., 2011; González-Vega et al., 2013). Bragg and Seier (1974) observed greater Se content and availability in CM compared to SBM. Only CMA had greater Se content than SBM in this experiment, which may have accounted for the increase in bone ash percent seen for pigs fed that diet. Research where CM completely replaced SBM indicated no effect on bone magnesium content (Leeson et al., 1987), but it is possible that ATTD of another mineral is better in CM than SBM, which may contribute to the increase in bone ash percent seen for pigs fed diets containing the other 2 CM.

Blood

The reduced serum concentrations of T3 and T4 in pigs fed CM compared with pigs fed the control diet are in agreement with results of previous research (Bowland, 1975; Aherne and Lewis, 1978; Busato et al., 1991). Reduced thyroid hormone levels in combination with increased thyroid gland weight are indicative of impairment of thyroid function due to

glucosinolates in the diets. The reason there was less of a difference in serum levels of T3 and T4 between diets containing CM-CV and the control diet, is that the CM-CV had a reduced concentration of glucosinolates compared with the high-protein CM. The thyroid hormones are responsible for normal muscle function, including growth and development, and a deficiency may result in growth depression (Hocquette et al., 1998). The decreased thyroid hormone concentrations from the CM diets that were observed in this experiment, however, were not low enough to reduce growth performance of pigs fed those diets.

There was no difference in CBC among treatments (Table 5.7). There was also no difference in BUN among treatments, indicating that all diets were balanced for AA to the same degree. Synthetic AA were used in diet formulation to obtain this balance.

In conclusion, high-protein or conventional CM can be included in diets for weanling pigs from 2 wk post-weaning at a minimum of 20% without reducing growth performance or negatively affecting organ, bone, or blood characteristics. However, greater inclusion levels may reduce performance due to reduced feed intake.

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TABLES

Table 5.1. Analyzed nutrient composition of ingredients (as-fed basis)¹

Item	Canola meal A	Canola meal B	Conventional canola meal	Soybean meal
GE, kcal/kg	4,450	4,403	4,172	4,222
DM, %	91.24	91.13	89.90	88.15
CP, %	45.69	46.97	35.10	46.67
Ash, %	6.88	6.10	7.98	5.57
Ether extract, acid hydrolyzed, %	3.48	3.28	3.77	2.48
NDF, %	18.32	17.90	25.04	8.23
ADF, %	12.66	10.95	17.53	4.81
Ca, %	0.64	0.51	1.25	0.29
P, %	1.26	1.16	1.16	0.57
Phytate, %	3.57	3.20	2.69	1.43
Phytate-bound P ² , %	1.01	0.90	0.76	0.40
Non-phytate-bound P ³ , %	0.25	0.26	0.40	0.17
Indispensable AA, %				
Arg	2.79	2.87	2.09	3.43
His	1.23	1.23	0.91	1.24
Ile	1.77	1.89	1.35	2.32
Leu	3.18	3.31	2.53	3.73
Lys	2.61	2.67	2.02	3.07
Met	0.91	0.91	0.68	0.69
Phe	1.80	1.90	1.38	2.45
Thr	1.85	1.84	1.49	1.82
Trp	0.67	0.71	0.46	0.68
Val	2.23	2.48	1.72	2.50
Dispensable AA, %				
Ala	1.88	1.92	1.50	2.02

Table 5.1 (cont.)

Asp	3.00	3.35	2.44	5.34
Cys	1.21	1.19	0.82	0.68
Glu	7.36	7.45	5.41	7.86
Gly	2.18	2.20	1.69	1.98
Pro	2.81	2.84	2.08	2.29
Ser	1.74	1.67	1.32	2.03
Tyr	1.20	1.24	0.96	1.75

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

³Non-phytate-bound P was calculated as the difference between total P and phytate-bound P.

Table 5.2. Analyzed glucosinolate composition of ingredients (as-fed basis)¹

Item, $\mu\text{mol/g}$	Canola meal A	Canola meal B	Conventional canola meal
Progoitrin	4.244	3.624	2.105
Glucoalyssin	0.909	0.582	0.423
Gluconapoleiferin	0.695	0.662	0.423
Gluconapin	1.952	2.276	1.393
4-hydroxyglucobrassicin	4.784	4.121	1.845
Glucobrassicinapin	0.790	0.752	0.591
Glucoerucin	0.935	0.928	0.955
Glucobrassicin	0.368	0.521	0.256
Gluconasturtiin	0.336	0.295	0.437
Neoglucobrassicin	0.473	0.456	0.266
Total Glucosinolates	15.486	14.217	8.694

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

Table 5.3. Ingredient composition of experimental diets (as-fed basis)¹

Ingredient, %	Diet											
	Control	Canola meal A				Canola meal B			Conventional canola meal			
	-	10%	20%	30%	40%	10%	20%	30%	10%	20%	30%	40%
Ground corn	55.61	51.54	47.42	43.33	39.11	52.01	48.35	44.62	49.55	43.36	37.19	31.05
Canola meal A	-	10.00	20.00	30.00	40.00	-	-	-	-	-	-	-
Canola meal B	-	-	-	-	-	10.00	20.00	30.00	-	-	-	-
Conventional canola meal	-	-	-	-	-	-	-	-	10.00	20.00	30.00	40.00
Soybean meal (47% CP)	28.00	21.00	14.00	7.00	-	21.00	14.00	7.00	22.50	17.00	11.50	6.00
Whey powder, dried	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Phytase premix ²	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choice white grease	-	1.25	2.55	3.80	5.15	0.75	1.55	2.40	1.90	3.90	5.90	7.85
Limestone	1.30	1.20	1.08	0.95	0.83	1.22	1.14	1.05	1.00	0.70	0.40	0.10
Lysine HCl	0.25	0.23	0.22	0.2	0.19	0.24	0.22	0.21	0.25	0.26	0.27	0.28
DL-Met	0.07	0.03	-	-	-	0.03	-	-	0.04	0.03	0.01	-

Table 5.3 (cont.)

L-Thr	0.05	0.03	0.01	-	-	0.03	0.02	-	0.04	0.03	0.01	-
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Mecadox premix ³	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin-mineral premix ⁴	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Phytase premix (Optiphos 2000, Enzyvia LLC, Sheridan, IN) at 0.02% inclusion provided 400 units of phytase per kilogram of complete diet.

³The Mecadox premix (Phibro Animal Nutrition, Teaneck, NJ, USA) provided 50 mg per kilogram complete diet of Carbadox.

⁴Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 5.4. Analyzed nutrient composition of experimental diets (as-fed basis)¹

Item	Diets											
	Control	Canola meal A				Canola meal B			Conventional canola meal			
	-	10%	20%	30%	40%	10%	20%	30%	10%	20%	30%	40%
GE, kcal/kg	3,875	3,981	4,062	4,140	4,274	3,869	4,085	4,056	3,989	4,142	4,376	4,436
DM, %	88.74	89.16	89.51	90.15	90.64	89.40	89.99	87.78	89.50	89.98	90.28	90.66
CP, %	22.64	21.22	22.68	24.98	25.81	21.82	22.19	25.32	22.46	23.48	22.44	22.37
Ash, %	5.20	5.50	5.77	6.01	5.66	5.93	6.07	5.88	5.39	5.77	5.53	5.68
AEE ² , %	2.59	3.01	5.85	6.18	7.20	3.07	4.56	4.73	2.93	3.72	8.27	9.71
NDF, %	6.00	7.50	8.36	9.85	11.05	7.20	8.43	9.39	7.38	9.11	10.66	13.04
ADF, %	2.87	3.53	3.93	4.67	5.52	3.03	3.62	4.37	3.55	5.25	6.12	8.00
Ca, %	1.13	1.01	1.02	1.03	0.96	1.06	0.98	0.97	1.02	0.84	0.79	0.75
P, %	0.48	0.53	0.58	0.64	0.73	0.51	0.58	0.63	0.54	0.56	0.62	0.66
Total glucosinolates ³ , µmol/g	0	1.55	3.10	4.65	6.19	1.42	2.84	4.27	0.87	1.74	2.61	3.48
Indispensable AA, %												
Arg	1.29	1.19	1.33	1.35	1.30	1.29	1.37	1.42	1.36	1.34	1.34	1.29
His	0.52	0.50	0.57	0.61	0.59	0.54	0.59	0.62	0.56	0.56	0.57	0.57
Ile	0.86	0.81	0.89	0.94	0.89	0.89	0.92	0.96	0.92	0.89	0.92	0.88
Leu	1.80	1.64	1.80	1.87	1.73	1.82	1.88	1.93	1.83	1.80	1.83	1.73
Lys	1.31	1.27	1.49	1.47	1.40	1.42	1.51	1.54	1.62	1.49	1.48	1.53

Table 5.4 (cont.)

Met	0.42	0.36	0.43	0.46	0.45	0.39	0.42	0.47	0.45	0.42	0.46	0.44
Phe	0.98	0.89	0.97	0.98	0.90	0.98	1.01	1.02	1.01	0.98	0.99	0.93
Thr	0.88	0.81	0.91	0.93	0.90	0.86	0.92	0.98	0.92	0.94	0.93	0.93
Trp	0.27	0.28	0.30	0.30	0.28	0.27	0.28	0.30	0.25	0.24	0.28	0.30
Val	0.98	0.95	1.07	1.16	1.13	1.04	1.11	1.20	1.05	1.06	1.10	1.09
Dispensable AA, %												
Ala	1.06	0.98	1.11	1.14	1.07	1.09	1.14	1.18	1.10	1.10	1.11	1.08
Asp	2.09	1.80	1.88	1.80	1.58	1.95	1.97	1.94	2.08	1.97	1.89	1.74
Cys	0.30	0.33	0.42	0.49	0.51	0.36	0.43	0.49	0.36	0.38	0.39	0.43
Glu	3.50	3.33	3.72	3.92	3.80	3.59	3.86	4.05	3.64	3.64	3.67	3.57
Gly	0.87	0.88	1.05	1.12	1.13	0.96	1.06	1.16	0.97	1.04	1.06	1.08
Pro	1.18	1.15	1.33	1.47	1.47	1.26	1.42	1.49	1.29	1.32	1.34	1.36
Ser	0.95	0.83	0.91	0.88	0.82	0.90	0.94	0.95	0.97	0.94	0.92	0.88
Tyr	0.68	0.59	0.65	0.64	0.60	0.67	0.68	0.68	0.69	0.68	0.68	0.65
Total AA	20.10	18.75	21.06	21.82	20.85	20.47	21.74	22.67	21.27	21.01	21.21	20.76

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²AEE = Acid hydrolyzed ether extract.

³Values for total glucosinolates were calculated rather than analyzed by multiplying the analyzed value in each source of canola meal by the inclusion of that canola meal in the diet. It was assumed corn and soybean meal contained no glucosinolates.

Table 5.5. Growth performance of weanling pigs fed diets containing graded inclusion levels of 3 different canola meals^{1,2}

Item	Diets													P-value					
													SEM	Canola meal A		Canola meal B		Conventional canola meal	
	Control	Canola meal A				Canola meal B			Conventional canola meal					Lin. ³	Q ³	Lin.	Q	Lin.	Q
	-	10%	20%	30%	40%	10%	20%	30%	10%	20%	30%	40%							
Initial BW, kg	9.9	9.9	10	9.9	9.9	10.1	10.0	9.8	10.0	9.8	9.8	9.9	0.56	0.983	0.886	0.853	0.703	0.926	0.930
ADFI, kg	0.96	1.00	0.94	0.87	0.84	1.00	0.94	0.88	0.98	0.94	0.90	0.84	0.04	<0.001	0.170	0.02	0.072	0.012	0.218
ADG, kg	0.56	0.60	0.60	0.55	0.54	0.59	0.57	0.54	0.59	0.61	0.58	0.57	0.02	0.136	0.056	0.254	0.173	0.951	0.108
G:F	0.59	0.60	0.64	0.64	0.64	0.59	0.60	0.62	0.60	0.65	0.65	0.68	0.02	0.031	0.599	0.133	0.430	0.001	0.960
Final BW, kg	21.8	22.4	22.6	21.5	21.2	22.4	21.9	21.3	22.3	22.6	22.1	21.8	0.90	0.417	0.304	0.466	0.388	0.965	0.404

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Data are least squares means of 8 observations for all diets.

³Lin. = linear effect of each canola meal; Q = quadratic effect of each canola meal.

Table 5.6. Organ, bone, and blood characteristics of weanling pigs fed diets containing graded inclusion levels of 3 different canola meals^{1,2}

Item	Diets													P-value					
	Control	Canola meal A				Canola meal B			Conventional canola meal				SEM	Canola meal A		Canola meal B		Conventional canola meal	
	-	10%	20%	30%	40%	10%	20%	30%	10%	20%	30%	40%		Lin. ³	Q ³	Lin.	Q	Lin.	Q
Organs, as % of BW																			
Liver	2.84	2.83	2.90	2.98	3.05	3.17	3.05	3.34	2.89	2.96	2.85	2.86	0.10	0.057	0.600	0.024	0.861	0.975	0.500
Heart	0.52	0.52	0.53	0.53	0.53	0.55	0.54	0.52	0.55	0.53	0.53	0.52	0.02	0.484	0.648	0.917	0.106	0.570	0.259
Kidneys	0.59	0.58	0.57	0.51	0.57	0.57	0.58	0.58	0.61	0.56	0.51	0.48	0.03	0.248	0.415	0.811	0.649	<0.001	0.423
T. Gl. ^{4,5}	12.1	11.3	13.1	13.4	15.0	12.7	12.0	15.7	12.6	14.0	14.4	12.4	1.20	0.040	0.445	0.106	0.259	0.556	0.189
Bones																			
Bone Wt., g	3.73	3.63	3.59	3.79	3.93	3.28	3.51	3.70	3.64	3.39	4.13	3.19	0.37	0.460	0.427	0.890	0.144	0.362	0.409
Ash Wt., g	1.72	1.71	1.71	1.86	1.91	1.58	1.72	1.81	1.77	1.68	1.96	1.59	0.16	0.099	0.506	0.286	0.211	0.831	0.231
Ash, %	46.1	47.7	48.3	49.3	48.8	48.5	49.5	49.2	48.5	49.9	47.5	50.2	0.86	0.017	0.251	0.003	0.069	<0.001	0.143
Blood																			

Table 5.6 (cont.)

T3 ⁶ , ng/mL	1.22	0.96	0.92	0.91	0.88	0.99	0.98	0.94	1.00	1.03	1.05	0.83	0.11	0.009	0.153	0.148	0.477	0.021	0.996
T4 ⁷ , ng/mL	38.0	36.5	33.7	33.4	31.2	36.3	36.4	33.3	37.3	36.1	36.0	35.4	2.82	<0.001	0.850	0.069	0.721	0.125	0.770
BUN ⁸ , mg/dL	11.5	10.1	10.2	11.2	11.6	11.4	10.2	10.9	9.6	10.6	8.9	9.3	1.01	0.676	0.152	0.375	0.577	0.115	0.651

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Data are least squares means of 8 observations for all diets.

³Lin. = linear effect of each canola meal; Q = quadratic effect of each canola meal.

⁴T. Gl. = thyroid gland.

⁵Weight of thyroid glands and SEM multiplied by 1000.

⁶T3 = triiodothyronine.

⁷T4 = thyroxine.

⁸BUN = blood urea nitrogen.

Table 5.7. Complete blood count for weanling pigs fed diets containing graded inclusion levels of 3 different canola meals^{1,2}

Item	Diets												<i>P</i> -value						
	Control	Canola meal A				Canola meal B				Conventional canola meal				Canola meal A		Canola meal B		Conventional canola meal	
	-	10%	20%	30%	40%	10%	20%	30%	40%	10%	20%	30%	40%	SEM	Lin. ³	Q ³	Lin.	Q	Lin.
WBC, 10 ³ /μL ⁴	24.3	15.1	17.8	17.7	17.2	19.2	17.8	21.9	17.4	17.9	20.4	19.3	2.74	0.21	0.18	0.54	0.15	0.37	0.17
NEU, 10 ³ /μL ⁴	7.08	6.54	6.90	6.15	6.59	5.50	6.80	7.07	6.50	7.56	6.81	6.09	1.25	0.84	0.96	0.76	0.30	0.58	0.63
LYM, 10 ³ /μL ⁴	16.6	7.65	9.75	10.8	9.64	12.9	10.1	13.5	9.85	9.28	12.1	11.6	2.15	0.17	0.12	0.34	0.21	0.39	0.08
MONO, 10 ³ /μL ⁴	0.62	0.69	0.76	0.59	0.60	0.66	0.78	1.14	0.81	0.85	1.05	1.08	0.25	0.81	0.62	0.10	0.45	0.27	0.85
NEU/LYM	0.48	0.98	1.07	0.85	0.86	0.52	0.79	0.63	0.90	0.86	0.68	0.60	0.25	0.45	0.21	0.17	0.40	0.95	0.15
RBC, 10 ⁶ /μL ⁴	6.80	6.45	6.57	6.58	6.62	6.47	6.76	6.57	6.53	6.48	6.64	6.84	0.20	0.80	0.27	0.64	0.69	0.70	0.03
HGB, g/dL ⁴	12.1	11.9	12.2	12.0	11.9	11.7	11.7	11.8	11.7	11.8	12.0	12.4	0.29	0.63	0.76	0.39	0.26	0.28	0.07
HCT, % ⁴	37.2	37.2	37.2	36.7	36.6	36.9	36.2	36.6	36.1	36.4	37.0	38.6	0.82	0.45	0.84	0.42	0.61	0.16	0.07
MCV, fL ^{4,5}	55.4	57.6	56.7	55.9	55.3	57.3	53.8	56.0	55.5	56.2	55.8	56.7	1.19	0.51	0.13	0.64	0.84	0.12	0.80
MCH, pg ⁴	18.1	18.4	18.6	18.2	17.9	18.1	17.3	18.0	18.0	18.2	18.0	18.3	0.39	0.72	0.13	0.60	0.36	0.30	0.76
MCHC, g/dL ⁴	32.6	31.9	32.8	32.6	32.5	31.7	32.3	32.2	32.5	32.5	32.4	32.2	0.27	0.40	0.85	0.78	0.12	0.18	0.77
RDW, % ⁴	25.0	22.0	24.3	22.8	23.4	23.1	23.7	21.6	23.7	23.3	23.3	22.9	0.80	0.33	0.23	0.02	0.90	0.08	0.43

¹Canola meal A and canola meal B were sourced from Dow AgroSciences LLC, Indianapolis, IN; conventional canola meal

and soybean meal were sourced from the Univ. of IL, Urbana, IL.

²Data are least squares means of 7-8 observations for all diets.

³Lin. = linear effect of each canola meal; Q = quadratic effect of each canola meal.

⁴WBC = white blood cell; NEU = neutrophil; LYM = lymphocyte; MONO = monocyte; RBC = red blood cell; HGB = hemoglobin; HCT = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width.

⁵fL= femtolitre (10^{-15} L).

CHAPTER 6

CONCLUSIONS

This research focused on the effects of feeding either of 2 high-protein CM to chicks and pigs compared to conventional CM and SBM. High-protein CM contains more CP and less fiber than conventional CM, indicating improved feeding value for swine and poultry. The intent of the experiments included in this thesis was to test that hypothesis by determining the P bioavailability and P digestibility of the high-protein CM, determining the effect of phytase on those measures, and determining the maximum inclusion levels in diets for pigs without reducing performance.

From the first experiment, conducted with chicks, it was concluded that P bioavailability was numerically greater in the high-protein canola meals and, thus, bioavailable P concentration was numerically greater in those meals as well. It was also concluded that inclusion of microbial phytase in diets can further increase the P bioavailability in high-protein canola meal. These results indicate better utilization of P in high-protein canola meals, allowing for less dietary P inclusion and reduced P excretion.

From the second experiment, conducted with pigs, it was concluded that there was no difference in apparent total tract digestibility (**ATTD**) of Ca or P or standardized total tract digestibility (**STTD**) of P between high-protein canola meals and conventional canola meal. However, it was also concluded that addition of microbial phytase to diets containing high-protein or conventional canola meal increased ATTD of Ca and P and STTD of P.

From the third experiment, utilizing weanling pigs, it was concluded that high-protein or conventional canola meals can be included in diets for weanling pigs at a minimum of 20% without reducing growth performance or negatively affecting organ, bone, or blood

characteristics. However, greater inclusions may result in reduced growth performance due to reduced feed intake.

Results of these experiments suggest that further breeding and selection for high-protein canola meals with reduced fiber and other anti-nutritional factors may result in improved performance and increased dietary inclusion levels compared with conventional canola meal.