STANDARDIZED AND TRUE TOTAL TRACT PHOSPHORUS DIGESTIBILITY IN CANOLA MEALS (*BRASSICA NAPUS* BLACK AND *BRASSICA JUNCEA* YELLOW) FED TO GROWING PIGS

By

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ABSTRACT

Two experiments were conducted to determine the apparent (ATTD), standardized (STTD) and true total tract digestibility (TTTD) of phosphorus (P) and ATTD of calcium (Ca) in *Brassica napus* black (BNB) and *Brassica juncea* yellow (BJY) canola meal (CM) fed to growing pigs. In Experiment 1, eight semi-purified diets containing graded levels of P i.e., 0.8, 1.6, 2.4 and 3.3 g/kg of DM, from either BNB or BJY, were fed to growing pigs with an initial BW of 19.9 ± 0.22 kg (mean ± SEM) in a randomized complete block design. The total and basal EPL estimated with the regression analysis and P-free diet methods were 665 ± 0.03 and 209 ± 96 mg/kg of DMI, respectively. The TTTD and STTD of P were determined to be 33.3 and 31.0% for BNB and 32.0 and 28.3% for BJY, respectively. In Experiment 2, the effect of high level of phytase supplementation on the ATTD of P and Ca and STTD of P in growing pigs was studied. Forty-two growing pigs with an initial BW of 19.8 ± 1.22 kg (mean ± SEM) were randomly allocated to 7 dietary treatments with 6 pigs per treatment according to a completely randomised design in a factorial arrangement with the factors being: 1) 2 types of CM (BNB and BJY) and 2) 3 levels of phytase (i. e., 0, 500 and 2,500 U/kg). The ATTD of P increased from 39.1 to 69.3, and 78.0% in BNB and from 46.0 to 71.4, and 78.0% in BJY as phytase levels were added at 0, 500 and 2,500 U/kg, respectively. The STTD of P increased from 40.0 to 70.0, and 78.3% in BNB, and from 46.3 to 72.1, and 78.5% in BJY as phytase levels were added at 0, 500 and 2,500 U/kg. The basal EPL estimate was 117 ± 23.4 mg/kg DMI. Fecal P excretion in BNB and BJY were reduced by average value of 50.3 and 61.0% with the addition of both 500 and 2,500 FTU phytase

respectively. Results from these two experiments show that the values obtained for STTD and TTTD of P in BNB and BJY were similar.

DEDICATION

I am very much proud to dedicate this thesis to my dear husband Mr. Roshan Adhikari, my parents Som Raj Acharya and Sita Acharya, and my sisters Sushma Acharya, Garima Acharya and Asu Acharya.

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FOREWORD

This thesis was written in a manuscript style and is composed of two manuscripts. Manuscript 1 was presented partially at the ASAS-ASDA Midwest Meeting in Des Moines, Iowa (March, 2013). Part of manuscript 1 was presented at the ASAS-ASDA Joint Annual Meeting, Indianapolis, Indiana (July, 2013) and also at Western Nutritional Conference, Winnipeg, Manitoba (September, 2012). Part of the first manuscript was presented at the Canola Cluster meeting, Winnipeg, Manitoba (September, 2012). Both manuscripts were formatted to meet the Guidelines for the Journal of Animal Science manuscripts preparation. The titles of manuscripts are: True and standardized total tract phosphorus digestibility in canola meals from *Brassica napus* black and *Brassica juncea* yellow fed to growing pigs; and Super-dosing of phytase on phosphorus and calcium digestibility in meals from *Brassica napus* black and *Brassica juncea* yellow fed to growing pigs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fiber
ADG	Average daily gain
ADP	Adenosine diphosphate
AME	Apparent metabolizable energy
AMP	Adenosine monophosphate
ANF	Anti-nutritive factor
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
ATTD	Apparent total tract digestibility
BJY	*Brassica juncea* yellow
BNB	*Brassica napus* black
BW	Body weight
Ca	Calcium

CCAC	Canadian Council on Animal Care
CF	Crude fat
CM	Canola meal
CP	Crude protein
Cr_2O_3	Chromic oxide
Cu	Copper
D_A	Apparent fecal P digestibility values
DCP	Dicalcium phosphate
DDGS	Distiller dried grains with soluble
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
D_T	True fecal P digestibility values
ECF	Extracellular fluid
EE	Ether extract
EECM	Expeller extracted CM

EPCM	Expeller pressed CM
EPL	Endogenous phosphorus loss
Fe	Iron
Fe_2O_3	Ferric oxide
FGF-23	Fibroblast growth factor-23
U	Phytase unit
GE	Gross energy
GIT	Gastro-intestinal tract
HCl	Hydrochloric acid
HNO_3	Nitric oxide
ICP	Inductively coupled plasma spectroscopy
IP_3	Inositol triphosphate
K	Potassium
MCP	Monocalcium phosphate
ME	Metabolizable energy
MSP	Monosodium phosphate
Mn	Manganese

mg	milligrams
N	Nitrogen
NDF	Neutral detergent fiber
NRC	National Research Council
NSP	Non-starch polysaccharides
P	Phosphorus
PA	Phytic acid
P_{Ai}	Apparent fecal digestible phosphorus in i^{th} diet
P_{Di}	Total phosphorus in i^{th} diet
P_E	Endogenous phosphorus level
P_f	Phosphorus in feces
P_i	Phosphorus intake
PO_4^{-3}	Phosphate
P_r	Phosphorus retention
PTH	Parathyroid hormone
RCF	Relative centrifugal force

RNA	Ribonucleic acid
SAS	Statistical Analysis Software
SBM	Soybean meal
SD	Standard deviation
SECM	Solvent extracted canola meal
SE	Standard error
SEM	Standard error of mean
STTD	Standardized total tract digestibility
TDP	Total digestible phosphorus
TTTD	True total tract digestibility
Zn	Zinc

1.0 GENERAL INTRODUCTION

Currently, canola production in Canada is increasing and sits at approximately 9 million tonnes of canola seed per year. The Canola Council of Canada is targeting an increment of upto 15 million tonnes of canola seed per year by 2015 (Canola Council of Canada, 2009). Canola is an offspring of rapeseed, which is bred to have low levels of erucic acid and glucosinolates. The majority (> 95%) of the seed produced in Canada is *Brassica napus* black (BNB) which is one of the most important oil seed crops in Canada. Canola seed is crushed to yield approximately 42% oil which is used for human consumption and 58% meal which is used for livestock feeding (Unger, 1990). Canola meal (CM) is known for its high quality proteins that make it a potential feed for swine diets. Compared to dehulled soybean meal (SBM), CM contains less gross energy (GE), less protein and over three times as much fiber as SBM (Bell, 1993). However, it is richer than SBM in most of the B-vitamins like choline, biotin, folic acid, niacin, thiamin, riboflavin as well as in essential minerals (Bell, 1993). Although CM has a high concentration of crude protein (CP), it contains some anti-nutritional factors like glucosinolates, fibre, phytate and tannins that limit its utilization in swine feed. After the process of genetic selection and different heat treatment methods, the nutritional value of CM is found to be improved. Canada has discovered new cultivar named *Brassica juncea* yellow (BJY) which has minimal anti-nutritional factors and low fiber (Simbaya, 1995). *Brassica juncea* yellow contains lower neutral detergent fiber (NDF), acid detergent fiber (ADF) but higher protein that BNB (Newkirk et al., 1997). The main criterion for successful incorporation of alternative feedstuffs like these is their digestible nutrient profile that is required for diet formulation that will act as a main component to ensure

that growth performance and feed cost per unit of output in animals are maintained (Zijlstra and Beltranena, 2007). However, nutritional characteristics including P digestibility in the new cultivar, BJY have yet to be determined. Determination of available or digestible phosphorus (P) in CM can provide the opportunity to enhance utilization of this meal as an alternative source of minerals like P together with its protein and energy content.

Phosphorus is an important mineral in the body and it is required for many biological functions in swine. However, its excess excretion in the environment in the form of undigested P has captured a major concern. Swine manure usually has high levels of P mostly in the form of phytate P which is not well utilized by pig's body due to insufficient level of phytase to break the phytates down. At the same time, addition of inorganic P has led to the problem of high cost of feeding pigs. The exact match of P requirement and P supply in animal diets can reduce the problem of pollution and consequently minimise feed costs. With an increased focus on both the environment and cost of swine diets, it is imperative that nutritionists know proper inclusion levels of P in various pig rations. To assist in this, accurate determination of digestibility values of P-containing feed ingredients is essential.

Among the several studies to determine P utilization by animals that have been proposed and carried out are digestibility method and the slope-ratio assay. Slope-ratio assay provides an estimation of both digestive and post absorptive utilization of P at the tissue level whereas digestibility measurement approximates the availability of P in feed ingredients through measurement of its digestive utilization. The slope-ratio assay has some limitations as it is expensive for routine determination of P and also is time

consuming. The apparent total tract digestibility (ATTD) values of P are variable even within the same feed ingredients which are influenced by the P content in assay diets and by endogenous P loss (EPL). For example, ATTD of P in corn ranges from 12 to 48% (Jongbloed, 1991; Weremko et al., 1997) in growing pigs. For growing pigs, ATTD of P in SBM ranges from 15 to 35% (Jongbloed et al., 1991). The reasons behind this are apparent digestibility values are not always additive in a mixture of feed ingredients (Fan and Sauer, 2002) and these values underestimate the true digestibility values of P (Fan et al., 2001).

The procedures of determining EPL and calculating true or standardized P digestibility procedure have been developed which is possible by using the regression analysis and P-free diet methods. Previously, Fan et al. (2001) developed the method of regression analysis for estimating EPL and true total tract digestibility (TTTD) of P associated with SBM. The concept of regression analysis is to establish linear relationship between apparent digestible and total intake of assay nutrients in diets. The EPL in ileal digesta or feces can be determined by extrapolating the dietary intake of P to zero P intake (Shen et al., 2002; Ajakaiye et al., 2003). Recently, a novel procedure has been proposed where a P-free diet is fed to an animal and basal EPL is calculated which thus gives a value called standardized total tract digestibility (STTD) of P (Petersen and Stein, 2006). Both of these procedures involve measurement of values for either TTTD or STTD of P by the determination of total or basal EPL. Basal EPL can be calculated from pigs fed a P-free diet (Petersen and Stein, 2006) and total EPL can be calculated by feeding graded levels of P in diet measured by regression analysis (Fan et al., 2001)

Both TTTD and STTD values of P calculated by regression analysis and P-free methods in individual ingredients are believed to be additive in mixed diets. It is, therefore, believed that diets formulated based on values for either TTTD or STTD of P will more accurately meet the P requirements of the animals and due to accurate supplementation it minimizes P excretion in manure. The TTTD of P in BNB was studied previously (Akinmusire and Adeola, 2009). However, there are no reference data available till date that has determined STTD and TTTD of P in BJY.

The addition of phytase to the diet improves P digestibility by stepwise hydrolysis of the phytate molecule (Simons, 1990) and decreasing P excretion (Jongbloed et al., 1992). Supplementation of diets with the exogenous phytase has been found effective in improving P digestibility and reducing the excretion of this mineral in the manure (Guggenbuhl et al., 2012). Also, improvements in Ca digestibility have been reported in pigs when diets are supplemented with microbial phytase (Mroz et al., 1994; Kornegay and Qian, 1996).

There are limited reports on the addition of phytase to improve P digestibility in pigs fed CM. In a study done by Akinmusire and Adeola (2009), the effect of phytase supplementation was studied on ATTD and TTTD of P in BNB CM. However, no recent information is available regarding addition of phytase both in BNB and BJY. Also, there is a dearth of information about the concept of super-dosing of phytase in the digestibility of feed ingredients. In this research, we studied the STTD of P by supplementing both standard (500 U/kg feed) and higher level (2,500 U/kg feed) of phytase in the digestibility of BNB and BJY.

Therefore, the overall objectives of this research were:

1. To use linear regression analysis to estimate TTTD of P in growing pigs fed BNB and BJY.
2. To use P-free diet to estimate STTD of P in growing pigs fed BNB and BJY.
3. To compare the digestibility values of P obtained by P-free and linear regression analysis between BNB and BJY.
4. To determine and compare the effectiveness of standard and super-dose levels of phytase in improving the ATTD and STTD of P in BNB and BJY.

2.0 LITERATURE REVIEW

2.1 PHOSPHORUS

2.1.1 Biological roles of phosphorus in animals

Phosphorus is an essential nutrient in swine diets serving important functions as part of structural compounds in bone and in cell membranes, as a source of high free energy bonds in nucleotides, as a structural component of nucleic acids, as a component of many enzyme cofactors, and as a component in many metabolic pathways. Phosphorus can exist either in a trivalent or penta-valent form. However, it is commonly found in the body as phosphate (PO_4^{-3}) form. Approximately 1% of the mature body weight of the pig has P in the form of tissues and organs (Peo, 1991). Approximately 60-80% of P is located in skeletal tissues while the remaining 20% is located in soft tissues (Crenshaw, 2001). Majorly, P is involved in bone mineralization and teeth formation. In nature, most of the P is combined with oxygen in the form of phosphate (Anderson et al., 2006). Calcium (Ca) and P both play an important role in the development and maintenance of the skeletal system as well as in performing different physiological functions in the body (Kornegay, 1985; Crenshaw, 2001). Phosphorus is also an important component of phospholipids in cell membranes (Crenshaw, 2001). It acts as a buffer and helps in energy transfer processes in the form of coenzyme like adenosine triphosphate (ATP) and creatine phosphate (Cashman and Flynn, 1999). It is involved in a range of metabolic processes and is used in the synthesis of deoxy-ribonucleic acid (DNA) and ribonucleic acid (RNA) (Anderson et al., 2006). Another function of P is the phosphorylation of glucose because glucose can undergo glycolysis only if it is phosphorylated (Anderson et al., 2006).

2.1.2 Digestion, absorption and regulation of phosphorus

Intestinal phosphatases, such as alkaline phosphatase and intestinal phytase, break the phytate bonds and release inorganic P at the brush border of the enterocytes of the intestine. Phosphorus can be absorbed in the small intestines only if it has been hydrolyzed to its inorganic form (phosphate), and absorption occurs through 2 main mechanisms: Na^+- dependent and Na^+- independent absorption (Anderson, 1991). Most P absorption takes place in the small intestine, mainly in the jejunum (Metzler and Mosenthin, 2008), and the transport of P from the gut lumen to the enterocyte can be either passive or active.

The intestine and kidney are two important organs in the regulation of P homeostasis (Berndt and Kumar, 2009). Phosphorus homeostasis occurs by the controlled interactions of the intestine, bones, and renal tubules (Taylor and Bushinsky, 2009). The regulation of P is also directly affected by the parathyroid hormone (PTH), vitamin D, and dietary phosphate level (Marks, 2006; Taylor and Bushinsky, 2009). Parathyroid hormone is secreted from the cells of the parathyroid glands and has the cells in bone and kidney as its major target. When serum P levels are low (hypophosphatemia), plasma Ca levels are elevated that decreases secretion of PTH. As a result, renal inorganic P excretion is reduced. Hypophosphatemia also causes an increase in the concentration of renal 1,25 dihydroxyvitamin D [1,25(OH)2D3] and calcitriol, which results in increased P mobilization from bone and soft tissues, and may result in increased intestinal inorganic P and Ca absorption as well. When serum P levels are high (hyperphosphatemia), there is a decrease in plasma Ca concentration resulting in increased PTH and increased renal excretion of P in the urine. In a recent review, however, Taylor and Bushinsky (2009)

demonstrated the role of other possible regulatory mechanisms. Homeostasis of P may be regulated by phosphatonins, such as fibroblast growth factor 23 (FGF-23), which is a phosphaturic peptide that reduces production of 1,25(OH)2D3 and increases the expression of an enzyme (24-hydroxylase) that converts 1,25(OH)2D3 into forms that are less biologically active. This in turn causes increased P excretion from the kidney and also a decreased absorption of P in the intestines. As a consequence, serum P levels are reduced (Berndt and Kumar, 2007), and P uptake by renal cells is decreased by other phosphatonins such as fibroblast growth factor 7, frizzled-related protein 4, and matrix extracellular phospho-glycoprotein (Taylor and Bushinsky, 2009).

2.1.3. Phosphorus requirement in swine

There are mainly two basic methods to determine the P requirements for swine. These are: empirical method and factorial calculations. Empirical method of determining P requirement works on the basis of one or more easily measured characteristics like feed utilization of animals and daily weight gain of animal. The empirical method is used most commonly. However, the factorial approach is believed to be more accurate because its measurements account for availability, retention, and also for the obligatory losses of P in the body (Weremko et al., 1997). Empirical measurements are most common and response criteria such as bone ash or bone breaking strength have been used for many decades to estimate P requirements for pigs (Cromwell, 2009). Initially, P requirements for pigs were reported as total P but in recent years, the concept of available/digestible P has been used in diet formulation. To address issues such as over-excretion of P in manure, diets should be formulated based on available/digestible P that meets the P

requirements more precisely. The alternative would be to formulate swine feeds on the basis of TTTD or STTD of P. For this, a database of P requirements on the basis of either TTTD or STTD is required for swine feed formulation. According to NRC (2012), the total P requirement of 11- 25 kg, 25-50 kg, 50-75 kg and 75-100 kg of pigs are 0.6, 0.56, 0.52 and 0.47% respectively, whereas STTD of P requirements are 0.33, 0.31, 0.27 and 0.24%, respectively. The requirement reported by NRC are the best available estimation but the accuracy may be limited (Knowlton et al., 2004).

2.1.4 Sources of phosphorus in swine diet

Phosphorus is found in many forms based on their sources that can be fed to pigs (Pointillart, 1991). They are from either plant, animal or inorganic sources. In comparison to plant sources, feed ingredients of animal origin have higher digestibility of P from 67 to 90% (Traylor et al., 2005). Nearly two thirds of total P in swine feed ingredients occur as phytate P which is poorly available to pigs (Cromwell, 1980). Mono-gastric animals like pig and poultry lack sufficient amount of phytate breaking enzyme called phytase and thus the utilization of phytate form of P from feed ingredients is hindered. Due to this reason, phytate bound P is undigested by animals and the excess gets excreted in manure. Swine manure is rich in P because diets fed to pigs are commonly over supplied with inorganic P (Cromwell, 2005). Land application of swine manure, therefore, is an issue because of the potential environmental effects of this practice. Because of these effects, regulations are made to limit the amount of P that can be applied in the field, and this has led the swine industry to find solutions to reduce P excretion (Cromwell, 2005). On the other hand, inorganic P is expensive to add to swine diets. Accurate determination of

available P in plant feed can help in minimizing the environmental pollution and also can save the wastage of such expensive mineral that would be added to swine diets. It is important to determine the level of P intake and also the optimum inclusion level in the diets in order to meet the maximum performance of the animal. Thus, it is vital to realize the levels of P utilization in all ingredients as they vary greatly between ingredients and also, P interacts with other factors within the diet (Cromwell et al., 1972).

2.1.4.1 Plant sources

Commonly, the availability of P from ingredients of plant origin is low for mono-gastric animals due to two main reasons: 1. intrinsic P is mainly bound to a complex called phytate/phytic acid (PA) (Figure 1) and 2. mono-gastrics lack the enzyme phytase in sufficient amount needed to degrade phytate. Apparent P digestibilities of commonly used plant ingredients vary from 10 to 60% (Jongbloed, 1991; Weremko et al., 1997). Of the total P in cereal grains and oilseeds, 60-85% is bound in phytate (Raboy, 1997). The main ingredients in swine diets are cereals, oilseeds, and their by-products (Godoy et al., 2005). Phytate P accounts for at least one half of the total P. The portion of total P present as phytate in cereals is about 59-70%, 20-46% in legume seeds, and 34-66% in oil seed meals (Eeckhout and De Paepe, 1994) (Table 1). According to a review done by (Ravindran et al., 1994), phytate P, as a percentage of the total P, is slightly higher in corn (68%) than it is in soybean meal (60%). However, Weremko et al. (1997) found that the highest phytate content is in maize and wheat (70-73%) and the lowest content in rye and oat grain (60%). Wheat P and barley P is more bio-available due to the presence of endogenous phytase in them (Pointillart et al., 1987).

Table 1. Total phosphorus, phytate phosphorus and non-phytate phosphorus content in some swine feed ingredients[1]

Ingredients	Total P (%)	Phytate P (%)	Non-Phytate P (%)
Field peas	0.42	0.17	0.25
Soybean meal, dehulled	0.71	0.38	0.33
Soybean meal, solvent extracted	0.64	0.36	0.28
Corn	0.26	0.21	0.05
Wheat	0.39	0.22	0.17
Barley	0.35	0.22	0.13
Fish meal	3.04	0.18	2.85
Canola meal, expelled	1.15	0.87	0.28
Canola meal, solvent extracted	1.08	0.65	0.43

[1]Values are from NRC (2012) for all ingredients with the exception of fish meal whose estimates are from NRC (1998).

2.1.4.2 Inorganic sources

Inorganic P usually has a higher digestibility coefficient for pigs than phytate bound P found in vegetable feed ingredients (Petersen et al., 2011). These are readily available to pigs for complete digestion in their body. Due to this reason, inorganic P can compensate for low digestibility of P in vegetable feed sources (Petersen et al., 2011). Three inorganic sources that are commonly used in the swine feed industry are monosodium phosphate (MSP), monocalcium phosphate (MCP) and dicalcium phosphate (DCP). Relative bioavailability of P in inorganic sources is expressed as a percentage of the standard, to MSP or MCP (Table 2.) Relative bioavailability of P for various inorganic phosphates ranges from 20 to 100% (Weremko et al., 1997).

Table 2. Relative bioavailability of phosphorus in various inorganic phosphorus sources

Phosphate source	% P	bioavailability of P[1], %
Bone meal	12.5	80-90
Dicalcium phosphate	18.5	95-100
Monocalcium phosphate	21.1	100
Rock phosphate	9.05	30-50
Monosodium phosphate	24.9	100

[1]Expressed as a percentage of monosodium phosphate or monocalcium phosphate
National Research Council (2012)

2.1.5 Bioavailability study of phosphorus

The bioavailability of P in various feedstuffs of plant origin varies from 10 to 60%. The concept of biological availability or 'bioavailability', which is generally applied to P, is a measure of the degree to which a P source can support the physiological processes of an animal (Waldroup, 1996). To measure bioavailability is a difficult concept because numerous factors are involved in both the absorption and utilization of the mineral. Thus, the bioavailability of P in feed ingredients is estimated as (1) percent digestibility, where the difference between the amount of P consumed and excreted in feces (or collected from the distal ileum) is assumed to be available, and (2) relative bioavailability, which estimates the availability of P by comparing to a known standard of a highly available form (Cromwell, 1992).

2.1.5.1 Slope ratio method

The slope ratio method provides a combined estimation of digestive and post-absorptive utilization of P at the tissue level (Fan et al., 2001). This method was developed by Cromwell (1980) to determine the availability of P in feed ingredients for pigs where they determined the values by bone strength. Relative bioavailability is measured by comparing a highly digestible P-source (standard P-source) to an unknown source (test diet) using a slope-ratio procedure. In the slope ratio method, there is a basal diet, two or three levels of test diets, and two or three levels of the standard diet containing the highly digestible P-source. The basal diet has a low P content, and is used to determine a common starting point. The test P-sources are added to the basal diet at two or three graded levels in such a way that all diets have a P concentration that is below the pigs' requirement for P. Likewise, the standard diets consist of the basal diet plus the

standard P-source at two or three levels of P fed below the pigs' requirement for P. All diets are fed for a period of 4 to 6 weeks. At the conclusion of the experiment, pigs are killed and one or more bones (femur, metatarsal, and metacarpal) are harvested, and the bone ash or bone breaking strength is determined. Bone ash or breaking strength is then regressed on P-intake for each source of P. The slope of the regression line for each P-source is determined and an equation is used to calculate the bioavailability:

Relative bioavailability = 100(slope B/ slope A) (1)

where slope B is the regression slope for the test diets, and slope A is the regression slope for the standard diets (Cromwell, 1992).

Therefore, the availability of P in the test ingredient is expressed relative to the availability of the highly digestible P-source in the standard diets. Commonly, MSP is used as the reference material where the availability is 100% to the animals (Fan et al., 2001). Bone parameters like ash, P content and bone breaking strength and blood parameters like plasma P and alkaline phosphatase levels are measured (Sulabo, 2003). However, there are some limitations of this technique. The first limitation is the high variability between results, and the second being underestimation of availability of some ingredients used compared to the other digestibility studies (Ketaren et al., 1993). The procedure is expensive as it involves sacrifice of large number of animals and is time consuming (Fan et al., 2001).

The digestibility procedures report a digestibility value for P in the test ingredient whereas the slope-ratio assays report the relative bioavailability of P. This means that one source of P is expressed as a percentage of another standard source. Therefore, the relative bioavailability values cannot be compared between studies if different P-sources

were used as the standard. In addition, the digestibility procedures allow for the estimation of the quantity of P excreted in the feces from the pigs, which cannot be calculated from data, obtained using the slope-ratio procedure.

2.1.5.2 Digestibility studies of phosphorus

2.1.5.2.1 Apparent total tract digestibility of phosphorus

Due to the amount of phytate P present in feedstuffs of plant origin and intrinsic phytase, the value of digestibility varies (Weremko et al., 1997). Digestibility values are measured by determining the difference between P-intake and fecal excretion which is then divided finally by the P intake (Jongbloed, 1991; Bruce and Sundstol, 1995). Two major methods are used to make this determination (i.e., the difference method and the direct method). Digestibility of P has been measured as ATTD of P (Jongbloed et al., 1992). These values, however, show high variability within the same ingredient (Fan et al., 2001). Another disadvantage of ATTD values is the fact that they do not account for EPL, which is believed to result in these values not to be additive in mixed diets (Fan et al., 2001; Shen et al., 2002; Ajakaiye et al., 2003; Akinmusire and Adeola, 2009). In calculation of the apparent total tract digestibility values, animals should spend some time consuming the diet of interest in order to adapt the gastrointestinal system to it. During this pre-experimental period, animals are expected to adapt not only to the composition and amount of feed that will be provided, but also to the confinement conditions of the trial (e g., metabolism crates). Apparent digestibility values are affected by assay criteria that are used to select the levels of P in the diets and also by the different levels of EPL. However, both true and standardized digestibility values are additive in a mixture of feed ingredients and they are uniform within a single feed type. All the digestibility methods

involve the assessment of the nutrient concentration in feed and feces. Table 3 summarises the apparent, true and standardized P digestibility in common feed ingredients fed to pigs.

Table 3. Apparent, true and standardized total tract phosphorus digestibility (%) in different feed ingredients fed to pigs[1]

Ingredient	Apparent total tract digestibility	True total tract digestibility	Standardized total tract digestibility	References
Soybean meal				
Dehulled, solvent	39.0	-	48.0	Dilger and Adeola, 2006; Fan et al.,
extracted	60.0	-	66.0	2001; NRC, 2012
Enzyme treated	39.0	-	48.0	
Expelled	60.0	-	66.0	
Fermented				
Corn				
Yellow dent	26.0	-	34.0	NRC, 2012; Shen et al., 2002
DDGS	60.0	76.5	65.0	
Corn germ	33.0	-	37.0	
Barley	43.1	-	-	Thacker et al., 2004
Hulless	39.0	-	45.0	NRC, 2012
Peas	65.9	72.3	-	Stein et al., 2006

Field peas	49.0	-	56.0	
Canola meal	28.3 to 32.7	34.3	-	Akinmusire and Adeola, 2009
Full fat	28.0	-	32.0	NRC, 2012
Expelled	28.0	-	32.0	
Solvent extracted	28.0	-	32.0	
Wheat	43.4	-	-	Nyachoti et al., 2005
Wheat DDGS	56.0	-	61.0	
Wheat bran	46.0	-	56.0	
Wheat middlings	46.0	-	56.0	

[1]The values are based on fecal digestible P.

2.1.5.2.2 Endogenous phosphorus losses

Endogenous P losses are the P excreted from the body that is not of dietary origin. The dominating route of such excretion is via feces through gastrointestinal (GI) tract (Rodehutscord, 2001). These EPL are P present in the feces that comes from salivary, gastric, and biliary juices, and also from pancreatic secretions and sloughed mucosal cells (Jongbloed, 1987). However, the amount of EPL seems to be conflicting in a number of studies and the amount secreted is influenced by numerous factors. High P intake resulted in a slight increase in fecal endogenous losses of P in pigs (Clark, 1968; Whittemore, 1973; Cramer and McMillan, 1980). The Ca to P ratio also affects the amount of EPL. However, the results are conflicting. Whittemore (1973) found a reduction in EPL when Ca to P ratio was lowered while Hermes et al. (1983) found an increase in EPL in similar condition.

In growing pigs fed under normal feeding conditions, daily fecal excretion of P is estimated at 9-10 mg of endogenous P/kg live weight (Jongbloed, 1987; Jongbloed, 1991) The EPL can be measured indirectly by using regression technique (Fan et al., 2001) or directly by feeding a P-free diet and measuring the amount of P excreted in feces (Petersen and Stein, 2006). For the utilization of whole body P and homeostasis of P in body in growing pigs, the activities like secretion, recycling and fecal loss are much important (Ajakaiye et al. 2003). This variation may be attributed to the dietary factors like high fiber diet, ANFs, or animal factor which can induce diet-dependent EPL (Petersen and Stein, 2006).

Previous data of total EPL calculations using the regression technique has demonstrated great variability with values ranging from 70 to 840 mg/kg DMI (Shen et al., 2002; Dilger and Adeola, 2006; Pettey et al., 2006). In contrast, estimation of basal

EPL from pigs that are fed a P-free diet seems to be less variable with values ranging from 139 to 211 mg/kg DMI (Petersen and Stein, 2006; Widmer et al., 2007). When EPL is measured using a P-free diet, basal EPL is obtained, which can be used to correct ATTD values to obtain STTD values. These values are believed to be additive in mixed diets. This can be accomplished by formulating a P-free diet, but such a diet has never been successfully formulated. However, if a P-free protein source is identified, it may be possible to formulate such a diet. By feeding the P-free source, the endogenous P can be directly determined by measuring the total P output. To obtain TTTD the endogenous losses that constitutes of both basal and diet specific loss of P, called total EPL is used. It is believed that digestibility coefficients based on TTTD are also additive in a mixed ration than are values based on ATTD (Fan et al., 2001).

2.1.5.2.3 Regression analysis technique

This technique has been employed in many previous studies. By using this technique, EPL have been found in the range of 90-630 mg/kg DMI (Ajakaiye, 2003); 70-840 mg/kg DMI (Shen et al., 2002); 140-320 mg/kg DMI (Fan et al., 2001); and 70-800 mg/kg DMI (Jongbloed, 1987). The concept was developed previously to determine true amino acid (AA) digestibility (Fan and Sauer, 1997). Most of the previous data on endogenous losses were obtained by regressing several levels of P back to zero P-intake. The EPL can be determined by calculating the linear relationship between the digestible fecal or ileal P intake and the total dietary P intake. Also, in this case the intercept of the linear regression gives the estimate of EPL.

In this technique, animals are fed at least three or four semi-purified diets containing graded levels of P which are recovered in feces or in digesta. Fan et al. (2001)

have shown that the EPL decreases as the dietary P content increases. So, it is mandatory to provide the feeds with P level lower than required amount by pigs. This helps in accurate estimates of EPL values as well as TTTD of P in pigs.

2.1.5.2.4 P-free diet

One of the recent practices to determine STTD of P is to formulate a feed devoid of P and use it to calculate endogenous loss. Previous research by Petersen and Stein (2006) has demonstrated that gelatin-based diets fortified with crystalline AA are highly digestible to pigs. By feeding the gelatin-based diet to pigs, the only P that will be excreted is P of endogenous origin. If a feed phosphate is added to this P-free diet, the only P in the diet will originate from the feed phosphate.

Some recent studies of P digestibility values in swine feed have been completed using the concept of P-free diet (Petersen and Stein, 2006: Stein et al., 2006; Widmer et al., 2007; Almeida, 2010; Petersen et al., 2011). The reason this approach has been used so far is that, by feeding P-free diet to pigs, we can determine the endogenous loss which is basal EPL that can be used to calculate STTD of several feed ingredients knowing their available P. The basal endogenous P loss (mg/kg of DMI) is measured from pigs fed the P-free diet according to the equation given by Petersen and Stein (2006):

$$EPL\ (mg/kg\ DMI) = ([Pf/Fi] \times 1{,}000 \times 1{,}000), \quad (2)$$

where, EPL is the endogenous P loss, Pf is the fecal P output and Fi is the total feed intake (gm) from start of collection day to end day of collection.

The STTD of P is calculated using the equation given by Petersen and Stein (2006):

$$STTD\ (\%) = ([Pi - (Pf - EPL)/Pi]) \times 100, \quad (3)$$

where, STTD (%) is the standardized total tract digestibility of P.

2.2 CALCIUM

2.2.1 Biological roles of calcium in animals

Approximately 99% of Ca in the body is located in the skeleton. Calcium in the skeleton is found in the structural complex hydroxyappetite along with P. It serves as a Ca reservoir for the rest of the body. The remaining 1% of total body Ca is in soft tissue and the extracellular fluid (ECF) space including blood. Calcium is essential for normal secretion of many hormones and also is an essential factor for blood coagulation. The Ca in a given feedstuff may already be in solution or it may be solubilized through the actions of gastric enzymes and peristalsis.

2.2.2 Metabolism of calcium

The degree to which dietary Ca can be solubilized in the intestinal lumen is affected by many constituents within the diet. Calcium absorption occurs throughout the small intestine via an active trans-cellular process or via a para-cellular process (Bronner et al., 1986). Although the highest rate of absorption occurs in the duodenum, the ingested Ca spends more time in the ileum and the jejunum and probably the greatest part of Ca absorption takes place there.

Calcium transport is an active process which occurs in three steps: uptake of Ca by the brush border membrane, transport of Ca through the cell, and movement of Ca out of the cell and into the blood stream. Calcium enters the cell through a Ca channel travelling down an electrochemical gradient (Bronner et al., 1986; Caffrey and Farach-Carson, 1989). Movement of Ca out of the cell occurs up an electrochemical gradient which requires energy in the form of ATP. For the transportation of Ca, two Ca transporters have been identified. The first is a Ca-ATPase enzyme, which utilizes the energy derived from the hydrolysis of ATP to move Ca up its electrochemical gradient

and out of the cell (Garrahan and Rega, 1990). This is also known as the primary transport mechanism. The second is Ca/Na antiport system. In this system, the inward movement of Na provides the energy necessary for Ca to be moved out of the cell (Reeves, 1990). Calcium is regulated by 3 hormones: 1) parathyroid hormone, 2) vitamin D, and 3) calcitonin. In addition, prostaglandins, reproductive steroids, and some other hormones can also affect Ca metabolism. It has been shown that the balance of Ca is regulated solely by the intestine whereas the balance of P responded in a similar manner but is also modulated by renal action (Fernandez, 1995).

2.3. FACTORS AFFECTING CALCIUM AND PHOSPHORUS ABSORPTION

There are some factors on which Ca and P absorption depends. They are; 1) supply/concentration of Ca and P 2) ratio of Ca and P in the diet or Ca and P interaction (Crenshaw, 2000; NRC, 2012). Regarding dietary concentration of Ca and P, the source is more significant for P than for Ca. The reason for this is that the P in plant and cereal grains are in the form of phytate which is less or non-available to animals. Mono-gastric animals like pig have insufficient enzyme phytase, which hinders P availability to the animal. The amount of digestible/available P varies in different cereal grains and vegetable proteins.

Calcium and P interaction is one of the major factors to consider in P digestibility. This interaction affects the determination of requirement of P in animals. The ratio of Ca to P influences dietary availability of Ca and P that enters the portal circulation. A narrower Ca to P ratio (less than 1:1) can help in efficient utilization of P (Wu, 2008). According to the studies performed by Liu et al. (2000), higher values of Ca:total P ratio

(1.5:1 or above) have been shown to reduce P utilization for growing-finishing pigs. The author found that wider Ca to total P ratio values may result in some detrimental effect like: 1) the extra Ca forms an insoluble phytate complex that is not accessible for hydrolysis by phytase and 2) high dietary Ca increases the pH of the intestinal contents that decreases microbial phytase activity. A wider ratio of Ca to P can lead to lower P absorption through which growth and bone calcification may be hindered (Eeckhout et al., 1995; Hall et al., 1991). The grams of fecal Ca excreted daily were reduced 54% by lowering the Ca to total P ratio from 1.5:1 to 1.0:1 due to the decrease in Ca intake and the increase in Ca digestibility (Liu et al., 1998). Calcium and P requirements for metabolic process can be supplied by formulating diets on the basis of ratio of Ca to TDP (Liu et al., 1998). It is essential to measure total P to Ca ratio value to guide diet formulation for minimizing P excretion (Ontario Pork Producers' Marketing Board, 2011). The ratio of total Ca to total P between 1:1 and 1.2:1 for diets containing PA (grain-SBM diet) is recommended for better growth and bone function in pigs. A total Ca to total P of 2:1 or 3:1 is optimal for growth performance and the efficiency of utilization of dietary P in growing pigs (Jongbloed, 1987; Yin, 2005).

However, at present the suggested ratio of total Ca to total P is between 1:1 and 1.25:1 (NRC, 2012). Due to the difference in available P between the diets, determination of Ca to total P cannot be similar and effective. A ratio of Ca to total digestible P was suggested as 2:1 for optimal growth performance and well dietary utilization by pigs (Yin, 2005).

2.4 METHODS OF COLLECTING FECAL SAMPLE FOR DIGESTIBILITY STUDIES

2.4.1 The total collection method (Conventional digestion trial)

This method is also called 'quantitative collection', conventional', or 'marker to marker' collection (Irwin and E. W. Crampton, 1951; Schurch et al., 1952; Clawson, 1955; Bakker and Jongbloed, 1994). As the calculations are based directly on the total amounts of the nutrient present in feed and feces, it is also called direct method (Schneider and Flatt, 1975).

This method involves the collection of the total amount of feces and urine produced during the sample collection days. It requires keeping accurate records on the total amount of feed actually ingested by the animals (feed offered minus feed rejected). The daily feed intake has to be held constant which is accomplished by restricting the allowance to a level lower than the voluntary feed intake of the animal if fed *ad libitum* (Schurch et al., 1952). For the process of collection of feces, animals are individually confined in a special crate were they can freely lie down and get up, but not turn around. This crate is intended to prevent coprophagy and to separate feces from urine. Urine collections jars and feces screen are placed under the crate for separate collection of urine and feces. To visually separate the feces resulting from the feed consumed during the collection period, easily distinguishable markers are added to the feed. The most widely used substances for this purpose are Indigo carmine (a blue substance), ferric oxide ($Fe_2O_{3)}$ and chromic oxide (Cr_2O_3) (Agudelo-Trujillo, 2005). Such substance must sharply demarcate the feces without diffusing and does not have any physiological effects on the animal (Schneider and Flatt, 1975).

2.4.2 The index method

The index method is also referred as the ‘indicator’, ‘marker’, ‘reference’, ‘inert reference substance’, ‘tracer’ method, or the ‘ratio technique’ (McCarthy et al., 1974; Miller et al., 1978). The index method has been named the indirect method, although this last term has been applied to a very different methodology known as ‘digestibility by difference’ (Schneider and Flat 1975). The basic difference between the total collection method and the index method is that the total collection method calculates the digestibility coefficients based on the total amount of the nutrient measured in feed and feces, while the index method calculates them based on the relative concentrations of the nutrient and an indicator or marker substance in both feed and feces. The indicator is added to the feed and its concentration in feed and feces is determined in order to calculate the percent of the nutrients digested and absorbed. In order to calculate the digestibility of a nutrient by the index method, the indicator: nutrient ratio is determined both in feed and in feces. So, to determine the apparent digestibility of a nutrient by this method it is only required to know the concentrations of the indicator and the nutrient in both the diet and in the feces.

The most effective markers will be inert materials that are not digested or absorbed. Also, they should be non-toxic, not having physiological or psychological effects, mix well with the feed and remain uniformly distributed in the digesta. It should be non-essential for the animal and regularly and completely voided in the feces (Adeola, 2001). Grab sampling is done in index method (Schneider and Flatt, 1975; Adeola, 2001).

2.4.3 Total collection versus index method

2.4.3.1 Advantages and disadvantages

The advantage of index method over the total collection method is that it reduces time, labor and costs of the trial. In addition, the index method does not require the housing of pigs in metabolic crates and can be conducted in ordinary, non- expensive pens. However, when index method is applied in pens, the problem of coprophagy can arise, which is not seen in animals that are in crates. This may affect the concentration of such indicator affecting the results of digestibility. In case of balance trial, fecal and urine collection is facilitated by total collection in metabolic crates. The amount of urine retention after absorption can be calculated easily in such crates.

On the other hand, pigs that are restrained for longer periods of collection in crates can have the risks of accidents, feed refusal, animal sickness or other similar circumstances like lack of physical exercise due to movement in regular pens. In order to test the suitability of Cr_2O_3 as an indicator in pig trials, researchers have compared different grab sampling procedures to the total collection method. One of the first studies that reported the use of Cr_2O_3 as a marker in pigs was done by Barnicoat (1945). In this study, he found out that the rate of fecal elimination of Cr_2O_3 was irregular throughout the collection period. In this study he found out that protein digestibility was 87.1 vs. 86.8% when comparing this method with total collection method.

2.5 INTRODUCTION TO CANOLA MEAL

Canola is an offspring of rapeseed (*Brassica campestris*) which belongs to family *Brassica* (Bell, 1984). The term canola has been registered and adopted in Canada to describe the oil, seeds, and plants obtained from the cultivars *Brassica napus* and

Brassica campestris. Brassica campestris was introduced to Canada in 1936 from Poland (Bell, 1984). Canola adds almost $14 billion annually to Canada's economy and most of the economic benefits stay in western Canada where the majority of canola is grown. Canola processing in Ontario and Quebec is worth $1.3 billion yearly (Canada Council of Canada, 2009). In early 1970's, canola was discovered using conventional plant breeding techniques by Canadian plant breeders to remove the anti-nutritional components namely erucic acid and glucosinolates so that it would be safer for human and animal consumption. Rapeseed oil contain around 25-45% erucic acid and 110-150 μmoles/g of aliphatic glucosinolates in the meal (Bell, 1993). The glucosinolates in rapeseed were reduced due to their toxicity and unpalatability to most animals, and therefore limit the inclusion level of rapeseed meal in animal feeds to very low levels.

The canola seed is small and round, 1-2 mm in diameter. It contains approximately 42-43% oil, which is extracted for use as a premium edible vegetable oil. The remaining canola meal is a widely used protein source in animal feeds. Canola meal has protein content of 36% on 88% DM basis (Newkirk et al., 2003). It is a good source of selenium and P. The P is present as phytate form with its bioavailibity being 30-40% of the total P level. The dietary inclusion level of canola meal has been found limiting upto 25% in grower (Brand et al., 2001) and finisher pigs (Mateo et al., 1998).

2.5.1 Canola meal types

2.5.1.1 *Brassica napus* black

Brassica napus or oilseed rape is the major oilseed crop (> 95%) produced in Canada (Canola Council of Canada, 2009). It is commonly called canola. The problem of

high level of erucic acid and glucosinolates has been eliminated with the invention of this cultivar (Bell, 1993). *B. napus* is bred through standard plant breeding techniques to have low levels of erucic acid (< 2%) in the oil portion and low levels of aliphatic glucosinolates (< 30 μmol/g) in the meal portion (Canola Council of Canada, 2009). Ileal digestibility studies of AA and N have been determined recently in yellow and black seeded *Brassica napus* (Sanjayan, 2013; Trindade Neto et al., 2012). However, there is no information available to date regarding either TTTD or STTD of P in BNB and BJY. The chemical composition of BNB is given in Table 4.

2.5.1.2 *Brassica juncea* yellow

B. juncea yellow contains low erucic acid and glucosinolates (Raney et al., 1995). Yellow or white mustard is mainly produced in western parts of the country. It is also known as canola quality yellow mustard (Canada Council of Canada, 2009) and for its pure yellow seed coat (Simbaya, 1995). According to Newkirk et al. (1997), the canola-quality mustards were found to contain oil characteristics similar to canola within the seeds. The high-fiber content of canola meal limits inclusion rates for canola meal into high protein animal feed, especially for pigs and poultry. *Brassica juncea* yellow, has some of advantages over BNB including greater tolerance to heat and drought, better seedling growth, and increased resistance to blackleg fungus or blackspot (Woods, 1991; Rimmer and van den Berg, 1992). Yellow-seeded lines have much lower meal fiber and cellulose contents and higher oil than meal from black-seeded lines which is due to their thinner seed coat (Burton et al., 2003; Rakow et al., 2007). Apart from these characteristics, BJY does adapt well to western Canadian weather condition and has high yield of oil and low chlorophyll. Therefore, with the developments of canola quality

mustard, the area suitable to grow canola quality *Brassica juncea* is greatly explored. The chemical composition of BJY is given in Table 4.

Table 4. Chemical composition of meals derived from *B. napus* black (BNB) and *B. juncea* yellow (BJY) canola meals (% of dry matter)

Components	BNB	BJY	SECM[1]	EECM[2]	EPCM[3]
CP	43.8	47.4	41.7	41.3	36.3
Fat	1.8	1.7	5.5	12.0	10.3
Ash	7.3	7.2	6.8	6.9	6.9
Starch	0.4	0.3	1.7	-	-
Sucrose	8.8	9.2	-	-	-
NSP	20.2	20.0	-	17.8	17.8
ADF	20.0	12.6	17.2	17.5	16.0
NDF	25.7	21.07	29.9	23.8	24.2
TDF	30.1	25.8	32.0	27.0	30.5
Glucosinolates (μmol/g)	30.7	18.8	3.8	23.2	10.8

[1]Solvent extracted CM
[2]Expeller extracted CM
[3]Expeller pressed CM
Slominski et al., 1999.
Woyengo et al., 2010.
Landero et al., 2012.
Khajali and Slominski, 2012.

2.5.2 Chemical composition of canola meal

Some of the major components of CM are protein, carbohydrates, crude fiber, lipids, minerals, amino acid, and ash (Table 5). Canola meal contains less protein and higher fiber compared to SBM. The amount of CP present in CM depends upon the type of canola species or the method by which they are processed. For e. g., BJY has higher CP (47.4%) than BNB (43.8%) on DM basis (Table 4). Also, SECM has CP of 34% similar to EECM which is 38.5% (Woyengo et al., 2010; Landero et al., 2011) (Table 4). Relative to the other protein supplement such as SBM, CM has well balanced AA profile (Bell, 1993) (Table 5). Canola meal has high content of methionine (2% of total protein as compared to 1.5% in SBM) and cysteine but is limited in other AA like lysine (10% lower lysine than SBM). One of the reasons why most of the feed supplied to pig and poultry are supplied with the mixture of both SBM and CM is to provide adequate AA and CP for better performance (Khajali and Slominski, 2012).

According to Bell et al. (1999), CM is one of the richest source of minerals with higher contents of Ca, P, Mg, S, Mn and Se and limited contents of Cu and K. It has high amounts of available Ca, Fe, Mn, P and Se compared to SBM (NRC, 2012). However, due to the binding action of phytate and formation of insoluble complexes, the availability of several minerals in CM is lower than SBM (Nwokolo and Bragg, 1977).

Canola meal is better than SBM with respect to several major vitamins like choline, biotin, folic acid, niacin, riboflavin, vitamin E except being lower in pantothenic acid (NRC, 2012). The limiting factor for lower energy in CM compared to SBM is the fiber that is present in higher amount. Except, the new cultivar of CM (BJY) which has been bred to have much lesser dietary fiber (Table 4), general CM has higher amount of

fiber. This restricts the metabolizable energy and protein digestibility in animals when fed with CM (Bell, 1993). Carbohydrates in CM are present in the form of simple sugars, starch, sucrose, oligosaccharides and are similar to these present in SBM (Table 5).

Table 5. Chemical characterization of canola meal and soybean meal (as fed basis)

Components	Canola meal	Soybean meal
Dry matter, %	90.0	90.0
Crude protein, %	36.5	45.6
Ether extract, %	3.6	1.3
Gross energy, MJ/kg	18.6	20.1
Carbohydrates, %		
Starch	2.5	0.7
Sucrose	6.0	6.2
Simple sugars	0.6	0.6
Oligosaccharides	2.5	5.3
Fibre, %		
Crude fibre	11.6	5.4
Non-starch polysaccharide	18.0	17.8
Neutral detergent fibre	26.0	12.0
Acid detergent fibre	18.2	7.5
Total dietary fibre	31.7	21.8
Amino acids, %		
Arginine	2.04	3.23
Lysine	2.00	2.86
Threonine	1.57	1.74
Methionine	0.74	0.65

Cysteine	0.85	0.67
Tryptophan	0.48	0.64
Minerals, %		
Calcium	0.7	0.3
Phosphorus	1.2	0.7
Magnesium	0.6	0.3
Sodium	0.08	0.01
Potassium	1.29	2.0
Vitamins, mg/kg		
Biotin	1.0	0.3
Folic acid	2.3	1.3
Niacin	169.5	29.0
Pantothenic acid	9.5	16.0
Riboflavin	3.7	2.9
Thiamine	5.2	4.5

Canola Council of Canada, 2009.
National Research Council, 2012.
Khajali and Slominski, 2012.

2.5.3 Anti-nutritive factors in canola meal

Nutritional value of CM is influenced by its content of different anti-nutritional factors. Some of the explanations for the low digestibility of CM include higher fiber content, tannins, pectin, lignin, phytate and glucosinolates (Table 6).

2.5.3.1 Fiber

The high fiber content of CM is a result of a large proportion of hull in relation to size of the seed. The hull represents about 16% of the seed weight, but increases to about 30% of the meal weight after oil extraction which is the main reservoir for non-starch polysaccharides (NSP) and lignin. Low levels of DE and ME in CM is due to the high level of fiber (Bell, 1993). Bell and Shires (1982) confirmed that yellow hull compared to brown hull of CM have lower crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber, or lignin. Likewise, yellow hulled CM is expected to have more CP, ash, Ca and P. With regards to new cultivars of CM, ADF and NDF contents of BJY (12.7% and 21.1%) are lower compared to those (20.1% and 25.7%) of BNB (Table 4). According to Simbaya (1996), the fiber components of canola meal include lignin, polyphenols, NSP and cell wall protein, which is not digested by mono-gastric animals. The low digestibility of fiber components is a key reason leading to low protein digestibility, amino acid availability and ME of CM (Slominski and Campbell, 1990). The insoluble polysaccharides in *Brassica* seed meals consist of pectins, cellulose, arabinans, arabinoxylans and galactomannan. Encouraging results in fiber reduction is due to dehulling, canola breeding for yellow seed coat, and dietary enzyme supplementation which are capable of degrading NSP in the gastrointestinal tract of the animals (Bell, 1993).

2.5.3.2 Glucosinolates

In comparison to the earlier rapeseed meal containing 110-150 μmoles of glucosinolates, CM breeders have developed new cultivars of CM that contain less than 30 μmoles of aliphatic glucosinolates (Table 4) (Bell, 1993). In order to be classified as canola, the oil of rapeseed must contain less than 2% erucic acid, while the meal must contain less than 30 μmoles of glucosinolates per gram of meal. Glucosinolates are very unpalatable reducing the voluntary feed intake in animals which result in a drop in animal performance. Glucosinolates act as an inhibitor of the thyroid gland which if present in high amounts, will cause goiter to occur. They are often referred to as goitrogens. These compounds have a very strong stringent taste associated with them and cause the rapeseed to be quite unpalatable. The three major glucosinolates in rapeseed meal are progoitrin or epiprogoitrin, gluconapin and glucobrassicanapin (Tyagi, 2002). An enzyme called myrosinase is present in rapeseed meal which is capable of breaking down these glucosinolates into a variety of toxic compounds including isothiocyanates, oxazolidinethiones, nitriles and inorganic thiocyanate ion (Paik et al., 1980). Similarly, other factors such as heat, low pH, anatomical and physiological structure of the gastrointestinal tract, digesta transit time and microbial activity cause glucosinolates to break down releasing harmful effects to animals like inhibition of thyroid hormone production and impairment in liver and kidney function (Mullan et al., 2000). When rapeseed meal was fed to animal, thyroid function was found to be depressed (FAO, 2012). However, after genetic selection, the glucosinolates content of canola meal has been reduced to about 15% of the level contained in traditional rapeseed meal (Bell, 1984).

2.5.3.3. Sinapine

Sinapine tastes bitter and mainly is a constituent of the seed embryo (Bell and Shires, 1982; Blair and Reichert, 1984). It is the choline ester of sinapic acid (Butler et al., 1982). Sinapine exceeding 0.1% inclusion in strains of brown laying hens' diet produces a fishy flavour in the eggs (Brand et al., 2007). The fishy flavour is due to the presence of a compound named trimethylamine in the yolk. The effect can be removed via hydrolysis with ammonia and steam (Bell, 1984) or via breeding techniques that discover cultivars resistant to various climatic conditions (Blair and Reichert, 1984). Canola meal contains approximately 1% of sinapine on a DM basis (Canola Council of Canada, 2009).

Table 6. Anti-nutritional composition of canola meal

Anti-nutritional components	Average
Crude fiber (%)	11.7*
Acid detergent fibre (%)	16.8*
Acid detergent lignin (%)	5.1*
Neutral detergent fibre (%)	26.0*
Total dietary fibre (%)	32.3*
Non-starch polysaccharides (%)	15.7**
Soluble NSP's (%)	1.4**
Insoluble NSP's (%)	14.4**
Oligosaccharides (%)	2.2
Tannins (%)	1.5-3.0***
Sinapine (%)	0.6-1.8***
Phytic acid (%)	3-6***
Glucosinolates (µmol/g)	7.2[†]

*Canola Council of Canada, 2009
**Simbaya, 1996
***Bell, 1993
[†]Newkirk et al., 2003

2.5.3.4. Phytate/Phytic Acid

2.5.3.4.1. Structure of phytate molecule

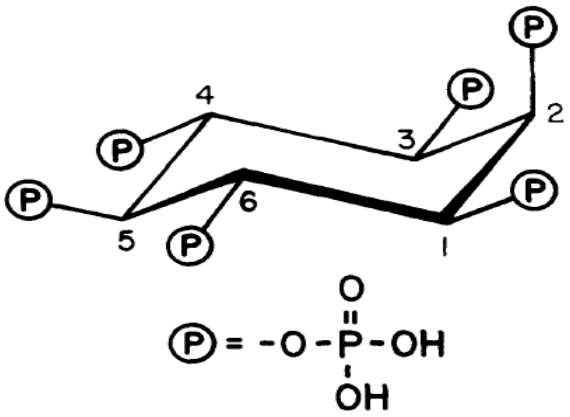


Figure 1. Structure of phytic acid (Graf and Eaton, 1990)

The chemical name of Phytate/PA molecule is myoinositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate (Figure 1). The molecule has six phosphate groups that are attached to a six-carbon molecule. Phytic acid can exist as free acid, phytate, or phytin depending on the physiological pH and the metal ions present. Such forms have been used interchangeably (Oatway et al., 2001). Phytates are regarded as primary storage of P and myoinositol in most seeds (Greenwood, 1990). The PA complex is the most important form of P storage in plants and their seeds, serving different functions during seed dormancy and germination such as initiation of dormancy, antioxidant protection during dormancy and storage of P, high energy phosphoryl groups and cations for use during germination (Ravindran et al., 1999). After hydrolysis of phytic acid, it liberates inositol and free P that is available to animals. Canola meal contains about 1.2% total P of which 0.7-0.8% is phytate bound (Canola Council of Canada, 2009). Moreover, when

high levels of CM are fed to monogastric animals like pigs, they can contribute to environment pollution due to abundant phytate P in the manure.

The amount of phytate varies not only among seeds, but also in the different parts of the kernel. In corn, about 90% of the phytate is concentrated in the germ portion of the kernel. The rest is mostly located in the aleurone tissues (O'Dell et al., 1972). For e. g., majority of phytate in wheat is bound to aleurone layer. In soybean and other dicotyledonous seeds, phytate is distributed throughout the kernel including cotyledon, endosperm, and embryonic axis (Raboy, 1997). Additionally, phytate is present in other plant tissues, such as roots, tubers, pollen and vegetative tissues (Raboy, 2001).

2.5.3.4.2. Effect of phytate on utilization of minerals, protein and amino acids

The complexes formed by phytate with the various minerals are insoluble at physiological pH making these minerals unavailable for intestinal absorption (Ravindran et al., 1999). When the intestinal pH is acidic, a weak binding occurs between minerals and phytate molecule, resulting in soluble mineral-phytate complexes. When the pH is basic, a very strong binding occurs between minerals and phytate molecule, resulting in insoluble mineral-phytate complexes (Reddy et al., 1982). According to Rimbach et al. (2008), phytate has the ability to form complexes with minerals like Ca, Zn, Cu, Co, Mn, Fe, and Mg, with Zn and Cu having the strongest binding affinity to bind to 6-phosphates in the PA chain.

From a study done by Saha et al. (1994), it has been found that phytate binding action makes a portion of dietary Ca unavailable to poultry and pigs by forming Ca-phytate complexes. Also, most feedstuffs fed to pigs and poultry are very low in Ca. For example, in corn, Ca level is 0.01 to 0.02% and in SBM, it is 0.25 to 0.35%. The total Ca

level of corn and soybean diets for swine and poultry is about 0.05%, which is approximately 5 to 10 % of the total dietary Ca requirement for pig and poultry (0.50 to 0.80 for pig and 0.50 to 1.00 for poultry). Therefore, Ca supplements in the diets are mandatory, and because they are relatively cheap, the problem of over-supplementation can occur in the diets of mono-gastric (Zhang, 1999). Among all the minerals, Zn is mostly affected (Kornegay, 2001). Binding of Zn to phytate may decrease Zn utilization and growth rate in the absence of Zn supplementation (Oberleas et al., 1962; Davies and Nightengale, 1975). The rate of absorption of Zn in the pig is decreased by the effect of phytate (Sandstrom and Sandberg, 1992).

Knuckles et al. (1989) and Kornegay (2001) showed that the bond formed between phytate with either protein or AA has the negative influences. The presence of phytate in phytate-rich diets interferes with optimal AA utilization from intact protein by 1) formation of indigestible protein-phytate complexes, 2) inhibition of digestive enzymes, and 3) decreased absorption of nutrients from the small intestine (Mroz et al., 1994). Moreover, interaction between phytate and protein depends on pH, forming binary protein-phytate complexes at low pH and ternary protein-phytate- mineral complexes at higher pH (Maenz, 2001) or towards neutrality. Different pH ranges in the pig's stomach facilitates the formation of phytin-protein complexes due to phytin-protein interactions (Singh and Krikorian, 1982). Also, phytin-protein complexes are insoluble in the aqueous environment of the GIT and are more difficult for proteolytic enzymes to hydrolyze these proteins as a result of which, protein digestion is reduced (Kies et al., 2006a).

2.6 PHYTASE

Phytase (myo-inositol hexaphosphate phosphohydrolase) is the enzyme that hydrolyses the phytate bonds and release phytate P. Pigs lack sufficient endogenous phytase to hydrolyze phytate P, and therefore, organic P of vegetable origin is not well digested by pigs (Nahm, 2004). Phytases have been fed to poultry and pigs to degrade plant phytate which would otherwise be excreted as intact form through the digestive tract releasing large amount of P resulting in high levels of it in manure. Currently, in poultry and pig diets, around 50%-80% total P is present in the form of phytate which is largely unavailable to animals. Mono-gastric animals have minimal levels of phytase activity in the brush border membrane of their digestive tracts (Maenz and Classen, 1998). Due to this reason, phytase is added to the diet to help in the hydrolysis of the phytate molecule. Addition of phytase enzyme to mono-gastric diets improves phytate digestibility (Augspurger and Baker, 2004; Snow, 2004; Augspurger et al., 2007). Phytate molecules are needed to be hydrolysed before mono-gastric can completely utilize the P it contains. The use of microbial phytase, especially in swine, has led to significant reductions in P excretion and P digestibility increment when fed CM (Akinmusire and Adeola, 2009). Phytase activity is defined in terms of phytase units, expressed as U/kg.

2.6.1 Mode of action of phytase

Phytase can be classified according to different mechanisms of action and optimum pH of activity (Bohn et al., 2008). According to Selle (2008), protein-phytate complexes are formed in the acidic conditions of the stomach. Most of the fungal, bacterial and plant phytate degrading enzymes have acidic pH values with the exception of the *Aspergillus fumigatus* enzyme, which is slightly basic in properties. However, in

comparison to enzymes from plant origin, microbial enzymes tend to show large pH stability. It has been found that microbial enzymes are even stable at pH values above 8.0 and below 3.0 whereas those of plant origin are least stable below pH 4.0 and above pH 7.5 (Konietzny and Greiner, 2002).

2.6.2 Sources of phytase

There are mainly 4 sources of phytase for pigs and poultry: (1) mucosal phytase produced by enterocytes of intestinal mucosa (2) intrinsic plant phytase present in certain plant ingredients, (3) microbial phytase originating from gut microbes, and (4) the addition of exogenous phytase (Selle, 2008)

2.6.2.1 Mucosal phytase

Mucosal phytase activity is pronounced in the duodenum section of small intestine (Applegate et al., 2003) where the efficacy is highest to dissociate Inositol triphosphate (IP3) by the action of increasing dephosphorylation (Lopez, 2000). The activity decreases progressively down the length of gut (Maenz and Classen, 1998). According to Selle (2008), the contribution of intestinal phytase in phytate hydrolysis is only of minimal importance in case of pig (gut) and chickens (crop, stomach and small intestine). Such a minimal production of mucosal phytase in pigs complements the use of exogenous phytase (Selle, 2008).

2.6.2.2. Plant phytase

Some plant phytate are found to be digested by animals without any phytase supplementation in diets (Maenz, 2001). The ability to hydrolyze phytate within the seed

of a plant feed varies between plants (Eeckhout and De Paepe, 1994).In some feed ingredients like wheat, barley, rye and triticale, considerable amount of intrinsic phytase activity is found (Weremko et al., 1997) but in ingredients like corn and SBM, there is lower intrinsic activity of phytase. However, when feed ingredients are pelleted under high heat, the intrinsic phytase activity is reduced (Liu et al., 1998). Due to the narrower pH range of plant phytase (5.0 to 6.0) as compared to microbial phytase (2.0 to 5.5), the performance of plant phytase is found to be poor (Selle, 2008).

2.6.2.3 Microbial phytase from gut microbes

There are a number of micro-organisms found in the hind gut of animal. Most of them are found in hind gut. These micro-organisms are able to produce their own phytase, which may contribute to phytate hydrolysis within the animal. Nevertheless, phytate hydrolysis in the large intestine is of little or no benefit to the animal or the environment (Selle, 2008).

2.6.2.4 Exogenous phytase

Currently, many microbial phytase are available for commercial use which is classified into 3 main categories as; 3-phytases, 6-phytases, and 5-phytases according to the site of initiation for hydrolysis of phytate/PA on the inositol ring. The 3-phytases are the most common category of phytases which are present in fungi and bacteria in the form of histidine acid phosphatases. Differences between fungal and bacterial phytase are: 1. how these organisms produce the enzyme and 2. specific substrate for the enzyme. According to Rao et al. (2009), fungal phytases are produced extracellularly and the bacterial (mainly gram-negative) phytases are produced intracellularly. Fungal phytases

have broad substrate specificity for PA, glucose, fructose, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and ATP, while bacterial phytases show high substrate specificity only for PA. Microbial phytase are commonly derived from either fungi (*Aspergillus niger*) or bacteria (*Escherichia coli*). These commercial phytase products can be added to the feed of mono-gastric animals to effectively hydrolyze phytate within the digestive tract. Phytases from different sources are unique in their appearance, physical and chemical properties, and also in enzyme activity.

2.6.3 Effect of phytase on phosphorus utilization in swine

Addition of microbial phytase to swine diets is based primarily on two facts; 1) a decrease in inorganic P added to the diet which reduces the cost of the diet and 2) improvement in P utilization by the pig's body which helps in reduction of large amount of P in manure and thus in the environment. The addition of phytase is found to improve the ATTD of P in many ingredients. Akinmusire and Adeola (2009) studied the addition of microbial phytase in canola and SBM based diet which improved both the ATTD and TTTD of P from 61 to 71%. After supplementation with 1,000 FTU of phytase, P digestibility in corn-SBM was around 61%, 80%, 52% and 75% in wheat-SBM, wheat-SBM-CM diet and barley-peas CM diet, respectively (Sheng F, 2006). Supplementing phytase at the level of 1,500 FTU in SBM improved ileal P digestibility value of around 74% (Traylor et al., 2001).

The effect of microbial phytase on STTD of P has been studied by Petersen and Stein (2006) and Almeida (2010) using field peas and corn co-products, SBM and distillers dried grains with soluble (DDGS), respectively. Both of those studies found that STTD of P increased due to the addition of phytase. Also, the addition of microbial

phytase decreases P excretion by 25 to 50% (Jongbloed et al., 1992) by increasing P digestibility (Kornegay and Qian, 1996). Similarly, Harper et al. (1997) found that supplying 500 U/kg phytase reduced fecal P excretion in pig by 21.5%. They also found that increasing levels of supplemental phytase in swine diets resulted in a linear increase in the ATTD of P. Mroz et al. (1994) reported that around 40-50% increment in ATTD of P was observed in pigs fed diets supplemented with microbial phytase.

Phytase supplementation can improve phytate P utilization both by increasing P availability and P retention which can ultimately lead to a reduction in P excretion (Jalal and Scheideler, 2001; Dilger et al., 2004; Rutherfurd et al., 2004; Onyango et al., 2005; Augspurger et al., 2007). According toWaldroup (2000), the reduction of P excretion in manure will result in a reduction in P pollution when phytase is supplemented in conjunction with reduced dietary P levels.

2.6.4 Effect of phytase on digestibility of nutrients other than phosphorus/ non-phosphorus effect of phytase

2.6.4.1 Calcium

Generally, a high positive correlation is observed between Ca and P absorption and utilization (Eeckhout and De Paepe, 1994). The microbial phytase improved the ATTD of Ca by 13 and 17% units when the diet was supplied with 250 and 500 U/kg (Radcliffe et al., 1998). Microbial phytase not only enhanced the ATTD of P, but also the ATTD of Ca (Kies et al., 2006b; Kornegay and Qian, 1996; Nyannor et al., 2007). It was estimated that an extra 0.8 g of P and between 0.4 and 0.7 g of Ca were absorbed with 500 U/kg of supplemental phytase. Supplementing microbial phytase improved Ca digestibility in pigs (Simons, 1990; Mroz et al., 1994; Li et al., 1998; Kuhn and Partanen, 2012) as well as in chickens (Qian et al., 1997; Ahmad et al., 2000; Tamim, 2004;

Onyango et al., 2005). Improvement in the ATTD of Ca, and total P in 45 kg canulated barrows by microbial phytase was observed in a study done by Mroz et al. (1994). In that study, the retention of Ca and P in pigs increased by 2.2 and 1.9 g/d, respectively.

2.6.4.2 Protein, amino acids and nitrogen

The action of phytate on protein and AA can be illustrated by the fact that phytate depresses protein/amino utilisation. Phytate forms de-novo binary protein–phytate complex, deteriorates endogenous amino acid flows and thus compromises intestinal uptakes of AA. Microbial phytase addition to phytate-containing diets could potentially improve AA digestibility by releasing phytate-bound AA in chickens (Ravindran et al., 1999; Snow et al., 2003). Phytase supplementation also has the potential to reduce P and N or AA excretion. Phytase, when supplemented at the rate of 500 U/kg was able to improve the utilization of DM, CP, Ca, P, energy and AA in a maize/rice and rapeseed/cottonseed meal based diet and reduced total output in manure. (Fan, 2005). Similarly, many of the other studies demonstrated that protein and AA digestibility in animals can be improved by addition of microbial phytase (Rutherfurd et al., 2002; Cowieson et al., 2004; Dilger et al., 2004; Rutherfurd et al., 2004; Kies et al., 2006b; Ravindran et al., 2006) .

However, there are some conflicting results regarding the effect of phytase on utilization of AA. Bruce and Sundstøl (1995) reported that phytase had no effect on the protein digestibility of pigs and Traylor et al. (2001) found out that phytase did not improve ileal digestibility of AA in SBM for pigs. Adeola and Sands (2003) found a lack of response in amino acid utilization (both pre- and post-absorptive) to microbial phytase supplementation, and Sands (2002) reported that microbial phytase supplementation of

low- or high-phytin diets did not improve ileal digestibility of AA in pigs fitted with a simple T-cannula. Based on growth rate and feed efficiency, the utilization of AA was improved from 15.5 to 19.5% when phytase (1,200 U/kg) was added to a low CP or high CP diet (Biehl and Baker, 1997). Similarly, high levels of phytase supplementation had no impact on protein digestibility in chickens (Augspurger and Baker, 2004) and also phytase did not improve digestibility of protein and AA in SBM (Peter and Baker, 2001). In regards to the effect of phytase to protein and AA digestibility, there are many factors associated; diet, animals, and intrinsic properties of ingredients/enzyme (Ravindran et al., 2006). In the diet factor, the concentration and source of phytin, protein quality, and mineral chelators are likely to affect protein and AA response to microbial phytase whereas with regards to the animal factors, species, genetics, and sex, are likely to impact response (Adeola and Sands, 2003). This may be influenced by such factors as GIT transit time and pH, as well as brush-border phytase activity regulation.

2.6.4.3 Energy

Phytase increases the availability of energy in diets of non-ruminants apart from Ca and P (Ravindran et al., 1999). Johnston (2000) and Williams (2001) reported that phytase increased gross energy (GE) digestibility and starch digestibility in pigs fed a corn-SBM diet. Starch digestibility can occur by the process of binding α-amylase or by chelating Ca^{2+}, which is needed for the normal activity of amylase. Shelton (2003) showed that phytase supplementation at the level of 500 U/kg of diet increased a small portion of energy availability in pigs fed corn-SBM diet. Similarly, in a corn-SBM diet fed to broilers, dietary phytase supplementation increased apparent digestibility of energy by a small amount (Rutherfurd et al., 2012; Shirley and Edwards, 2003). Ravindran

(2001) suggested that when phytase was added to a wheat-SBM-sorghum-based diet, AA and energy responses were responsible for the performance improvements. The response plateaued at a phytase dose of 750 U/kg feed. Apparent fecal digestibility of energy was higher in phytase-supplemented piglets, which could be attributed mainly to higher protein and fat digestibilities (Kies, 2005). Newkirk and Classen (2001) found out that apparent metabolizable energy (AME) in broiler chicks was improved when the diet was supplemented with phytase. However, in a study by Onyango et al. (2004), no improvements in AME was seen. It was explained that the reason for the variation in energy retention could be the differences in phytase source and dietary ingredients that were used in the studies.

2.7 PHYTASE SUPER-DOSING

In recent years, despite feeding phytase at a normal standard level, it has been suggested that the application of phytase can be done in a higher dose, known as 'super-dosing'. Conventional doses of phytase are believed to be from 500 to 1,000 FTU. There is a dearth of information about the supplementation of phytase doses higher than 1,500 U/kg (Kies et al., 2006a). Super-dosing is defined as the level of phytase supplementation at or above 2,500 FTU/ kg of feed (Adeola and Cowieson, 2011). Nelson et al. (1971) reported the dose of 950 FTU/g to 7,600 U/kg of *Aspergillus ficuum* in broiler chicken where they found that phytate P disappearance was increased from 38.9% with 950 U/kg to 94.4% with 7,600 U/kg. Simons (1990) concluded that an addition of 1,000 U/kg diet of microbial phytase to broiler diets provides levels of performance which are as good or better than broilers fed diets supplemented with phosphate. Later on, Shirley and Edwards (2003) observed a quadratic increase in phytate P disappearance from 42% to

95% when they supplied 93 U/kg to 12,000 U/kg of phytase, respectively, in a poultry diet. In their study, phytase not only aided in P digestibility, but also increased AME and N retention when a dose of 12,000 U/kg was supplied. However, before Shirley and Edwards (2003), some previous studies included such doses of phytase (Zhang et al., 2000).

The effect of higher doses of phytase (1,000, 5,000, and 10,000 U/kg) in chickens fed P-AA deficient diets was also studied (Augspurger and Baker, 2004; Brana et al., 2006; Pirgozliev et al., 2007). They recommended that using 10-20 times the normal dose of phytase can release upto 100% of the phytate-bound P in a corn-SBM broiler diet. Digestive utilization of P or Ca in pigs fed phytase at 16,500 U/kg was observed in a P-deficient diet which also improved growth performance and indices of P utilization in pigs (Nyannor et al., 2007). Using graded and higher dose of microbial phytase not only increases the digestibility of P but also various minerals like Mg, Na, K and Cu in pigs (Kies et al., 2006b). Average daily feed intake (ADFI), average daily gain (ADG), feed to gain ratio, feed efficiency together with the mineral digestibility was increased with increasing phytase levels (Zyla, 2000; Lan et al., 2002; Shirley and Edwards, 2003; Dilger et al., 2004; Onyango et al., 2005)). Digestibility of P increased from 34% in the basal diet to 84% in the diet supplemented with 15,000 FTU, generating 1.76 g of digestible P per kilogram of feed. Dietary phytase supplementation beyond present day standards (500 U/kg) could further help to restrict dietary mineral inclusion levels and also reduces the mineral output to the environment (Kies et al., 2006a).

According to Adeola and Cowieson (2011), the probable mechanism of higher doses of phytase could be one the following; 1) more P is liberated or P/Ca proportion is

restored in animals, 2) less residual phytate (destruction of anti-nutritive effect of phytate), and 3) generation of *myo*-inositol which is the completely de-phosphorylated form of phytate that also has vitamin-like/lipotropic effect.

2.8 CONCLUSION

Several studies have been conducted in the past to determine the nutritive value including P digestibility in newly developed feed ingredients used in pig diets. Due to the oversupply of inorganic P in diets of animals like pig which would result in the concerns related to environmental pollution, the practice of exact formulation on the basis of available/digestible P is mandatory. Both regression and P-free methods have been used to determine TTTD and STTD of P in many feed ingredients. The basic concept in both of these methods is the measurement of EPL. However, there are not adequate information on either STTD or TTTD of P in BNB and BJY. Also, no study has been done to compare between the STTD and TTTD of P in such meals.

The objective of the first experiment was to determine and compare TTTD and STTD in BNB and BJY fed to growing pigs by using both regression analysis and P-free diet. The objective of the second experiment was to determine and compare the STTD of P in BNB and BJY when they are supplemented with phytase.

3.0 MANUSCRIPT 1

True and standardized total tract phosphorus digestibility in meals derived from *Brassica napus* black and *Brassica juncea* yellow fed to growing pigs

3.1 ABSTRACT

A study was conducted to determine the true (TTTD) and standardized (STTD) total tract digestibility of phosphorus (P) in canola meal (CM) from *Brassica napus* black (BNB) and *Brassica juncea* yellow (BJY) in growing pigs using a P-free diet or the regression method. Fifty four barrows with an initial BW of 19.9 ± 0.22 kg (mean ± SEM) were randomly allocated to one of 9 dietary treatments, with 6 replicates per treatment in a randomized complete block design. Dietary treatments were cornstarch-based with increasing concentrations of P, i. e., 0.8, 1.6, 2.4 and 3.3 g/kg (as fed basis) from either BNB or BJY as the sole source of P. A gelatin based P-free diet was used to measure basal endogenous P losses (EPL). Limestone was added to maintain a Ca:total P ratio of 1.2:1 in dietary treatments. Daily feed allowance was based on the BW of pigs at the beginning of each period and was calculated to supply 2.6 times the maintenance energy requirement of the growing pigs and offered in two equal portions at 0800 and 1600 h as a dry mash. Pigs were kept individually in metabolism crates equipped with a feeder and a nipple drinker and were fed experimental diets for 14 d, including 9 d for adaptation to feed and environmental conditions and 5 d for total, but separate collection of feces and urine. The TTTD of P was determined by regression analysis as the P concentration increased from 0.8 to 3.3 g/kg of DM. The STTD of P was calculated for the diet with the highest level of either cultivar (around 30% in the diet) where the P concentration was 3.3 g/kg of DM. The total and basal EPL estimates obtained with

regression analysis and the P-free diet were 665 ± 0.03 mg/kg DMI and 209 ± 96 mg/kg of DMI, respectively. The TTTD and STTD of P measured by regression analysis and the P-free diet were 33.3 and 31.0% for BNB and 32.0 and 28.3% for BJY. The results showed that the STTD and TTTD of P in BNB and BJY are similar and the regression analysis and feeding a P-free diet give the same estimates of P digestibility in CM.

3.2 INTRODUCTION

Phosphorus is the third most expensive nutrient in pig diets and the utilization of plant P by pig is known to be poor (Akinmusire and Adeola, 2009). Most of the P in pig diets is supplemented by inorganic P and it is necessary to minimize the usage of inorganic P sources by replacing them with organic P obtained from several plant ingredients. It is necessary to match the true supply of P in feed with the requirement of pig body in order to minimize the problem of excess excretion of P and thus subsequent environmental issues. This is possible by determining the bioavailability of P in different plant feed ingredients.

Digestibility studies have been used to estimate the bioavailability of P in feedstuffs. The ATTD of P is not always additive in a mixture of feed ingredients when used in diet formulation and the digestibility values are variable within same feed ingredients (Dilger and Adeola, 2006). The reason behind determining either TTTD or STTD of P would be to find out the digestibility value that is less variable within a single feed ingredient. Regression analysis method has been used previously to determine the TTTD of P in different feedstuffs (Fan et al., 2001; Shen et al., 2002). The P-free diet

method has been used to calculate STTD of P in inorganic and organic P sources (Petersen and Stein, 2006; Widmer et al., 2007).

There is no adequate information available on the STTD and TTTD of P in most of the pigs feed ingredients (NRC, 2012). Canola meal, a by-product of canola oil production is widely used as a protein source in pigs (Bell, 1993; Mullan et al., 2000). *Brassica napus* black has been commonly used in pig diets in for years. However, BJY, a new cultivar of CM with reduced fiber content has been developed recently (Slominski, 1997). In a recent study with growing pigs, Akinmusire and Adeola (2009) reported the TTTD of P in CM (34.3%). However, there is no adequate information available on BNB and BJY with regards to STTD and TTTD of P. It is necessary to determine TTTD/STTD of P in BNB and BJY to effectively utilize P in pigs. Thus, the objectives of this study were to determine the TTTD and STTD of P in CM derived from BNB and BJY in growing pigs using regression analysis and P-free diet methods.

3.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled in accordance with the guidelines described by the Canadian Council on Animal Care (CCAC, 2009).

3.3.1 Animals and housing

Fifty-four crossbred barrows [(Yorkshire × Landrace) × Duroc; Genesus Inc., Oakville, MB, Canada] with an average initial BW of 19.9 ± 0.22 kg (mean ± SD) were obtained from the University of Manitoba Glenlea Research Unit for use in the study. Pigs were individually housed in metabolic crates (118 cm × 146 cm) in a temperature

controlled room (21°C ± 2°C) and had free access to water. The metabolic crates had smooth plexi-glass sides that allowed visual contact with pigs in adjacent crates. Fecal and urine collection tray was fitted under the crate floor for the sample collection.

3.3.2 Experimental diets

Canola meals derived from BNB and BJY were obtained from a local crushing plant at Bunge Canada (Altona, Manitoba, Canada). Nine cornstarch-based diets consisting of a P-free diet and 4 diets containing 0.8, 1.6, 2.4 and 3.3 g/kg of DM of P either from BNB or BJY as the only sources of P were formulated (Table 7). Limestone was added to maintain Ca:total P ratio of 1.2:1 in all diets. All diets were supplemented with minerals and vitamins to meet or exceed recommended specification for growing pigs (NRC, 1998).

Table 7. Composition and analyzed values of experimental diets (as-fed basis)

Item, %	Diets/P-content								
	P-free	*B. napus* black				*B. juncea* yellow			
		0.8	1.6	2.4	3.3	0.8	1.6	2.4	3.3
Ingredient, %									
B. napus	0.00	7.90	16.00	24.00	32.00	0.00	0.00	0.00	0.00
B. juncea	0.00	0.00	0.00	0.00	0.00	7.60	15.35	23.10	30.80
Cornstarch	48.42	56.48	48.23	40.10	32.00	56.78	48.90	41.02	33.20
Dextrose	0.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Vegetable oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	0.80	0.12	0.27	0.40	0.50	0.12	0.25	0.38	0.50
Solka floc[1]	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vit-Min premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Potassium carbonate	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Magnesium oxide	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Pork gelatin[3]	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AA mixture[4]	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Analyzed values									
DM, %	93.92	93.00	93.00	93.32	93.72	93.00	93.00	93.00	93.39
GE, kcal/kg	4,096	3,985	4,025	4,029	4,108	3,968	3,969	3,982	4,115
CP, %	17.70	2.20	4.19	6.81	12.31	2.00	3.50	6.66	12.17
Ca, %	0.27	0.06	0.12	0.26	0.42	0.07	0.08	0.21	0.45

P, %	0.00	0.08	0.16	0.24	0.33	0.08	0.16	0.24	0.36

[1]International Fiber Corp., North Tonawanda, NY.

[2]Provided the following quantities of vitamins and minerals per kg of a complete diet: Vitamins: A, 2000 IU, $D_{3,}$ 200 IU, E, 40 mg, K, 2 mg, B_1, 1.5 mg, B_2, 7 mg, B_6, 2.5 mg, B_{12}, 25 µg, calcium pantothenate, 14 mg, folic acid, 1 mg, niacin, 21 mg, biotin, 70 µg. Minerals: Cu, 10 mg (as copper sulphate), iodine, 0.4 mg (as potassium iodine), iron, 120 mg (as ferrous sulphate), Mn, 10 mg (as manganous oxide), Se, 0.3 mg (as sodium selenite), Zn, 110 mg (as zinc oxide).

[3]Pork gelatine obtained from Gelita Gelatine USA Inc., Sioux City, IA.

[4]Provided the following quantities (%) of AA per kg of complete diet: DL-Met, 0.27; L-Thr, 0.08; L-Trp, 0.14; L-His, 0.08; L-Ile, 0.16; L-Val; 0.05.

3.3.3 Experimental design and general conduct of the study

The experiment was conducted in a randomized complete block design to give 6 replicates per treatment in 3 consecutive periods. Each period consisted of 14 d; the first 9 d were for adaptation and the last 5 d for separate and total collection of feces and urine. Pigs were fed the diets at 2.6 times the maintenance energy requirement (NRC, 1998) based on their BW at the beginning of each period. The daily feed allowance was offered in 2 equal portions at 0800 and 1600 h. The total collection of feces and urine was done as described by Ragland et al. (1998). Briefly, on d 10, each pig received 5 g of ferric oxide (Fischer Scientific, Ontario, ON) as an indigestible marker in 100 g of feed which was fed in the morning. The remaining portion of feed was offered after all the marked feed was consumed. Fecal collection commenced when marker appeared in feces. On the morning of d 15, pigs were offered 100 g of marked feces as described above, and collection of feces ended when marker appeared in feces. Total collection of urine started on morning of d 10 and ended in the morning of d 15. Fecal samples were collected from the trays underneath metabolism crates into plastic sample bags (Ziplock, 26.8 cm × 27.3 cm) once daily in morning, weighed and stored frozen at -20°C. Urine was collected once daily in morning (in jugs containing 10 mL of 6 N HCl to minimize N losses) and weighed, and a subsample (10% of total weight) was obtained, strained through glass wool, and stored frozen at -20°C.

3.3.4 Sample preparation and chemical analyses

Fecal samples were dried in a forced air oven at 60°C to remove moisture and pooled per pig. Diets, ingredients and fecal samples were finely ground by using 2-mm mesh screen in a Thomas Wiley Mill (Thomas model 4 Wiley Mill; Thomas

Scientific) and thoroughly mixed before analysis. All analyses were performed in duplicates. Analyses for DM in diets and fecal samples were carried out according to AOAC (method 934.01; 1990). For DM analysis, 1 g of sample was weighed in a pre-weighed silica dish and dried in an oven for overnight at 104°C. Next day, the sample was removed, cooled down in desiccator and was re-weighed. Gross energy in experimental diets was determined using an adiabatic oxygen bomb calorimeter (Model 6300; Parr Instrument Co., Moline, IL) with benzoic acid as the calibrating standard. Ingredients and diets were also analyzed for CP (AOAC, 2007) using an N analyzer (Leco Corporation, St. Joseph, MI).

Total P and Ca analyses in ingredients, feed and feces were digested according to the procedures described (method 985.01; AOAC, 1990) and were analyzed using an inductively coupled plasma (ICP) spectroscopy (Varian Inc., Palo Alto, CA). For Ca, P analyses, around 1 g sample was weighed in a labelled Pyrex tube without screw and ashed overnight in a furnace at 600°C. After ashing, tubes were allowed to cool and were removed from the furnace for acid digestion. 10 mL of 5N HCl/ HNO_3 (1%v/v) was added to those tubes, capped with screw and were digested for 1 hour in a sonication bath that was preheated to 70°C. After cooling down the samples from sonication bath, 2.5 ml of the sample (1 ml for feces) was pipetted and diluted with deionized water using 100 ml volumetric flask. After that, the samples were filtered through Q5 filter paper into a 20 ml scintillation vials.

Urine samples were thawed and pooled for each pig for analysis. Urine analysis was done by diluting the frozen urine samples. For this, the urine samples were thawed and from the total amount of urine, 10 ml (duplicate) was pipetted. The samples were

then centrifuged at an RCF of 3,000 *g* for 10 minutes so as to settle down the organic matters. About 4 ml of supernatant fluid was harvested from the tube and were diluted with deionized water to make it 20 ml. The dilution ratio was 1:5. The samples were then filtered through Q5 filter paper into scintillation vials to get rid of any residues. Then the samples were sent for ICP analysis.

3.3.5 Calculations and statistical analyses

The ATTD of fecal P in each of the P-containing diets (for both CM types) was calculated as outlined by Petersen and Stein (2006) using the following equation:

$$ATTD = ([Pi – Pf]/Pi) \times 100, \qquad (4)$$

where ATTD represents the apparent total tract digestibility values in %; Pi represents the total P intake (g) from d 10 to d 14 of the experimental period, and Pf represents the total fecal P output (g) that originated from the feed fed from d 10 to d 14. The ATTD of DM and Ca in all diets based on BNB and BJY were also calculated using the equation (4).

Basal EPL were determined using the P-free diet and expressed on a DMI as outlined by Petersen and Stein (2006) using the following equation:

$$EPL_{basal} = ([Pf/Fi] \times 1{,}000 \times 1{,}000), \qquad (5)$$

where EPL_{basal} is the basal endogenous P losses (mg/kg DMI); and Fi is the total feed intake (g) from d 10 to 14. The daily basal EPL (mg/d) in pigs fed the diets containing P was calculated by multiplying the calculated EPL (mg/kg DMI) by the DMI of each pig for whole 5-d collection period and was divided by 5.

The STTD of P was calculated as outlined by Petersen and Stein (2006) using the following equation:

$$STTD\ (\%) = ([Pi - (Pf - EPL_{basal})/Pi] \times 100, \qquad (6)$$

where STTD is the standardized total tract digestibility of P (%); and EPL_{basal} was calculated as in equation (5).

Phosphorus retention was calculated as outlined by Petersen and Stein (2006) using the following equation:

$$Pr = ([Pi - \{Pf + Pu\}]/Pi) \times 100, \qquad (7)$$

where Pr is the retention of P (%); Pu is the urinary output of P over the 5 d collection period (g). The retention of Ca was similarly calculated using equation (7).

The ATTD of P, Ca and DM in BNB and BJY were calculated using equation 4. Total fecal P output was regressed against dietary P intake for each CM cultivar (fig 2A and 2B). The analysis of total EPL by this method depends upon establishing linear relationships between apparent digestible and total intake of assay nutrients in diets (Ajakaiye, 2003; Fan et al., 2001; Shen et al., 2002). If there is linear relationship between apparent fecal digestible intake and total intake of dietary P, the endogenous P loss in feces can be directly determined by extrapolating the dietary input of P to zero (Ajakaiye, 2003). The intercept of the linear regression analysis provide an estimate of the amount of endogenous P that a pig would excrete if it were fed zero P in the diet. The apparent digestible P intake can be calculated by using following equation:

$$P_{Ai} = P_{Di} \times D_A \qquad (8)$$

where P_{Ai} represents the apparent fecal digestible P in the ith diet (g/kg DMI); P_{Di} is the total P in the ith diet (g/kg DMI); and D_A is the apparent fecal P digestibility values in the ith diet (%).

Linear relationships between apparent digestible P intake and the graded levels of total P in the diet can be established using following equation (Ajakaiye, 2003):

$$P_{Ai} = P_E + [(D_T/100) \times P_{Di}] \qquad (9)$$

where P_{Ai} and P_{Di} are as defined in equation 5; P_E is the endogenous P levels in the feces (g/kg of DMI); and D_T is the true fecal P digestibility values (%) in the P-containing assay ingredient. In equation 8, P_{Ai} is the dependent variable; P_{Di} is the independent variable; and P_E and D_T are the regression coefficients.

Data were subjected to ANOVA using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). The model included dietary treatment as the fixed effect and replicate as a random effect. For the estimation of endogenous loss at zero P intake, P absorption and P intake were analyzed for linear relationship using PROC regression procedure in SAS. The pig was the experimental unit for all analyses. Contrast statements were used to analyze linear and quadratic effects of increasing levels of P. Differences were considered significant at $P < 0.05$ and trends were considered at $P < 0.10$.

3.4 RESULTS

All pigs remained visibly healthy and readily consumed their daily ration throughout the experimental period. The mean final BW of pigs in this trial was 28.2 ± 1.0 kg (mean ± SEM). The analyzed composition of CM types used in the present study is given in Table 8. The analyzed phytate content in BNB and BJY (as fed basis) was 0.64% and 0.66%, respectively. The analyzed values for the dietary P were closer to the

calculated for all diets (Table 7). The analyzed dietary P concentrations were 0.8, 1.6, 2.4, and 3.3 (g/kg of DM) irrespective of CM cultivar. The concentration of CP increased from 2.2% to 12.3% and 2.0% to 12.2% as the dietary inclusion of BNB and BJY increased. Dry matter content for CM based diets averaged 93.26 ± 0.34% for BNB and 93.09 ± 0.2% for BJY.

Table 8: Analyzed and calculated composition of canola meal (CM) types

	CM	
Item	*B. napus* black	*B. juncea* yellow
DM, %	93.00	92.30
CP, %	37.20	37.70
NDF, %	24.20	16.00
GE, kcal/kg	5,415	5,648
Analyzed Ca, %	0.63	0.68
Analyzed total P, %	1.01	1.05
Analyzed phytate P, %	0.64	0.66
[1]Analyzed non-phytate P, %	0.37	0.39

[1]Calculated as the difference between total P and phytate P.

3.4.1 Apparent total tract digestibility of P and Ca

Daily P intake, total fecal P excretion, P retention, P retained as a percentage of P intake, and P absorption increased (linear, $P < 0.001$) as levels of P was increased in all diets based on BNB and BJY. The ADG increased as levels of P increased (linear, $P < 0.01$) from 0.8 to 3.3 g/kg in the diets. The ATTD of P increased linearly ($P < 0.001$) with the increasing dietary P content (Table 10). The ATTD values of P determined with the regression and P-free diet methods are given in Table 10. The ATTD of P increased from 19.0 to 30.0% for BNB and from 17.3 to 28.3% for BJY as dietary P content increased from 0.8 to 3.3 g/kg. The urine P output was not influenced by dietary treatments. Similarly, daily Ca intake, total fecal Ca excretion, Ca retention, Ca retained as percentage of P intake and Ca absorption increased (linear, $P < 0.05$) as levels of P increased in both CM types (Table 10). However, Ca intake also showed quadratic response (quadratic, $P < 0.01$) in diets as dietary P levels increased. However, there was no significant variation ($P > 0.05$) in the ATTD of Ca between all diets and the value ranged between 39.0 to 53.0% in both meals.

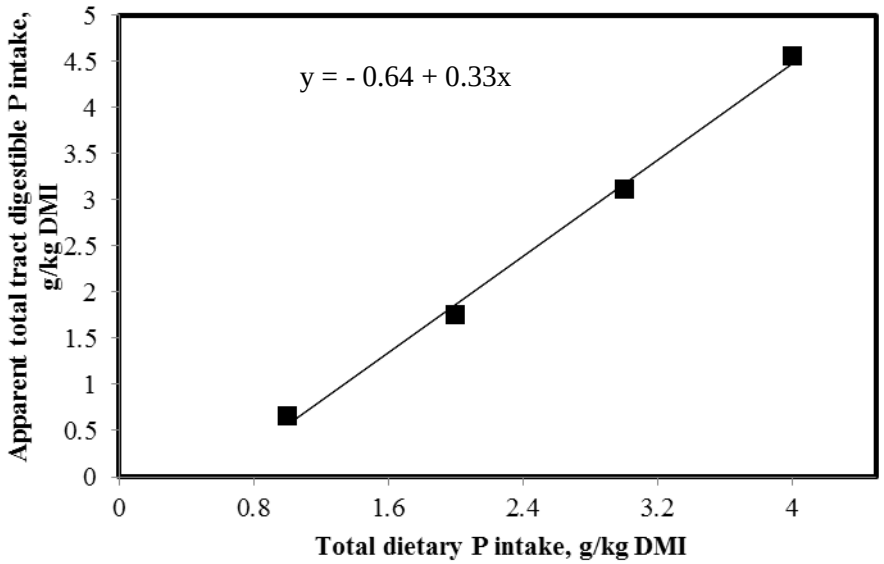
3.4.2 Endogenous P losses

The total EPL values estimated with the regression analysis were similar between CM types and averaged 665 ± 0.03 mg/kg DMI (Table 9). The basal EPL estimated with the P-free diet averaged 209 ± 96 mg/kg DMI (Table 10).

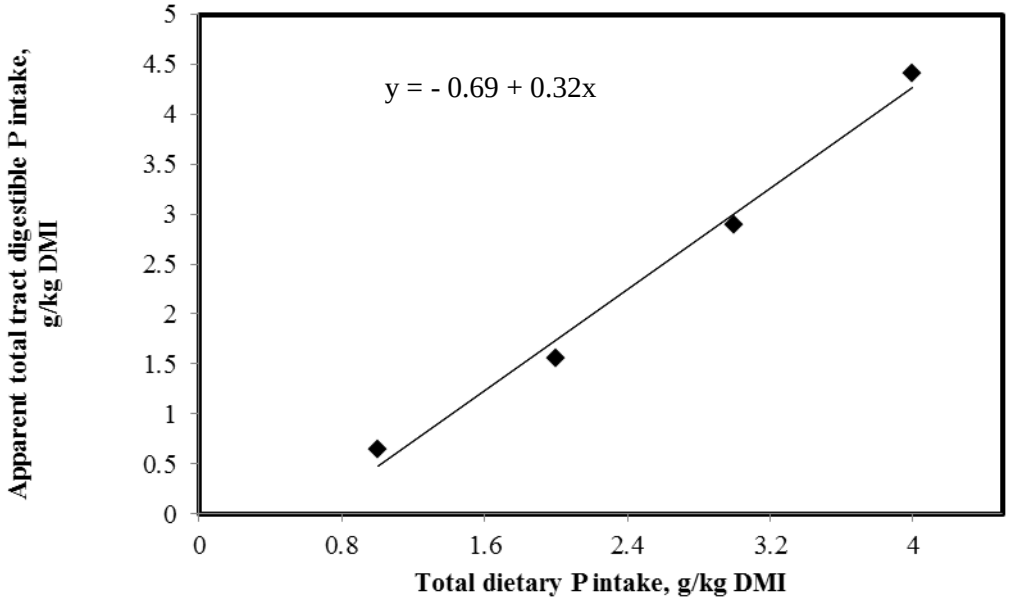
3.4.3 True and standardized total tract digestibility of P

The regression for P intake level in both CM types is shown (Fig. 2). The slopes and intercepts for both CM were similar when determined using the regression procedure.

There were no differences ($P > 0.05$) between the TTTD values (33.3 vs. 32.0%) of P in BNB and BJY as determined using the regression analysis. The TTTD of P (%) in BNB and BJY are given in Table 9. Similarly, there were no differences ($P > 0.05$) between the STTD values (30.7 vs. 28.3%) of P in BNB and BJY when determined using the P-free diet.



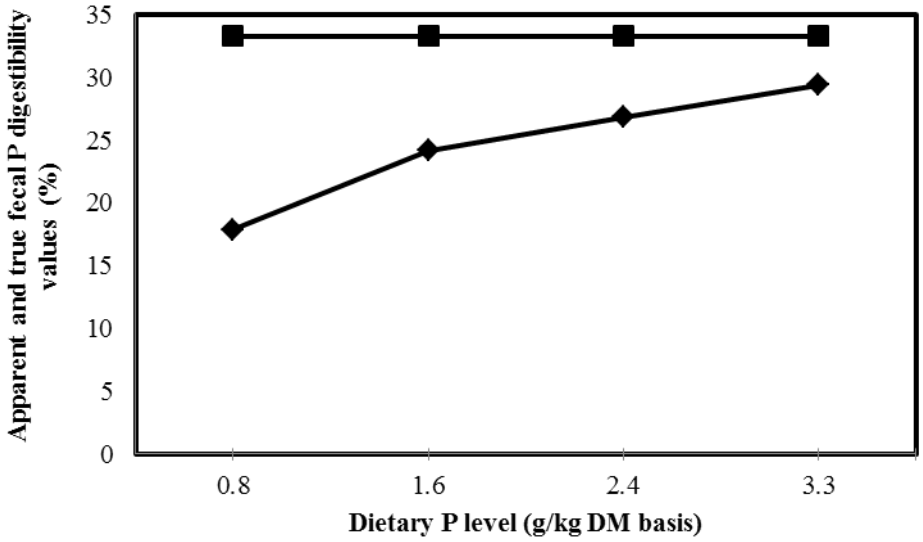
(A)



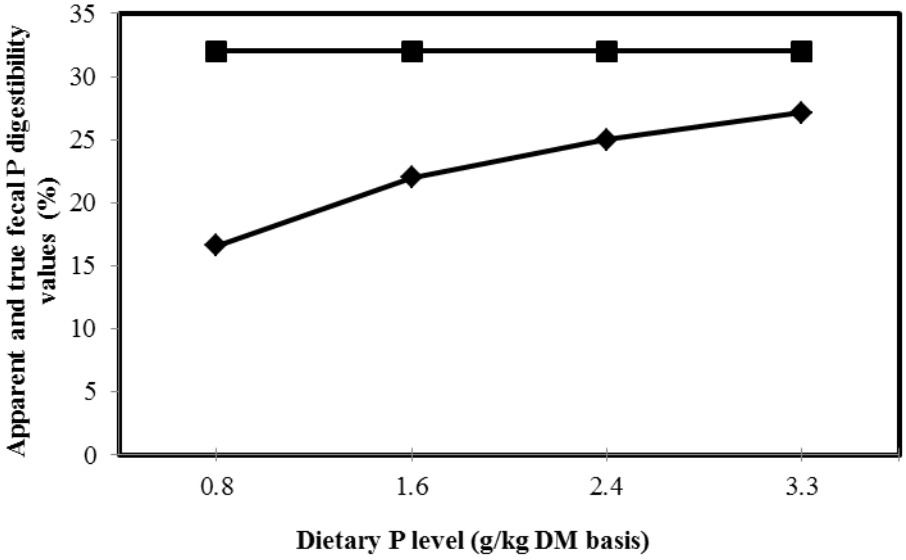
(B)

Figure 2. Linear relationship between the apparent fecal digestible phosphorus (P) (y: g/kg DMI) and the dietary total P input (x: g/kg DMI) in growing pigs fed *Brassica napus* black (A) and *Brassica juncea* yellow (B) diets with increasing P content from 0.8 to 3.3

g/kg DMI. (A) In *Brassica napus* black*:* linear relationship analysis, y=1.305x - 0.74, n = 24, r^2=0.99, $P < 0.05$ for both intercept and slope of the equation. (B) In *Brassica juncea* yellow*:* linear relationship analysis, y=1.26x – 0.78, n = 24, r^2=0.98, $P < 0.05$ for both intercept and slope of the equation.



(A)



(B)

Figure 3. Effects of dietary levels of P (g/kg DMI) on apparent (♦) and true (■) total tract digestibility of P in growing pigs fed *Brassica napus* black (A) and *Brassica juncea* yellow (B) diets with increasing P content from 0.8 to 3.3 g/kg DMI.

Table 9: Linear relationship between total tract phosphorus (P) output and dietary intake of P in growing pigs fed canola meal derived from Brassica napus black (BNB) and Brassica juncea yellow (BJY)

Item	Regression equation[1]	SE of the linear term[2]	SE of the intercept[2]	r^2	Estimated EPL, mg/(kg of $BW^{0.75}$.d)	Estimated TPD, %
B. napus	y= 0.6407 + 0.6671x	0.02	0.21	0.96	0.64	33.3[u]
B. juncea	y = 0.6959 + 0.6812x	0.03	0.33	0.95	0.69	31.9[w]

[1]Regression of total tract output [mg of P/(kg of $BW^{0.75}$.d)] against dietary P intake determined from feeding growing pigs either *B. napus* black or *B. juncea* yellow CM.

[2]Standard error of the regression components (n=54).

[u]Calculated as [(1-true P indigestibility) × 100] for *B. napus.*

[w]Calculated as [(1-true P indigestibility) × 100] for *B. juncea.*

Table 10. Phosphorus (P) and calcium (Ca) balance and digestibility of P and Ca in growing pigs fed 2 types of canola meal (CM)[1]

	B. napus black, g/kg DMI				*B. juncea* yellow, g/kg DMI				SEM	P-values[4]			
										B. napus black		*B. juncea* yellow	
Item	0.8	1.6	2.4	3.3	0.8	1.6	2.4	3.3		L	Q	L	Q
ADG, kg/d	0.45	0.68	0.96	0.92	0.56	0.74	0.80	0.80	0.1	0.01	0.21	0.01	0.21
P intake, g/d	3.6	7.3	11.5	15.5	3.8	7.1	11.4	15.8	0.3	0.00	0.05	0.00	0.06
P excreted, g/d	3.1	5.52	8.40	11.30	3.24	5.52	8.65	11.6	0.2	0.00	0.77	0.00	0.67
P absorbed, g/d	0.7	1.7	3.1	4.6	0.6	1.6	2.9	4.4	0.3	0.00	0.05	0.00	0.05
ATTD DM, %	93.0	89.0	78.0	75.0	93.0	90.0	83.0	79.0	1.3	0.34	0.06	0.32	0.06
ATTD[2] P, %	19.00	27.0	27.0	30.0	17.3	25.0	27.0	28.3	2.0	0.00	0.65	0.00	0.63
P retention, g/d	0.1	0.4	0.6	0.9	0.1	0.3	0.6	1.7	0.1	0.00	0.14	0.00	0.10
P retention, % of intake	15.7	25.5	26.6	29.2	14.5	21.3	24.6	27.0	2.1	0.00	0.56	0.00	0.32
STTD[3] P, %	23.0	27.0	28.5	31.0	21.3	24.6	27.0	28.3	2.0	0.01	0.53	0.01	0.51
Ca intake, g/d	4.7	9.7	14.1	19.1	4.8	9.4	14.0	20.6	0.2	0.00	0.01	0.00	0.01
Ca excreted, g/d	2.4	4.5	7.6	10.0	2.9	4.5	7.2	9.8	0.4	0.00	1.00	0.00	1.00
Ca absorbed, g/d	2.3	5.2	6.6	9.1	1.2	5.0	6.8	10.9	0.5	0.00	0.15	0.00	0.15
ATTD Ca, %	49.0	53.1	46.5	47.1	39.0	52.0	49.0	53.0	4.3	0.10	0.40	0.81	0.68
Ca retention, g/d	0.4	0.9	1.2	1.6	0.3	0.7	1.20	1.9	0.1	0.00	0.94	0.00	0.94
Ca retention, % of intake	45.6	48.7	43.1	43.21	36.0	40.13	42.8	48.40	4.9	0.01	0.820	0.01	0.65

[1]Data are means of 6 observations per treatment.

[2]ATTD = apparent total tract digestibility.

[3]STTD P = standardized total tract digestibility of P. This value was calculated by correcting ATTD for the endogenous P loss (209 ± 96 mg/kg DMI) that was calculated from pigs fed a P-free diet.

[4]L = linear contrast; Q = quadratic contrast.

3.5 DISCUSSION

The objective of this study was to determine the TTTD and STTD of P in BNB and BJY fed to growing pigs. The new cultivar, BJY has considerably lower lignin, glycoprotein and total dietary fiber compared with BNB (Simbaya, 1995; Slominski et al., 2012). The analyzed phytate and non-phytate P in BNB were slightly lower than in BJY (0.64 vs. 0.66% and 0.37 vs. 0.39%, respectively). The ATTD values of P vary with the phytate-P content of the ingredients and there were similar phytate and non-phytate-P content in BNB and BJY. Montoya and Leterme (2009) reported that there were no differences between BNB and BJY with regards to ATTD of DM and energy. Such report could be considered to see if there are any differences between the P digestibility values in our study. Overall, the average ATTD of P from BNB and BJY were 25 ± 4.3%, which is close to the value of 28.6 ± 3.1 reported by Akinmusire and Adeola (2009) for BNB. However, the ATTD of P was increased when P content was increased from 0.8 to 3.3 g/kg DMI in both CM types in our study. This was due to the fact that the contribution of EPL decreases exponentially as dietary P content increases (Fig. 3). Also, intrinsic differences among feed ingredients could cause this divergence in ATTD of P.

In the past, both regression analysis and the P-free diet methods have been used to estimate TTTD and STTD of P in various feed ingredients. The use of regression analysis was developed when endogenous loss of N and AA from pig was calculated by Fan et al. (1995). Later on, it was validated by Fan et al. (2001) to calculate the true P digestibility in SBM. The application of regression analysis technique requires some considerations. One of those conditions is that pigs should be supplied with the dietary P below their requirement (Fan et al., 2001). However, such choice of P levels should also be within the linear response range (Dilger and Adeola, 2006). Also, for the regression curvature, there

is a greater dependence on the lowest P level supplied in the diet (Dilger and Adeola, 2006). Due to the linear relationship between apparent fecal digestible P and total dietary intake of P, the endogenous P outputs could be calculated by the simple linear regression analysis method. Linear regression analysis method has been used to determine TTTD of P and EPL associated with CM in pigs (Akinmusire and Adeola, 2009). In their study, the TTTD of P was reported to be 34% in BNB. In that study, they used 3 levels of CM containing 132, 264 and 396 g/kg (as fed basis). In our study, the TTTD of P in BNB and BJY were 33.3 and 32.0% which are similar to the study by Akinmusire and Adeola (2009). Similar to our study, two SBM cultivars were used in a study to compare the TTTD of P between fecal and ileal level using regression analysis method (Dilger and Adeola, 2006). This method has also been used to determine TTTD in SBM (Fan et al., 2001; Ajakaiye et al., 2003; Akinmusire and Adeola, 2009).

The P-free diet method has been used to determine the STTD of P in different feed ingredients. The approach of using gelatin based P-free diet was developed previously by Petersen and Stein (2006), where they studied STTD of P in different levels of inorganic P fed to growing pigs. In the current study, STTD (%) of BNB and BJY were found to be 31.0 vs. 28.3. The value of STTD of P in BNB and BJY were near to the TTTD of P (34%) calculated by regression analysis method (Akinmusire and Adeola, 2009). In another study by Rodehutscord (1997), P digestibility in rapeseed meal was 24% and the diet was potato starch based basal diet which was highly deficient in P and similar to our study. The value of STTD of P was higher in our study as compared to that of Rodehutscord (1997). The diets were semi-purified in our study and is comparable to those used by Dilger and Adeola (2006) and also our diets were based on cornstarch

(Petersen and Stein, 2006). Similar to previous studies, a 7 to 9 d adaptation period was used in this study which was sufficient to meet steady state conditions for pigs to get adapted to metabolism crates and the environment (Stein et al., 2006; Widmer et al., 2007; Almeida, 2010; Stein, 2011; Almeida and Stein, 2012). To our knowledge, no effect on daily feed intake was observed in pigs fed with P-free diet as they properly consumed their daily ration.

The value of total EPL in our study is higher than that of a study conducted by Ajakaiye (2003), where they used regression analysis and found the value of 210 mg/kg of DMI. However, our value of total EPL falls between the study by (Fang, 2007), where EPL associated with SBM and wheat middling were 450-1020 and 620-920 mg/kg DMI, respectively. Also, our value is similar to that reported by Shen et al. (2002), where EPL associated with corn was 670 mg/kg DMI. Similarly, basal EPL from our study was closer to values reported in previous studies where the value ranged between 139 and 207 mg/kg DMI when a P-free diet was fed to growing pigs (Petersen and Stein, 2006; Widmer et al., 2007; Almeida, 2010). The reasons behind higher values of EPL determined by regression analysis method could be due to EPL from both animal's body and specific diet-dependent EPL. The variations in total EPL between the current and previous studies could be due to the selection of graded level of P in the experimental diets (Jongbloed, 1987). According to Jongbloed (1991), when P intake is significantly less than requirement, low EPL values can be expected. This can be supported further by the study done by Rodehutscord (1997), where they have reported that a reduction in EPL becomes obvious at a more deficient level of P supply in the diet. According to Petersen and Stein (2006), intrinsic phytase activity or anti-nutritional factors (ANFs) may induce

diet dependent P loss which thus increases total EPL in diets. The variability of the EPL among pigs would be also due to the luminal and physiological factors such as age, sex, developmental stages, or breed of pigs (Fang, 2007). The values for STTD of P are lesser than TTTD because only basal EPL values were corrected in STTD whereas, total EPL values were corrected in TTTD (Almeida, 2010). To our knowledge, no determination of TTTD and STTD of P has been done using BJY. Thus, it would be interesting to know the values of both TTTD and STTD of P in BJY compared to BNB.

Also, data obtained from the urine analysis showed that there is a little excretion of P. One of the reasons for this would be that the diets supplied to animals were lower than their P requirements. The values of retention and absorption of urine P are comparable to some of the published results (Stein, 2011).

In conclusion, the results of this experiment showed that the BNB and BJY used in the current study had similar values of TTTD and STTD of P in growing pigs; hence, both values may be used to calculate P supply for growing pigs. Newly developed cultivar of CM, BJY has similar P digestibility to that of BNB which means either of the meal could be supplemented as a P source in pig diets. The TTTD and STTD of P evaluated in BNB and BJY in the current study could be used to formulate growing pig diets.

4.0 MANUSCRIPT 2

Super-dosing effect of phytase on phosphorus and calcium digestibility in canola meals from *Brassica napus* black *and Brassica juncea* yellow fed to growing pigs

4.1 ABSTRACT

A study was conducted to investigate the effect of high level of phytase enzyme supplementation on apparent total tract (ATTD) and standardized total tract digestibility (STTD) of phosphorus (P) and ATTD of calcium (Ca) in *Brassica napus* black (BNB) and *Brassica juncea* yellow (BJY) in growing pigs. A total of 42 barrows with an initial BW of 19.8 ± 1.22 kg (mean ± SEM) were fed one of 7 experimental diets in a completely randomised design in a factorial arrangement with the factors being i) 2 types of canola meal (i. e., BNB and BJY) and ii) three phytase levels [(0, 500 and 2,500 phytase units (U/kg)]. A gelatin-based P-free diet was formulated to measure the basal endogenous P losses (EPL). Limestone was added to maintain a Ca:total P ratio of 1.2:1 in dietary treatments. Daily feed allowance was based on BW at the beginning of the experiment and was calculated to supply 2.6 times the maintenance energy requirement of the pig and was offered in two equal portions at 0800 and 1600 h as a dry mash. Pigs were kept individually in metabolism crates equipped with a feeder and a nipple drinker and were fed experimental diets for 14 d, including 9 d for adaptation and 5 d for total but separate collection of feces. The ATTD of P was increased ($P < 0.01$) from 39.0 to 69.3, and 78.1% in BNB and from 46.0 to 71.4, and 78.0% in BJY when phytase was added at 0, 500 and 2,500 U/kg of diet. Supplementation of phytase linearly increased ($P < 0.01$) the STTD of P in BNB from 40.0 to 70.0, and 78.3%, and also linearly increased ($P <$

0.01) the STTD of P in BJY from 46.3 to 72.1, and 78.5%. Also, the ATTD of Ca was increased from 57.6 to 75.0, and 73.8% in BNB and from 66.0 to 78.0, and 80.0% in BJY when phytase levels were increased. Pigs fed 2,500 U/kg diet had similar ATTD and STTD of P as pigs fed 500 U/kg. The basal EPL calculated from pigs fed P-free diet was 117 ± 23.3 mg/kg DMI. These results indicate a STTD of 40 or 46% in growing pigs fed BNB or BJY and showed that the addition of phytase at 500 and 2,500 U/kg improves STTD of P in BNB by 75.0 and 95.0%, respectively and in BJY by 55.0 and 70.0%.

4.2 INTRODUCTION

About two-thirds of P from plant feedstuffs is poorly digested by animals like pigs and poultry due to phytate bound P and lack of phytase that digest phytate P (Eeckhout and De Paepe, 1994; Selle, 2008). Oilseed meals such as CM contain large amount of phytate P which cannot be properly digested by pigs (Jongbloed, 1991). To improve P digestibility, diets are therefore supplemented with phytase sources or inorganic P. Phytate binds nutrients like AA, protein and energy and reduces the bio-availability of P as well as of other minerals like Ca, Mg, and Zn (Selle, 2008). According to Jones (2010), phytase is commonly used in pig diets at a level of 500 U/kg. However, it has been found that dietary phytase supplementation at higher rates (i. e. beyond 500 U/kg) can further improve utilization of minerals like Ca and P and reduces excretion of such minerals both in pigs and poultry (Adeola, 1995; Augspurger and Baker, 2004; Kies et al., 2006b).

Canola meal (i. e. BNB) is recognized for its well-balanced AA profile and high level of protein (Bell, 1993). However, due to its high fiber content and anti-nutritional factors that cause problems with high inclusion in pig diets, canola breeders have

developed a new cultivar of canola named BJY (Simbaya, 1995; Slominski, 1997). In a study by Akinmusire and Adeola (2009), addition of microbial phytase at 1,000 units/kg of diet increased the TTTD of P up to 61.4%. Akinmusire and Adeola (2009) studied the effect of phytase supplementation on TTTD of P in growing pigs fed BNB. However, there is no information on the STTD of P in BNB and BJY for growing pigs and the effect of phytase on STTD of P. It was hypothesized that addition of phytase at a level higher than standard (500 U/kg) will increase both ATTD and STTD of P. Hence, the objectives of the research were to determine the effect of high phytase supplementation (2,500 U/kg phytase) on ATTD and STTD of P in BNB and BJY and also to determine the basal EPL to calculate the STTD of P.

4.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled in accordance with the guidelines described by the Canadian Council on Animal Care (CCAC, 2009).

4.3.1 Animals and housing

Forty-two crossbred barrows [(Yorkshire × Landrace) × Duroc; Genesus Inc., Oakville, MB, Canada] with an average initial BW of 19.8 ± 1.22 kg (mean ± SEM) were obtained from the University of Manitoba Glenlea Research Unit for the study. Pigs were housed individually in adjustable metabolism crates (118 cm × 146 cm) with smooth transparent plastic sides and plastic covered expanded metal sheet flooring in a temperature controlled room (21 ± 2°C). Fecal and urine collection trays were fitted under the crate floor for sample collection.

4.3.2 Experimental diets

Canola meal derived from BNB and BJY were obtained from a local crushing plant at Bunge Canada (Altona, Manitoba, Canada). Seven cornstarch-based experimental diets were formulated (Table 12). Three diets contained 30% (as fed basis) BNB and either 0, 500 or 2,500 U/kg of diet. The other 3 diets contained 30% (as fed basis) BJY and either 0, 500 or 2,500 U/kg of diet. A P-free diet was formulated to determine the basal EPL from growing pigs. *Brassica napus* black or BJY were the only source of P in the respective diet. Limestone was added to maintain Ca:total P ratio of 1.2:1 in all diets. All diets were supplemented with amino acids, minerals and vitamins to meet or exceed the recommended specification for growing pigs (NRC, 2012).

Table 11. Analyzed composition of canola meal (CM) types

	CM	
	B. napus black	*B. juncea* yellow
DM, %	92.90	92.30
CP, %	38.00	43.40
NDF, %	23.20	17.05
GE, kcal/kg	5,414	5,648
Analyzed Ca, %	0.63	0.68
Analyzed total P, %	1.08	1.12
Analyzed phytate P, %	0.58	0.59
Analyzed non-phytate P[1], %	0.50	0.53

[1]Calculated as the difference between total P and phytate P.

Table 12. Composition and analyzed values of experimental diets (as fed-basis)

	Diets						
Ingredient, %	P-free	BNB + 0 U/kg	BNB + 500 U/kg	BNB + 2,500 U/kg	BJY + 0 U/kg	BJY + 500 U/kg	BJY + 2,500 U/kg
BNB[a]	0.00	32.00	32.00	32.00	0.00	0.00	0.00
BJY[b]	0.00	0.00	0.00	0.00	30.80	30.80	30.80
Cornstarch	49.80	26.80	26.80	26.80	27.60	27.60	27.60
Dextrose	0.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	20.0	20.00	20.00	20.00	20.00	20.00	20.00
Vegetable oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	0.80	0.40	0.40	0.40	0.37	0.37	0.37
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Solka floc[c]	4.00	0.00	0.00	0.00	0.00	0.00	0.00
Potassium carbonate	0.40	0.00	0.00	0.00	0.00	0.00	0.00
Magnesium oxide	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Pork gelatin[d]	20.0	6.53	6.53	6.53	6.86	6.86	6.86
Vit-Min premix[e]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys-HCl	0.20	0.20	0.20	0.20	0.30	0.30	0.30
DL-Methionine	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Threonine	0.24	0.03	0.03	0.03	0.03	0.03	0.03
Tryptophan	0.15	0.05	0.05	0.05	0.05	0.05	0.05
Iosleucine	0.16	0.00	0.00	0.00	0.00	0.00	0.00
Valine	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Histidine	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Analyzed values							
DM, %	93.00	92.40	92.40	93.00	92.41	92.40	92.60
CP, %	18.00	16.00	16.00	16.40	19.00	19.00	19.06
GE, kcal/kg	4,018	4,057	4,056	4,064	4,073	4,054	4,060

Ca, %	0.52	0.43	0.42	0.44	0.50	0.45	0.40
P, %	0.01	0.40	0.40	0.42	0.44	0.40	0.40

[a]BNB=*Brassica napus* black; [b]BJY= *Brassica juncea* yellow

[c]International Fiber Corp., North Tonawanda, NY.

[d]Pork gelatine obtained from Gelita Gelatine USA Inc., Sioux City, IA.

[e]Provided the following quantities of vitamins and minerals of a complete diet: vitamin A, 1000000 IU/g; vitamin D_3, 500000 IU/g, vitamin E, 500 IU/g, vitamin K, 440 mg/g as Menadione; thiamine, 980 mg/g; riboflavin, 800 mg/g; Calcium Pantothenate, 980 mg/g; choline chloride, 700,000 mg/kg; niacin, 995 mg/g as nicotinic acid; vitamin B_6 , 985 mg/g as pyridoxine; vitamin B_{12}, 10,000 mg/kg; biotin, 20,000 mg/kg; folic acid, 970 mg/g; calcium iodate 615 mg/g; copper sulphate, 252 mg/g; ferrous sulphate, 300 mg/g; manganese oxide, 600 mg/g; sodium selenite, 2 mg/g; and zinc oxide, 720 mg/g.

4.3.3 Experimental design and general conduct of the study

The experiment was conducted in a completely randomised design in a 2 × 3 factorial arrangement with the factors being i) 2 CM types (i. e., BNB and BJY) and ii) 3 levels of phytase (i. e., 0, 500, 2,500 FTU). The experimental diets were fed to 42 pigs to give 6 replicates per diet. Daily feed allowance was based on the BW at the beginning of experimental period and was calculated to supply 2.6 times the estimated maintenance energy requirements for growing pigs (NRC, 2012). The pigs were fed twice daily at 0800 and 1600 h. Pigs were allowed *ad libitum* access to water throughout the experiment. The experimental period consisted of 14 d; the first 9 d were for adaptation and the last 5 d for separate but total collection of feces and urine. The total collection of feces and urine was done as described by Ragland et al. (1998) using the procedures described in Manuscript I. Briefly, on d 10, each pig received 5 g of ferric oxide (Fischer Scientific, Ontario, ON) as an indigestible marker in 100 g of feed which was fed in the morning. The remaining portion of feed was offered after all the marked feed was consumed. Fecal collection commenced when marker appeared in feces. On the morning of d 15, pigs were offered 100 g of marked feces as described above, and collection of feces ended when marker appeared in feces. Total collection of urine started on morning of d 10 and ended in the morning of d 15. Fecal samples were collected from the trays underneath metabolism crates into plastic sample bags (Ziplock, 26.8 cm × 27.3 cm) once daily in morning, weighed and stored frozen at -20°C. Urine was collected once daily in morning (in jugs containing 10 mL of 6 N HCl to minimize N losses) and weighed, and a subsample (10% of total weight) was obtained, strained through glass wool, and stored frozen at -20°C.

4.3.4 Sample preparation and chemical analyses

Fecal samples were dried in a forced air oven at 60°C to remove moisture and pooled per pig. Diets, ingredients and fecal samples were finely ground by using 2-mm mesh screen in a Thomas Wiley Mill (Thomas model 4 Wiley Mill; Thomas Scientific) and thoroughly mixed before analysis. All analyses were performed in duplicates. Analyses for DM in diets and fecal samples were carried out according to AOAC (method 934.01; 1990). For DM analysis, 1 g of sample was weighed in a pre-weighed silica dish and dried in an oven for overnight at 104°C. Next day, the sample was removed, cooled down in desiccator and was re-weighed. Gross energy in experimental diets was determined using an adiabatic oxygen bomb calorimeter (Model 6300; Parr Instrument Co., Moline, IL) with benzoic acid as the calibrating standard. Ingredients and diets were also analyzed for CP (AOAC, 2007) using an N analyzer (Leco Corporation, St. Joseph, MI).

Total P and Ca analyses in ingredients, feed and feces were digested according to the procedures described (method 985.01; AOAC, 1990) and were analyzed using an inductively coupled plasma (ICP) spectroscopy (Varian Inc., Palo Alto, CA). For Ca, P analyses, around 1 g sample was weighed in a labelled Pyrex tube without screw and ashed overnight in a furnace with 600°C. After ashing, tubes were allowed to cool and were removed from the furnace for acid digestion. 10 mL of 5N HCl/ HNO_3 (1%v/v) was added to those tubes, capped with screw and were digested for 1 hour in a sonication bath that was preheated to 70°C. After cooling down the samples from sonication bath, 2.5 ml of the sample (1 ml of feces) was pipetted and diluted up to 100 ml with deionized water. After that, the samples were filtered through Q5 filter paper into a 20 ml scintillation

vials. Phytate phosphorus was determined using the procedure described by Haug and Lantzsch (1983).

4.3.5 Calculations and statistical analyses

The apparent total tract digestibility (ATTD) of P in all diets was calculated using the following equation given by Petersen and Stein (2006):

$$ATTD = ([Pi - Pf]/Pi) \times 100, \quad (10)$$

where ATTD represents the apparent total tract digestibility values in %; Pi represents the total P intake (g) from d 10 to d 14 of the experimental period, and Pf represents the total fecal P excretion (g) that originated from the feed fed from d 10 to d 14. The ATTD of DM and Ca in the diets were also calculated using the above equation.

Basal EPL were determined using the P-free diet and expressed on a DMI as outlined by Petersen and Stein (2006) using the following equation:

$$EPL_{basal} = ([Pf/Fi] \times 1{,}000 \times 1{,}000), \quad (11)$$

where EPL_{basal} is the basal endogenous P losses (mg/kg DMI); and Fi is the total feed intake (g) from d 10 to 14. The daily basal EPL (mg/d) in pigs fed the diets containing P was calculated by multiplying the calculated EPL (mg/kg DMI) by the DMI of each pig for whole 5-d collection period and was divided by 5.

The STTD of P was calculated as outlined by Petersen and Stein (2006) using the following equation:

$$STTD\ (\%) = ([Pi - (Pf - EPL_{basal})/Pi] \times 100, \qquad (12)$$

where STTD is the standardized total tract digestibility of P (%); and EPL_{basal} was calculated as in equation (11).

Data were analyzed as a 2 × 3 factorial arrangement in completely randomized design using the GLM procedure of SAS (SAS software release 9.1, SAS Inst., Inc., Cary, NC) with the main effects being 2 CM types and added 3 levels of phytase (0, 500 and 2,500 U/kg respectively). Tukey's test was used to separate treatment means when a significant treatment effect was observed. Differences were considered significant at $P < 0.05$ and trends were considered at $P < 0.10$.

4.4 RESULTS

The analyzed values of total P and phytate P was 1.08 and 0.58% and 1.12 and 0.59% for BNB and BJY, respectively (Table 11). The analyzed dietary P values for all diets were similar to the calculated values (Table 12). All pigs remained visibly healthy and readily consumed their daily ration throughout the experiment.

Dry matter content for CM-based diets averaged 92.54 ± 0.29% for BNB and 92.45 ± 0.10% for BJY. The effects of dietary treatments on P intake, total fecal P output, ATTD and STTD of P, Ca intake, total fecal Ca output, and ATTD of Ca without or with added phytase are given in Table 13. There were no differences between P intake (g) when pigs were fed with diets containing either 0 or 500 U/kg of diet. However, P intake increased linearly ($P < 0.05$) as phytase level increased from 500 to 2,500 U/kg in both

BNB and BJY. The total fecal P output (g) in feces was reduced ($P < 0.001$) as level of phytase increased from 0 to 500 and 2,500 U/kg in both CM types. No differences in fecal P output were detected between the two CM types as the level of phytase was increased.

Brassica napus black had similar ($P > 0.05$) ATTD values for DM, P and Ca compared to BJY at different levels of phytase. There was an increase in ATTD of P from 39.1 to 70.0, and 77.7% for BNB and from 45.7 to 71.4, and 77.8% for BJY when phytase was added at 0, 500 and 2,500 U/kg, respectively. Similarly, STTD of P increased from 40.0 to 70.0 and 78.3% for BNB and from 46.3 to 72.1 and 78.5% for BJY when phytase level was increased consecutively. The value of EPL was determined from pigs P-free basal diet and was calculated to be 117.28 ± 23.3 mg/kg DMI.

The ATTD of Ca was affected individually by both CM and phytase ($P < 0.001$). The ATTD of Ca increased from 57.6 to 74.8 and 73.7% for BNB and from 65.7 to 78.0, and 79.5% for BJY.

4.5 DISCUSSION

4.5.1 Phosphorus and phytate P in ingredients

The concentration of total and phytate P determined in BNB and BJY is in agreement with the values reported by NRC (2012). In the current study, the analyzed total P were 1.08 for BNB and 1.12% for BJY. The values are closed to 1.31 and 0.90% reported for CM by Rodehutscord (1997) and Liu et al. (1998), respectively. Also, our values of total P were lesser than those of the results by Slominski et al. (2012) where total P for BNB and BJY were 1.30 and 1.23, respectively. However, the value of phytate P was a little lower in a previous study using conventional CM done by Akinmusire and

Adeola (2009). Also, analyzed values for Ca (0.63 for BNB and 0.64 for BJY) and total P in our study were higher than a study done by Landero et al. (2012) and Seneviratne (2010) using EPCM where Ca and P values were 0.56 and 1.06, and 0.65 and 1.10, respectively. However, in a study conducted by Landero et al. (2011) values for Ca was higher (0.88) and for P is lower (0.98) where they used SECM.

4.5.2 ATTD and STTD values of P and Ca without phytase

The nutrient output was determined by using total collection method which can be compared to previous studies by Petersen and Stein (2006), and Almeida et al. (2012). The values for the ATTD of P in BNB and BJY measured in this study (39.1 vs. 45.7 %) was higher than the values for SECM, EPCM, and full fat CM reported by NRC (2012) where the value was 28% for all three CM. The ATTD of P values without phytase were found varying between 28 and 33% (Akinmusire and Adeola, 2009; NRC, 2012). In this study the values are higher than any other previously reported values with ATTD of P in BJY being higher than BNB. The reasons behind this could be due to slightly higher values of non-phytate or available P in both meals used in our study. Likewise, the values for the ATTD of Ca in BNB and BJY in this study were 57.5 and 66.0%, respectively. We were not aware of any other studies that estimated the ATTD of P and Ca in new CM cultivar, BJY. In one of the study by Dilger and Adeola (2006), due to similarities in phytate P concentrations between two SBM cultivars, it is reasonable to assume that effects upon P digestibility were a direct result of differences in total P and phytate P content.

The value for basal EPL determined in this study was very close to previous value of 139 ± 18 mg/kg DMI reported by Petersen and Stein (2006) which was determined by

feeding P-free diet. However, the value was slightly lower than some of the other studies (Stein et al., 2006; Widmer et al., 2007). Values ranging from approximately 70 mg/kg of DMI (Dilger and Adeola, 2006; Pettey et al., 2006) and up to 670 mg/kg of DMI (Shen et al., 2002) have been reported. It has been suggested that the EPL depends upon the dietary P concentrations (Jongbloed, 1987). If the intake of P is lower than the P requirement, we can expect low values of EPL. This corresponds to the fact that the values obtained using a P-free diet would be expected to represent the lowest possible values of EPL. Also, the variation in the values of EPL would be large if determined by using regression analysis method.

This value of EPL was used to correct ATTD of P to calculate STTD values of P. Values for STTD of P calculated in our study for BNB and BJY were 40.0 and 46.3%. These values of STTD of P were higher (average 43.0%) than of previous study done by Akinmusire and Adeola (2009) where they determined TTTD of P in BNB CM as 34.3% using regression analysis method.

4.5.3 ATTD and STTD values of P and Ca with phytase

When diets were supplied with phytase, increased ATTD and STTD of P was found. This can be explained by the fact that phytate P is hydrolysed by addition of phytase which liberated P in gut of pigs improving overall P digestibility (Selle, 2008). As BNB and BJY were similar in their phytate content, the similar ATTD of P could be expected. From a study by Akinmusire and Adeola (2009), addition of microbial phytase increased TTTD of P in conventional CM from 34.3 to 64%.

Table 13. Apparent (ATTD), standardized (STTD) total tract digestibility of P, and ATTD of Ca in *Brassica napus* black (BNB) and *Brassica juncea* yellow (BJY) supplemented with 0, 500 or 2,500 U/kg of diet[1]

	BNB			BJY				Significance (*P* value)		
Item	0 U/kg	500 U/kg	2,500 U/kg	0 U/kg	500 U/kg	2,500 U/kg	SEM[d]	CM[e]	Phytase	CM×Phytase
Feed intake (g)	829	834	854	841	818	846	15.50	0.79	0.32	0.66
P intake (g)	17.10^{b}	18.00^{b}	19.30^{a}	20.04^{a}	18.00^{b}	18.00^{b}	0.33	0.13	0.04	< 0.0001
Fecal P output (g)	10.43^{a}	5.52^{b}	4.34^{b}	11.00^{a}	5.10^{b}	4.00^{b}	0.51	0.76	< 0.0001	0.64
ATTD P, %	39.11^{b}	69.30^{a}	78.00^{a}	46.00^{b}	71.42^{a}	78.00^{a}	2.47	0.15	<0.0001	0.42
STTD P, %	40.00^{b}	70.00^{a}	78.30^{a}	46.30^{b}	72.10^{a}	78.50^{a}	2.47	0.15	<0.0001	0.43
Ca intake (g)	19.00	19.00	20.00	21.40	20.00	19.30	0.36	0.00	0.16	0.00
Fecal Ca output (g)	71.42	82.40	70.43	69.55	75.13	91.41	32.38	0.88	0.94	0.90
ATTD Ca, %	58.00^{c}	75.00^{ab}	74.00^{ab}	66.00^{bc}	78.00^{ab}	79.55^{a}	3.07	0.03	<0.0001	0.72
ATTD DM, %	39.00	40.20	44.00	49.00	43.00	42.00	3.78	0.20	0.66	0.30

$^{a, b, c}$ within a row, means without a common superscript differ (P < 0.05)

[1]Data are means of 6 observations per treatment.

[d]Pooled standard error of mean.

[e]Canola meal cultivar.

To our knowledge, no determination of STTD or TTTD of P with the addition of phytase was found in new cultivar, BJY. While comparing STTD of P between BNB and BJY, we found that digestibility of BJY was higher than that of BNB in both 500 and 2,500 U/kg addition of phytase. However, there were no significant differences between levels of phytase on ATTD and STTD of P in both meals when it was increased from 500 to 2,500 U/kg. Also, our data shows higher STTD of P when phytase was added at 500 U/kg (average 70% for BNB and BJY) as compared to the previous research by Akinmusire and Adeola (2009), where they have found TTTD of P of only 61% in CM. It is likely that low concentration of phytate bound P in CM in their study may reduce the effectiveness of phytase in order to improve STTD of P. This could be one of the reasons for the reduced effect of phytase in the previous study. Our hypothesis of increasing STTD of P when there is super-dosing of phytase was not true as there were no significant differences in the STTD of P when phytase was increased from 500 to 2,500 U/kg. In one of the previous study by Almeida and Stein (2012), while phytase was added at 0, 500, 1000 and 1,500 U/kg, there was a quadratic increase in the STTD of P in corn and corn co-products. They also reported that a reduced concentration of phytase may be used if corn or corn co-products do not need a maximum release of P from those ingredients. Also, in high protein DDGS, effect of phytase on STTD of P was minimal when it was increased from 500 to 1,500 U/kg. If we would have selected 4 different levels of phytase in our study, i. e., 0, 500, 1,000 and 1,500 phytase units, we could compare the STTD of P by establishing a regression equation to determine the effect of graded levels of phytase. With regards to phytase efficacy, it can be described on basis of granule size of some phytase products which can prevent complete hydrolysis of

substrate in upper gut (Slominski, 2011). According to the study by Slominski (2011), due to uneven enzyme-substrate distribution within the feed matrix and due to larger particle size of phytase products, phytate P release could be delayed. Our results shows that the addition of only 500 FTU (standard level of phytase) could be recommended as there is no remarkable increase in both ATTD and STTD of P by addition of 2,500 U/kg of phytase which is also on the other hand, cost effective for pig diets.

Phytase supplementation tended to improve the ATTD of Ca from 57.5% in the basal diet to 74.8 and 74.7 % in BNB and from 65.7% in the basal diet to 78 and 79.5% in BJY, but not significantly. Also, both levels of phytase (500 and 2,500 U/kg) increased ATTD of Ca in BNB and BJY but there were no marked differences in increment between 500 and 2,500 U/kg. Also, in BJY, the addition of super-dose level (2,500) did not show any difference in ATTD of Ca compared to the addition of 500 U/kg.

In conclusion, the results of this experiment showed that both BNB and BJY used in the study had greater digestible P contents than used in the previous experiment, realizing that BNB or BJY could be a better source of P for growing pigs. The STTD of P values that were evaluated in the current study could be used when formulating growing pig diets containing feedstuffs so as to minimize the cost of feeding.

5.0 GENERAL DISCUSSION

It is critical to precisely determine the nutrient digestibility in feed ingredients that are used in swine industry. Phosphorus (P) is the third most important nutrients in feed cost of pig diets whose accurate digestibility values needs to be determined in several feedstuffs. By determining the accurate digestibility values of P in feed ingredients, we can: 1) minimize the cost of inorganic P that would be included in large amount in swine ration, 2) match the demands of P in pig's body with its dietary supply, and 3) minimize the environmental impact of P due to undigested P excretion in manure. New feed ingredients have been introduced in pig diets with a motive to supply the available nutrients. *Brassica napus* black (BNB) has been used as a cost effective protein and energy source in pig diets (Bell, 1993). Canola breeders have developed new cultivar of CM named *Brassica juncea* yellow (BJY). Both of these cultivars contain lesser anti-nutritive factors like glucosinolates and erucic acid, whose level otherwise would be more in general CM.

Although previous studies have been conducted to determine the nutritive value of BNB and BJY in pigs, especially in energy and protein digestibility (Zhou et al., 2013), there is still lacking studies regarding P digestibility in such meals. However, true total tract digestibility (TTTD) of P was determined by Akinmusire and Adeola (2009), where they have used conventional CM. Therefore, the primary objective of this thesis was to determine standardized (STTD) and TTTD of P in BNB and BJY fed to growing pigs. Supplementation of microbial phytase in order to improve TTTD of P digestibility in BNB CM have been studied by Akinmusire and Adeola (2009). However, no study has reported to date regarding phytase supplementation in BJY and also the super-dosing of

phytase in these meals. Thus, the secondary objective was to determine STTD of P in BNB and BJY with supplementation of phytase at a standard level (500 FTU) and a super-dose level (2,500 FTU).

In an initial experiment, P digestibility and endogenous P loss (EPL) was compared between BNB and BJY fed to growing pigs using P-free and regression equation technique. The regression technique has been previously used to determine TTTD of P in corn-SBM based diet (Fan et al., 2001) whereas the P-free diet was introduced by Petersen and Stein (2006), where they determined STTD of P in different inorganic sources of P. However, there are no studies to date that compares these two methods which are both used to measure P digestibility in feed ingredients. In this study, 4 different graded levels of P were included from test diets; BNB and BJY to measure TTTD of P using the regression technique. We concluded that STTD and TTTD of P in BNB and BJY were similar with the values averaged 30%. A linear relationship was estimated between apparent digestible P intake and total dietary P intake in the pigs fed both BNB and BJY. The estimated value of total EPL by regression technique was found to be comparable to some of the values determined in previous studies. The apparent total tract digestibility (ATTD) of P in BNB and BJY were also found to increase as the inclusion of P increased. However, there were no differences in ATTD of Ca in both CM types when the inclusion level of P increased.

Similarly, addition of phytase improved both ATTD and STTD of P in BNB and BJY. However, the values of ATTD and STTD of P were higher in the second study compared to the first one. The reason behind this would be that the meals were from different batches and the total and phytate P did differ between the batches when

analyzed (Table 8 and Table 12). Also, in second study, much lower NDF values in BJY (23 vs. 17%) could be another reason for both ATTD and STTD of P being higher than that of BNB.

6.0 SUMMARY AND CONCLUSION

Brassica napus black and BJY had similar ATTD (26.0 vs. 24.4%), STTD (31.0 vs. 28%) and TTTD (33.0 vs. 32.0%) of P. However, ATTD of Ca was found similar between the different inclusions levels of P fed to growing pigs. When measured by regression analysis and the P-free method, the differences were observed in EPL where the value was higher when measured from the regression analysis. Both techniques can be used to measure either true or standardized P digestibility. Likewise, the ATTD increased from 39.0, to 70.0 and 78.0% and from 46.0, to 71.0 and 78.0% and STTD increased from 40.0, to 70.0 and 78.3 % and from 46.3, to 72.1 and 78.5% in BNB and BJY when phytase was added at 0, 500 and 2,500 U/kg, respectively. It shows that addition of phytase was able to increase both ATTD and STTD in BNB and BJY, but there were no significant increases in such values when phytase was increased from 500 to 2,500 FTU (super-dose level). Similarly, ATTD of Ca increased from 57.0, to 74.0 and 73.0% and from 66, to 77 and 79% when phytase level was added at 0, 500 and 2,500 U/kg.

Recommendation for further research:

1. Supplementation of multi-carbohydrase enzyme on STTD of P in CM types (BNB and BJY).
2. Determination of the effect of feeding BNB or BJY on carcass quality and characteristics.
3. Effect of phytate:non-phytate P levels on the ATTD and STTD of P.
4. Use of both marker method and total collection method for the sample collection to compare the P digestibility values between 2 meals.

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