GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, ORGAN MASS AND WHOLE-BODY OXYGEN CONSUMPTION IN GROWING PIGS FED DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS)-CONTAINING DIETS SUPPLEMENTED WITH A MULTICARBOHYDRASE ENZYME

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ABSTRACT

The aim of this study was to determine growth responses and apparent total tract nutrient digestibility in growing pigs fed diets containing graded levels of distillers dried grains with solubles (DDGS) and to determine the effect of a multicarbohydrase enzyme (MC) supplementation on visceral organ mass and fasting whole-body oxygen consumption (FWBOC). In Experiment 1, 48 pigs (25.5 ± 0.14 kg BW) were assigned on the basis of sex and BW (2 pigs per pen) to 4 diets (6 pens per diet) based on corn-barley-soybean meal with 0, 10, 20 and 30% DDGS in a 42-d trial. Apparent total tract digestibilities (ATTD) of energy and other nutrients were determined using acid insoluble ash (AIA) as the indigestible marker. Inclusion of DDGS in the diets decreased (linear, $P < 0.05$) average daily gain (ADG) and tended to decrease gain to feed ratio (G:F) (linear, $P = 0.067$) but not average daily feed intake (ADFI) ($P > 0.10$); however, pigs fed the 20% DDGS diet had similar ADG as pigs fed the 0% DDGS diet. Increasing DDGS content in the diet linearly decreased ($P < 0.01$) the ATTD of DM, energy and CP (cubic, $P < 0.05$) but not ($P > 0.10$) P and Ca; however, ATTD of neutral detergent fiber (NDF) increased (linear, $P < 0.01$) when DDGS was included in the diet. In Experiment 2, 24 pigs (19.9 ± 0.46 kg BW) were randomly assigned to 3 corn-soybean meal-basal diets (8 pigs per diet): Control (C, 0% DDGS); basal diet with 30% DDGS (C+30% DDGS) or C+30% DDGS supplemented with MC (C+30% DDGS+MC). On d 15, WBOC during the 24- to 30-h postprandial period was determined using an open-circuit indirect calorimeter on 4 pigs per diet. On d 28, pigs were killed to determine visceral organ mass and intestinal morphology. There was no diet effect ($P > 0.10$) on final BW, FWBOC,

and liver, spleen, pancreas, heart, stomach and caecum weights relative to empty BW. However, pigs fed the C and C+30% DDGS+MC had heavier (P = 0.05) empty BW compared with those fed unsupplemented 30% DDGS-containing diet. Small intestine, colon plus rectum and the portal-drained viscera (PDV) were heavier (P < 0.05) in DDGS-fed pigs compared with C; supplementing with MC substantially decreased the effect of DDGS on the weight of these visceral organs. Feeding pigs DDGS tended to decrease (P < 0.10) villous height (VH) and VH:CD in the ileum. Results show that including DDGS in grower pig diets up to 20% has no effect on performance although ATTD of energy, DM, and N may be reduced. Inclusion of DDGS at 30% increased PDV mass and reduced dressing percentage but these effects were overcome by a multicarbohydrase enzyme supplementation.

DEDICATION

This thesis is dedicated to my father, Mr. Emmanuel K. Agyekum of blessed memory.

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FOREWORD

This thesis was written in a manuscript format and it is composed of two manuscripts. Manuscript I and Manuscript II have been submitted to the Canadian Journal of Animal Science and Journal of Animal Science, respectively. Part of the work in Manuscripts I and II were presented at the ASAS–ADSA Midwest meeting in Des Moines, Iowa (March, 2011). Authors to manuscript I are A. K. Agyekum, T. A. Woyengo, B. A. Slominski and C. M. Nyachoti whereas authors to manuscript II includes all the above mentioned authors except T. A. Woyengo. In this thesis, the manuscripts are formatted according to the guidelines of the American Society of Animal Science manuscript preparation.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

4.0 MANUSCRIPT II

5.0 GENERAL DISCUSSION

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AA	Amino acid(s)
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AIA	Acid insoluble ash
AOAC	Association of Official Analytical Chemists
ANF	Anti-nutritional factors
ATTD	Apparent total tract digestibility
BW	Body weight
CD	Crypt depth
CF	Crude fat
CP	Crude protein
d	Day
DC	Digestibility coefficient
DDG	Distillers dried grains
DDGS	Distillers dried grains with solubles
DDS	Distillers dried solubles
DM	Dry matter
DS	Distillers solubles
EE	Ether extract
Exp	Experiment

FWBOC	Fasting whole-body oxygen consumption
G:F	Gain to feed ratio
GE	Gross energy
GIT	Gastrointestinal tract
h	Hour
MC	Multicarbohydrase enzyme blend
ME	Metabolizable energy
MNSD	Minnesota-South Dakota region
NDF	Neutral detergent fiber
NRC	National Research Council
NSP	Nonstarch polysaccharides
PDVO	Portal vein-drained visceral organ
SAS	Statistical Analysis System
SEM	Standard error of the mean
SID	Standardized ileal digestibility
VH	Villous height
wcDDGS	wheat-corn distillers dried grains with solubles
WB	Whole-body

1.0 GENERAL INTRODUCTION

The common factor that swine producers consider to be of great importance is the cost of feed since it accounts for almost two-thirds of the variable cost of production. The feedstuffs used in formulating swine diets have been based primarily on cereal grains and high quality protein supplements. However, in recent years, the global feed industry has seen cereal grain price hikes which has been attributed to several factors, the most important of which is the introduction of ethanol production into the demand and supply equation of these cereal grains, especially, in North America (Coyle, 2007; CBO, 2009). Indeed, in 2007, the global biofuel production was estimated to have tripled from 18 billion to 60 billion litres since the year 2000 (Coyle, 2007) and continues to increase. Reasons often adduced by governments to justify their support for the biofuel industry include; addressing energy cost, energy security, global warming concerns (UNCTAD, 2008) and achieving rural development objectives (Coyle, 2007). Apart from Brazil, which uses sugar cane, cereal grains (particularly, corn and wheat) are mostly used as feedstock in other countries for ethanol production (UNTCAD, 2008; Walburger et al., 2006). For instance, the bio-refinery plants in the United States are estimated to have the capacity to produce over 18.5 billion litres of ethanol whereas Canadian plants have the capacity to produce over a billion litres of ethanol, annually, using mostly corn and wheat or corn as the feedstock, respectively (Government of Alberta, 2010). Taken together, the trend and estimates of cereal grains for biofuel production elicit the need for swine producers to reconsider the feedstuffs for swine diet formulation in order to reduce feed cost.

Indeed, recent reports (Johnston et al., 2003; Zijlstra, and Beltranena, 2007; Agudelo- Trujillo, 2009; Lackey, 2010) indicate increasing interest in the use of alternative feedstuffs which, by definition, are crop residues or by-products (co-products) from the food industry not consumed by humans but suitable for feeding livestock. The use of these alternative feedstuffs does not only lower production cost but also serves as an eco-friendly approach to the disposal of the enormous amounts of by-products generated. The main problem with these alternative feedstuffs is the limitations on their inclusion level, especially in the growing pig's diet due to their bulky and fibrous nature.

One such alternative feedstuff is distillers dried grains with solubles (DDGS) which is a co-product of ethanol production and its availability in North America has become wide spread due to the reasons indicated above. Since most of the starch is removed from the grain used during the fermentation process, DDGS has higher concentrations of the remaining nutrients such as crude protein, fat, minerals and gross energy compared with the published values of its corresponding grain (NRC, 1998; Spiehs et al, 2002; Stein and Shurson, 2009) which makes it an attractive feedstuff for swine feed but because of the high fiber content, the digestibility of these nutrients is low (Nyachoti et al., 2005; Lan et al., 2007; Widyaratne and Zijlstra, 2007). Dietary fiber enhances dry matter flow and endogenous losses of nutrients leading to a decrease in ileal and fecal digestibilities of energy and other nutrients (Li et al., 1996; Nyachoti et al., 1997; Souffrant, 2001; Wenk, 2001) when added to pig diets resulting in reduced BW gain and efficiency of gain (Jørgensen, et al., 1996; Noblet and Le Goff, 2001). Including DDGS in swine diets may reduce the need for

supplemental phosphorus from inorganic sources and also decrease phosphorus excretion because of its high phosphorus bioavailability (Stein and Shurson, 2009).

Although corn is used as the main feedstock in North America and wheat, mostly, in western Canada and Europe (Shurson et al., 2004; Nyachoti et al, 2005; Cozannet et al., 2010), it is a common practice for the ethanol plants in western Canada to use a blend of corn and wheat with the proportions for fermentation dictated by the cost and availability. Given the vast information on DDGS in the literature, there is no doubt that using DDGS can be a money saver for North American pork producers. Therefore, the question that arises is definitely not whether DDGS should be used but instead, how much of it can be included in swine diets, particularly, the growing pig's diet before the negative effects start to show and what can be done to overcome these adverse effects for more of DDGS to be used or included in grower diets.

Recent experiments evaluating the effects of DDGS and enzyme supplementation on growth performance and nutrient digestibilities in pigs have not produced consistent results. Some studies (DeDecker et al., 2005; Widmer et al., 2007; Xu et al., 2010) observed no effect on body weight (BW) whereas others (Whitney et al., 2006; Thacker, 2006; Feoli, 2008; Linneen et al., 2008) observed a reduction in BW gain when 30% or more DDGS was included in growing-finishing pig diets. Enzyme supplementation targeting nonstarch polysaccharides improved growth performance and/or nutrient digestibilities in pigs fed 30% or more DDGS in some studies (Opapeju et al., 2006; Foeli, 2008; Emiola et al., 2009) but not others (Foeli, 2008; Jacela et al., 2009; Jones et al., 2010). The variations in the chemical composition of the DDGS samples used, basis for diet formulation, amount of substrate (i.e., the

various nonstarch polysaccharides) and the concentration of enzymes in the feed are often cited as reasons for the discrepancies in results between studies. However, the effect of these carbohydrase enzyme preparations in DDGS-containing diets particularly, on the gastrointestinal tract of pigs and the metabolic adaptation of pigs to DDGS-containing diets alone or supplemented with carbohydrase enzymes remains to be known. Besides, it is not known whether the lack of response in growth rate (based on live weight gain) to supplemental enzyme will still hold if carcass weight gain is used as the performance indicator given the confounding effect of gut mass and fill in pigs fed high-fiber diets on live weight (Kyriazakis and Emmans, 1995; Whittemore et al., 2003).

On the other hand, several authors have reported a marked effect of a high-fiber ingredient or diet on gut anatomy, development and function (Pond et al., 1989; Jin et al., 1994; Jørgensen et al., 1996; Nyachoti et al., 2000; Ma et al., 2002). In general terms, dietary fiber intake leads to an increase in the size and length of the visceral organs. The visceral organ mass which makes up 15% or less of the total body mass accounts for a large proportion of the body's energy expenditure (heat production) therefore, the increase in the gut mass due to high dietary fiber intake will result in an "extra" increase in maintenance energy requirement resulting in less energy retained for body growth (Pond et al., 1989; Yen, 1997). This gut hypertrophy is often associated with modifications of the gut epithelial morphology (Jin et al., 1994), which is an energy consuming process.

To this end, information on the effects of feeding growing pigs DDGS-containing diets without or with carbohydrase enzymes on energy expenditure, changes in

visceral organ mass and intestinal morphology remains to be known. Such information will help in understanding the metabolic adaptation of growing pigs to diets containing DDGS (a high-fiber feedstuff) and the consequences on maintenance energy and nutrient requirements, and how dietary manipulation may overcome these negative effects to maximize nutrient utilization of DDGS-based diets in the growing pig.

Therefore, the objectives of the studies reported in this thesis were:

1) To determine the effects of increasing dietary DDGS content on performance and energy and nutrient digestibility in growing pigs.
2) To determine the effects of DDGS and a multicarbohydrase enzyme blend on visceral organ mass and whole-body oxygen consumption in growing pigs.

The experiments were conducted using DDGS resulting from cofermentation of equal proportions of wheat and corn.

2.0 LITERATURE REVIEW

2.1 Introduction

Traditionally, pigs are fed diets that contain substantial amounts of cereals (e.g. corn, wheat and barley) and meals derived from oilseeds (e.g. soybean) to supply their nutrient (e.g. energy and protein or amino acids) requirements. However, trends in the demand and supply of these conventional feedstuffs for swine in the last few years necessitate producers around the world to seek alternative feedstuffs to feed their hogs. Specifically, the price of cereals (the main feed ingredient for swine feed formulation) keep rising and has become very unpredictable, nowadays. To the North American swine industry, competition from the ever increasing human population for the demand of these cereal grains is not the cause for cereal grain price hike since it is somewhat predictable because this has always been an important factor influencing the demand and price of these grains. Thus, the introduction of ethanol production into the supply and demand equation of cereal grains appears to be responsible for the price hike. Indeed, in a recent report prepared by United States Congressional Budget Office (CBO, 2009), it was estimated that approximately 3 billion bushels of corn were used to produce ethanol in the United States in 2008; this estimate represented an increase of nearly a billion bushels of the previous year resulting in the price of corn to rise by more than 50% between April 2007 and April 2008 (CBO, 2009). The same can be said for Canada and other parts of the world where ethanol production is embraced (Auld, 2008; Lann et al., 2009). Unfortunately, the price and demand of cereal grains for ethanol production is expected

to go up due to the increasing interest for renewable fuel production and governments' support for the ethanol industry (Coyle, 2007; Miranowski, 2008).

In the production of ethanol, a co-product, dried distillers grain with solubles (DDGS), is produced which can be used as an alternative feedstuff in swine diet. This product is the dried residue remaining after the starch fraction of the grain is fermented with selected yeasts and enzymes to produce ethanol and carbon dioxide (Rausch and Belyea, 2006). As a result of starch removal, the concentration of the remaining nutrients such as protein, fat, fiber and minerals in the grain increases by three-fold or more compared with the corresponding grain (Table 2.1). The choice of feedstock for ethanol production depends on the geographical climate, supply, price, and production efficiency. In North America, corn has been the primary substrate for ethanol production and the resulting corn-derived DDGS has been extensively studied as a feedstuff for swine (Spiehs et al., 2002; Pedersen et al., 2007; Stein and Shurson, 2009) although sorghum is used at times in the United States (Wang et al., 2008; Urriola et al., 2009). In western Canada and Europe, wheat is often used (Nyachoti et al., 2005; Zijlstra et al., 2007; Cozannet et al., 2010); however, co-fermentation with corn is common, particularly, in western Canada to increase production efficiency and also serve as a risk management for the supply and price of grain.

In this chapter, a review of the literature pertaining to the present study is provided. Thus, an overview on the production and nutritive value of DDGS and information on DDGS for pigs in the growing-finishing phase is discussed. In the literature review presented herein, the abbreviation, DDGS, refers to corn- and wheat-

Table 2.1. Concentration of energy and nutrients in DDGS and corresponding grain (DM basis).

Item	Component[a]						
	DM (%)	GE (kcal/kg)	CP (%)	EE (%)	NDF (%)	ADF (%)	P (%)
NRC, 1998							
Corn	89	-	8.3	3.9	9.6	2.8	0.28
Corn-DDGS	93	-	27.7	8.4	34.6	16.3	0.77
Pedersen et al., 2007							
Corn	86.7	4,483	8.3	3.3	7.7	2.6	0.23
Corn-DDGS[b]	87.6	5,427	32.2	11.7	27.6	11.6	0.69
Nyachoti et al., 2005							
Wheat	92.4	4,362	14.4	1.6	12.7	5.2	0.37
Wheat-DDGS[c]	95.8	5,071	42.3	3.7	30.8	12.9	0.90
Widyaratne and Zijlstra, 2007							
Wheat	88.2	-	19.8	1.8	9.4	2.7	0.40
Wheat-DDGS	91.9	-	44.5	2.9	30.3	21.1	1.10

[a]DM = Dry matter; GE = Gross energy; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; P = Total phosphorus.
[b]Average of 10 corn-based DDGS samples from South Dakota and Minnesota ethanol plants.
[c]Average of winter wheat- and mixed wheat-based DDGS samples.

derived DDGS unless otherwise stated. Discussions on the effect of dietary fiber on visceral organ changes and energy expenditure are also included.

2.2 The use of distillers co-products in swine feed

The literature contains enough evidence to suggest that distillers co-products (distillers dried grains, DDG; distillers dried solubles, DDS; and distillers dried grains with solubles, DDGS) have been used in swine diets for more than 50 years. In the reviews by Shurson et al. (2004) and Stein and Shurson (2009), a chronology on the history of research on distillers co-products in the United States is provided. It appears that majority of the early studies focused on evaluating the feeding values of these co-products based on their effect on the growth performance of pigs (e.g. Fairbanks et al., 1944, 1945; Krider et al., 1944). Subsequent studies in the late 1950s were more interested in evaluating distillers co-products as a source of "unidentified growth factors" (e.g. Conrad and Beeson, 1957; Jeter et al., 1960; Gage et al., 1961), although some researchers (Conrad et al., 1959; Spree et al.,1967) did not find any evidence of such activities.

There was a dramatic shift in the annual production of the various distillers co-products during the period indicated above. The widely used co-product during the 1940s was DDG, followed by DDGS and DDS; however, from the 1970s, DDGS became the primary co-product (Newland and Mahan, 1990). The construction of large-scale ethanol plants during the 1970s and 1980s further increased the production and availability of DDGS for research on the maximum inclusion levels of this co-product in swine diets as

well as research on the amino acid (AA) composition of DDGS (Stein and Shurson, 2009). Discussions in this chapter are related to information on DDGS only.

2.3. Distillers dried grains with solubles (DDGS)

2.3.1 Overview of DDGS production

As indicated above, DDGS is a co-product from ethanol production. Fuel ethanol can be produced from cereal grains either by the wet mill or dry grind method but the process that produces DDGS as co-product of ethanol production is the dry grind method (Bothast and Schliche, 2005; Rausch, and Belyea, 2006). Figure 2.1 provides a schematic presentation of the distillation process that result in the production of ethanol and DDGS.

Upon arrival of grain in the plant, the whole kernel is ground into powdery form using a hammer or roller mill for ease of water penetration during the subsequent cooking period. The ground grain is then mixed with water to form a mash or slurry and cooked. During the process of cooking, the enzyme, α–amylase, is added to hydrolyze all the starch polymers by breaking down the α-1, 4-glycosidic bonds (Bothast and Scliche, 2005). Fermentation of the slurry involves two steps. The first step, termed saccharification, involves the addition of glucoamylase to the mixture to release the glucose monomers from the non reducing end of the carbohydrate chains (Rausch, and Belyea, 2006). In the second step, yeast (*Saccharomyces cerevisiae*) is added to the mixture and then allowed to ferment for a period of time. The resulting material after complete fermentation of the mixture is composed of ethanol, water, and solids that were not fermented (Rausch, and Belyea, 2006; Husky Energy, 2011). Ethanol is then distilled

off using distillation columns, a stripping column and a molecular sieve to remove any remaining water molecules (Figure 2.1). The ethanol produced is then denatured by adding a small amount of gasoline to produce fuel–grade ethanol. The carbon dioxide (CO_2) produced after the fermentation process is either released into the atmosphere or collected for later use in the beverage industry. The solid and liquid fraction remaining after distillation (i.e., the whole stillage) is drawn from the distillation unit and centrifuged to produce wet grains and thin stillage. An evaporator condenses the thin stillage into distillers solubles (DS) which is added to the wet grains and dried to form DDGS (Figure 2.1: Bothast and Scliche, 2005; Rausch, and Belyea, 2006). Thus, DDGS consists of all the distillers grains and more than 70% of the condensed solubles produced after fermentation (Stein, 2008).

2.3.2 Chemical composition of DDGS

The literature contains a plethora of information on the nutritional profile of DDGS. Basically, the nutritional profile of DDGS is a reflection of the grain used in the fermentation process. Thus, factors such as grain type, variety and quality, and soil and climatic conditions influencing the nutrient concentration in grains will ultimately affect the chemical composition of the DDGS produced (Spiehs et al., 2002; Shurson et al., 2004). Nonetheless, the concentration of the nutrients in DDGS (except starch) is three-fold or more when compared with the parent grain as indicated earlier. Distillers dried grains with solubles also has a high bioavailability of phosphorus due to the fact that most of the phytate-bound phosphorus are hydrolyzed during the fermentation process

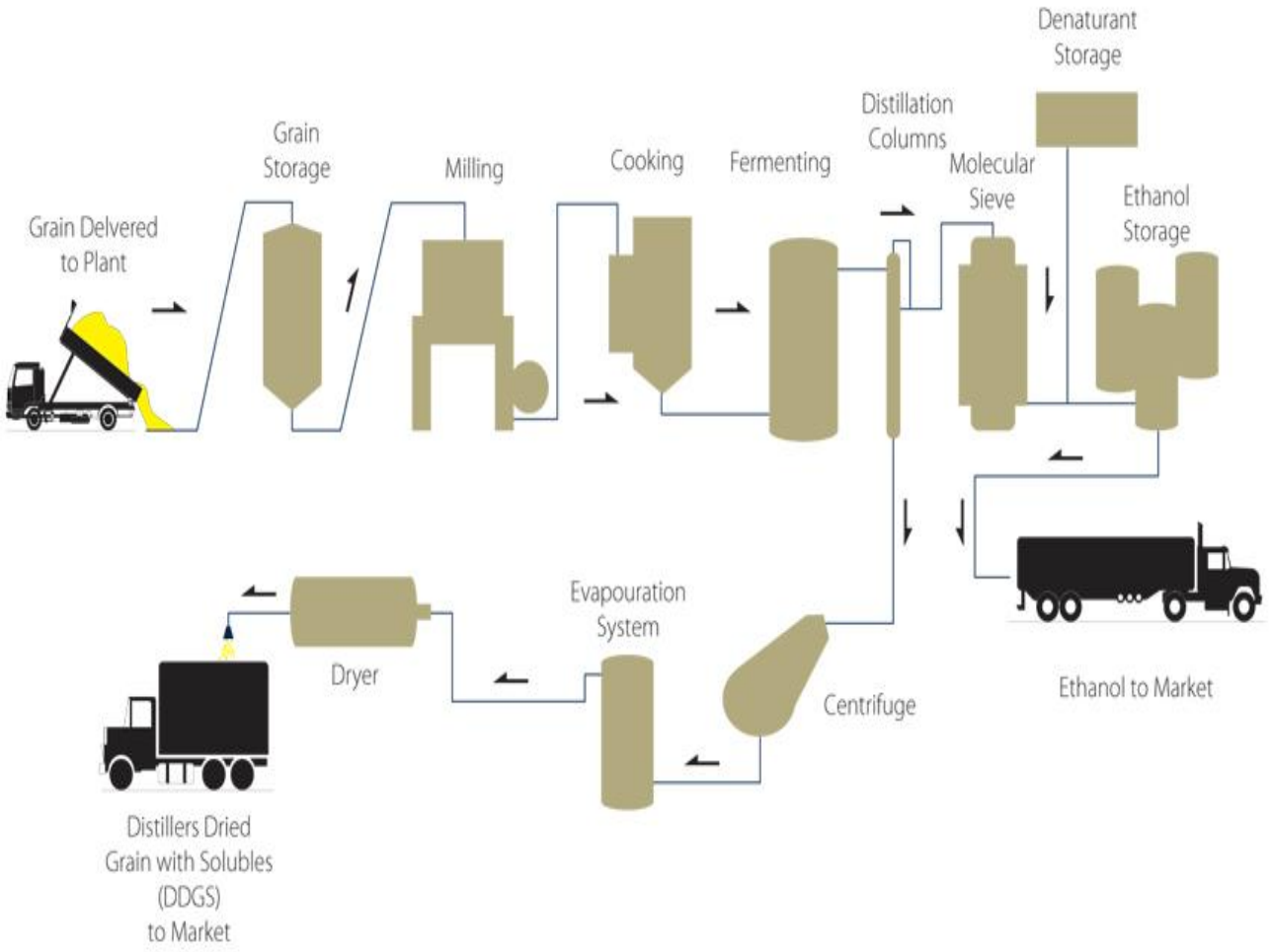

Figure 2.1. Overview of the ethanol production process (adapted from Husky Energy, 2011; http://www.huskyenergy.com/)

(Cromwell, 1993; Stein and Shurson, 2009) thus, making it a good source of phosphorus.

Majority of the studies evaluating DDGS as a swine feed have focused on corn-derived DDGS. This is due to the fact that most of the ethanol plants, especially, the ones in North America use corn as the main feed stock because of its abundance and high yield of ethanol (Berg, 2004; Shurson et al., 2004; Bothast and Schlicher, 2005). However, wheat is used as the main feedstock particularly, in western Canada and Europe and some times, a blend of these two grains are used with their proportions depending on grain availability and price.

In Table 2.2, the nutritional profile of DDGS reported by several researchers is shown. There appears to be considerable differences in the macro nutrient profile between corn- and wheat-derived DDGS. Wheat has a higher crude protein (CP) but lower crude fat contents than corn (NRC, 1998). It is therefore, not surprising for wheat DDGS to have a higher concentration of CP (on average; 40.6 vs. 30.3%) and a lower ether extract (EE: on average; 4.8 vs. 10.9%) when compared with corn DDGS (Table 2.2). On average, wheat DDGS appears to have a higher dry matter (DM, 92.3 vs. 89.8%) and slightly higher neutral detergent fiber (NDF, 36.3 vs. 34.9%) and acid detergent fiber (ADF, 15.3 vs. 14.2%) contents than corn DDGS. In the review by Zijlstra et al. (2007), higher levels of DM, CP and nonstarch polysaccharide (NSP) and lower EE values for wheat DDGS were reported when compared with corn DDGS. The macro nutrient profile of wheat-corn DDGS is intermediate (on average: CP, 36.9%; EE, 5.5%; ash, 4.9%) (Table 2.2).

Table 2.2. Chemical composition of distillers dried grains with solubles (DM basis)

Item	Composition[a]						References
	DM (%)	CP (%)	EE (%)	NDF (%)	ADF (%)	Ash (%)	
Corn-based DDGS							
1	93.0	27.7	8.4	34.6	16.3	-	NRC, 1998
2[b]	88.9	30.2	10.9	44.5	16.5	5.8	Spiehs et al., 2002
3	88.2	30.3	12.8	31.2	14.6	4.8	Widyaratne and Zijlstra, 2007
4[c]	87.6	32.3	11.7	27.6	11.6	4.4	Pedersen et al., 2007
5[d]	91.3	31.1	11.1	36.5	12.1	3.0	Urriola et al., 2009
Wheat- based DDGS							
1	91.9	44.5	2.9	30.3	21.1	5.3	Widyaratne and Zijlstra, 2007
2	93.9	37.2	5.0	46.5	13.2	4.3	Beliveau and McKinnon 2008
3	91.6	45.8	4.6	28.9	19.5	-	Gibb et al., 2008
4[e]	92.6	36.1	4.6	29.2	12.0	5.2	Cozannet et al., 2010
5	91.7	39.2	7.0	46.8	10.5	5.5	Oryschak et al., 2010
Wheat-corn DDGS[f]							
1 -	94.8	37.3	4.6	39.5	19.8	4.9	Nyachoti et al., 2005
2 (4:1)	92.0	42.4	4.7	30.6	19.5	5.0	Widyaratne and Zijlstra, 2007
3 (1:1)	91.4	31.0	7.1	39.8	11.1	4.9	Yáñez et al., 2011

[a]DM = Dry matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

[b]Average of 10 DDGS samples from 10 new Minnesota and South Dakota ethanol plants production.

[c]Average of 10 DDGS samples from South Dakota and Minnesota ethanol plants.

[d]Average of 8 DDGS samples.

[e]Average of 10 DDGS samples from 7 European ethanol plants.

[f]DDGS produced from cofermentation using different proportions of wheat and corn (wt/wt).

2.3.3 Variations in the chemical composition of DDGS

One major concern of using DDGS is its variability in nutrient composition even if the DDGS comes from the same source (Spiehs et al., 2002). The differences in the chemical content between wheat- and corn-derived DDGS are due to the differences in the chemical compositions of the individual intact grains as discussed above. There are also variations in nutrient composition even when DDGS is derived from the same grain. For instance, Pedersen et al. (2007) chemically analyzed 10 corn-based DDGS samples from ethanol plants in the Minnesota-South Dakota (MNSD) region and reported that the concentration of CP differed from 25.9 to 32.4%; gross energy (GE), from 4,571 to 4,820 kcal/kg; starch, from 4.1 to 11.4%; EE, from 8.6 to 12.4%; P, from 0.51 to 0.74% and; ADF and NDF, from 8.6 to 12% and 20 to 26.6%, respectively. Another chemical analysis of 118 corn DDGS samples conducted by Spiehs et al. (2002), however, found a lower coefficient of variation (CV, < 10%) among and within 10 ethanol plants from the MNSD region over a 3-year period for DM, CP, EE and crude fiber but higher CV for the 10 essential AA (EAA, 25.7 %) and minerals (80.4%) analyzed. Of the 10 essential AA analyzed in the corn DDGS samples, lysine (CV = 17.7%) was the most variable, followed by methionine (CV = 16.3%) which agrees with the report by Cromwell et al. (1993).

As indicated earlier in this review, much of the variation in the nutrient content of DDGS is due to the inherent variations among grain varieties and the geographical location where the cereal grain is grown. In addition, processing factors such as the grinding procedure used, type of yeast for the fermentation process, efficiency of starch extraction from the grain, amount of solubles added back, and temperature and time of

drying all contribute to further variability in the chemical composition and physical appearance (especially, color) of the DDGS produced (Cromwell et al., 1993; Shurson et al., 2004). The process of standardizing the nutrient composition and quality of DDGS has been very slow because DDGS is a co-product of a process intended for the production of fuel ethanol and thus, not the main focus of the ethanol plants. It is therefore, imperative for swine nutritionists to conduct frequent chemical analyses of the different batches of DDGS samples received for diet formulation to ensure consistency in the nutrient concentrations of swine diets.

2.3.4 Effect of DDGS on nutrient digestibility in growing-finishing pigs

The digestibility of DM, energy and CP has been reported by several researchers to be adversely affected when DDGS is included in the diet of a growing-finishing pig. For instance, Nyachoti et al. (2005), Widyaratne and Zijlstra (2005) and Thacker (2006) reported lower digestibilities of DM, CP and energy in diets with wheat DDGS compared with pigs fed the wheat-based control diets. Feoli (2008) and Urriola and Stein (2010) reported a decrease in the digestibilities of DM, energy and NDF in pigs fed a corn DDGS-based diet compared with pigs fed a corn-soybean meal-based control diet. The adverse effect of DDGS on nutrient digestibility is probably due to its high-fiber content because it is well known that, in pigs, dietary fiber enhances DM flow and reduce transit time in the gut as well as increases endogenous nitrogen losses leading to a decrease in ileal and fecal digestibilities of energy and nutrients such as starch and protein (Souffrant, 2001; Wenk, 2001; Montagne, 2003).

In the above-mentioned nutrient digestibility experiments, dietary inclusion of DDGS had no effect on P and Ca digestibilities. The similarities in the digestibility of P in DDGS based diets compared with the control diets is probably due to the high bioavailability of the organic P in DDGS due to the fact that most of the phytate-bound P is hydrolyzed during the fermentation process in the ethanol plants (Stein and Shurson, 2009) and since Ca and P are both required for bone tissue synthesis (NRC, 1998), Ca digestibility and retention is influenced by the level of available P in the body as such including DDGS in the diets of pigs may not have a negative effect on Ca digestibility. The high bioavailability of organic P in DDGS implies that when DDGS is included in the diets of pigs, the need for supplemental inorganic P (dicalcium phosphate or mono calcium phosphate) and exogenous phytase enzymes will decrease leading to a reduction in the cost of swine diet as well as the quantity of P excreted in the manure (Pedersen et al., 2007; Stein and Shurson, 2009; Almeida and Stein, 2010).

2.3.5 Performance characteristics of growing-finishing pigs fed DDGS diets

Feeding growing-finishing pigs high-fiber diets has been shown to reduce growth performance because a greater proportion of the nutrients ingested from the high-fiber diets are excreted as indicated in the preceding discussion. The result is that less nutrients are retained and utilized for whole body growth therefore, prolonging the period to the target market weight.

The effects of including DDGS from new generation ethanol plants in diets fed to growing-finishing pigs have been described in several recent studies conducted (Table

2.3). These recent studies (Table 2.3) have shown variable results in pig responses to the inclusion of DDGS in their diets. Cook et al. (2005) reported that feeding growing-finishing pigs (42 to 116 kg) diets with up to 30% DDGS from a new generation ethanol plant has no effect on average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) when compared with pigs fed no DDGS diets. DeDecker et al. (2005) also observed no differences in ADG, ADFI and G:F among growing-finishing pigs fed 0, 10, 20 or 30% DDGS diets. Whitney et al (2006), however, conducted an experiment to investigate the effect of including 0, 10, 20, and 30% DDGS on growth performance of pigs (28 to 115 kg) using a 5-phase feeding program and concluded that ADG, and G:F tend to decrease when pigs are fed diets containing more than 10% DDGS, even though ADFI was not adversely affected. Hastad et al. (2005) investigated the influence of DDGS on feed intake and diet palatability compared to a corn-soybean meal control diet and observed that including 30% DDGS in the diet reduced ($P < 0.05$) ADFI and that pigs preferred corn-soybean diets to diets containing DDGS. It is, however, not known whether pigs' preference will change when they are fed only varying levels of DDGS-containing diets (up to 30%). Linneen et al. (2008) concluded that including DDGS up to 20% of the diets of growing-finishing pigs in a commercial production setting have the tendency to decrease ADG and ADFI as such, only 10 to 15% DDGS should be included in growing-finishing pig diets under commercial production conditions for optimum growth performance.

Table 2.3. Effect of distillers dried grains with solubles on performance characteristics in growing- finishing pigs[1,2]

	References[3]						
Item	1	2	3	4	5	6	7
Inclusion rate, %[4]	30	15	20	20	30	30	20
Duration of study, d	100	29	114	78	100	103.5	-
Average Initial BW, kg	22.1	86.5	22	49.8	43.7	28.4	33.2
Final BW	No effect	No effect	No effect	No effect	No effect	Reduced	No effect
ADFI	Reduced	No effect	No effect	Reduced	No effect	No effect	No effect
ADG	No effect	No effect	No effect	Reduced	No effect	Reduced	No effect
G:F	Increased	No effect	No effect	Increased	Reduced	Reduced	No effect
Dressing percentage, %	Reduced	No effect	No effect	Reduced	Reduced	Reduced	No effect

[1] DDGS = distillers dried grains with solubles from new generation ethanol plants derived from corn.

[2]Information reported is based on diet with the highest DDGS inclusion level versus the control diet.

[3]1) Xu et al., 2010; 2) Yoon et al.; 2010; 3) Widmer et al., 2008; 4) Linneen et al., 2008; 5) Gaines et al., 2007; 6) Whitney et al., 2006; 7) McEwen, 2006.

[4]Maximum inclusion level of DDGS in the diet.

A recent study conducted by Xu et al. (2010) in which growing-finishing pigs were fed corn-soybean meal based diets without or with 10, 20 and 30% DDGS from a new generation ethanol plant in a 3-phase feeding program, showed that ADG was not affected and G:F increased but ADFI decreased in a linear manner when DDGS increased in the diets.

Three reasons can be adduced to the inconsistencies in the growth performance results. Firstly, the variations in the chemical composition (Spiehs et al., 2002; Stein and Shurson, 2009) and nutrient digestibilities, especially, AA (Fastinger and Mahan, 2006; Stein et al., 2006) of the DDGS samples used in the various experiments. Thus, using a source of DDGS that has a low digestibility of AA may reduce pig performance. Secondly, the basis on which the diets were formulated in the various experiments is not the same. In some of the experiments in which poor performance was observed (e.g. Whitney et al. 2006) experimental diets were formulated on total AA basis instead of standardized ileal digestibility (SID) basis as was done in the experiment by Widmer et al (2008) who observed no adverse effect on growth performance from dietary DDGS inclusion in the diets of growing-finishing pigs. Given that most proteins and AA are not fully digested and absorbed (NRC, 1998), formulating swine diets on total AA may not reflect the bioavailability of AA in the diet for tissue protein synthesis and thus, body growth compared with formulating diets on SID AA which provide a more accurate measure of the bioavailability of AA in a swine diet. Lastly, in many of the studies in which DDGS-containing diets resulted in poor performance (e.g. Whitney et al., 2006; Linneen et al., 2008), the diets containing DDGS had higher CP contents than the control. It is therefore, not possible to ascertain whether the poor growth performance was as a

result of DDGS inclusion in the diets or the excess CP because energy is required for deamination and excretion (energy dependent processes) of the excess CP (AA) in the DDGS-containing diets thus, reducing the amount of energy available for growth (Chen et al., 1999; Kerr et al., 2003). Excessive dietary CP can also reduce the feed intake of pigs (Goerl et al., 1995; Chen et al., 1999). It, therefore, follows that experimental diets among studies in which DDGS is to be evaluated on the performance of growing-finishing pigs should be formulated on the same nutritional basis and the nutrient contents of the experimental diets for each study should be as similar (i.e. isocaloric and isonitrogenous) as possible to reduce confounding effects in order to draw valid conclusions on the optimal level of inclusion of DDGS in diets for pigs in the growing-finishing phase.

2.3.6 Effect of DDGS on carcass weight

Inclusion of DDGS in the diets of growing-finishing pigs has been reported by several researchers to decrease carcass weight (i.e. dressing percentage), especially, when DDGS is included at 30% or more of the diet when compared with the control (Table 2.3) even when slaughter weights are similar (Gaines et al., 2007; Xu et al., 2010). In the experiment by Linneen et al. (2008), feeding growing-finishing pigs a 20% DDGS diet reduced carcass weight by 1.82 kg when compared with pigs fed no DDGS control diet. Whitney et al. (2006) observed a 5.1-kg reduction in carcass weight for pigs fed a 30% DDGS diet compared with the control diet. Recently, Xu et al. (2010) also observed a

1.7-kg reduction in carcass weight when pigs were fed 30% DDGS diets compared with those fed the control diet even though slaughter weights were similar.

Adding increasing amounts of DDGS to the diet increases the fiber content because DDGS is a high-fiber feedstuff. This leads to an increase in visceral organ mass and gut fill as shown in several studies in which pigs were fed high-fiber diets (e.g. Pond et al., 1988; Jørgensen et al., 1996) which ultimately contributes to the live weight gain. This may be the reason why some researchers (Gaines et al., 2007; Xu et al., 2010) found no effect of including 30% DDGS in growing-finishing pigs diets on final BW even though carcass weight reduced in the DDGS fed pigs. This implies that measurements based on carcass weight may provide a more accurate interpretation of performance data on DDGS-containing diets than using live weight gain (i.e. ADG) as a performance indicator as suggested by Kyriazakis and Emmans (1995) and Whittemore et al. (2003). Besides, in the North American context, live BW will not be a better indicator of growth performance in pigs fed high-fiber or DDGS-containing diets since the non-carcass (offal) component has less economic value compared with the carcass.

2.3.7 Economics of DDGS inclusion in swine diets

One of the objectives of the swine industry is to formulate diets to meet pig's requirements for optimum performance at the least possible cost. As indicated in section 2.3.2 of this review, DDGS has high levels of energy, protein and digestible P which implies that DDGS can be used as a partial replacement for corn, soybean meal and inorganic P. Indeed, it has been estimated by Thaler (2002) that with 90 kg of DDGS and

1.35 kg of limestone, 80 kg of corn, 8.55 kg of 46% soybean meal and 2.7 kg of dicalcium phosphate can be replaced per ton of a complete feed. There is also some evidence to confirm that including DDGS in swine diet formulas results in a significant reduction in the cost of feed formulation. For instance, Thaler (2002) reported that including 10% DDGS in swine diets could save $2.08 of feed on per ton basis. In that sense, increasing the dietary inclusion rate of DDGS is expected to cause further reduction in feed cost. Thus, Lee et al. (2011) reported that including 20% DDGS in finisher diets significantly reduces ($P < 0.05$; by 430 won/kg) the feed cost per kg BW gain when compared with the control diet (1251 vs. 821 won/kg; Control vs. 20% DDGS-base diet, respectively). However, for DDGS to be cost effective when included in swine diets, several factors will have to be considered. Thus, hindsight on the nutrient level and digestibility, dietary inclusion rate, cost of competing ingredients (especially, corn and soybean meal) and the basis for diet formulation (total vs. available nutrient basis) (Shurson and Pomerenke, 2008) should be considered. Moreover, given the negative effect of increasing levels of dietary DDGS on carcass weight reported in section 2.3.6 of this review, it will be imperative to consider the effects of DDGS inclusion rates on carcass yield when the economics of DDGS in swine diet is to be evaluated to ensure no adverse effect on pig revenue.

2.3.8 Enzyme supplementation of DDGS-containing diets

Pigs and poultry have a digestive system that is suitable for the utilization of simple carbohydrates (e.g. starch) and therefore, lack the ability to efficiently utilize the

more complex carbohydrate components of feedstuffs, such as the non-starch polysaccharides (NSP, e.g. arabinoxylans, cellulose and β-glucans) which constitute the major part of dietary fiber because these group of animals do not secret the enzymes responsible for hydrolysing these NSP (Bedford, 2000; Zijlstra et al., 2010). In general diets with high-fiber levels are associated with reduced nutrient digestibility and growth performance in pigs (as discussed above) and poultry (Guenter, 1997; Bedford, 2000). Furthermore, pigs and poultry are not able to utilize all the P in feedstuffs, the majority (almost two-thirds of P) of which are bound to phytic acid, because they do not produce a sufficient amount of the enzyme which liberates P from phytic acid (Bedford, 2000). Thus, NSP and phytic acid are considered as anti-nutritional factors (ANF) in pig and poultry nutrition because they interfere with the digestion and utilization of energy and other nutrients. In this context, several exogenous enzyme preparations have been developed over the years to specifically target and break down these ANF to improve nutrient digestibility and utilization in diets fed to pigs and poultry (Guenter, 1997; Liu and Baidoo, 1997; Bedford, 2000). Enzymes can be defined as biologically active proteins that break down specific chemical bonds in order to liberate nutrients for further digestion and absorption (Bedford, 2000). Enzymes that break down phosphate groups from phytic acid are called phytases and the NSP degrading enzymes target specific NSP, e.g., xylanase degrades xylans, cellulase degrades cellulose and β-glucanase degrades β-glucans (Bedford and Schuzle, 1998; Bedford, 2000). Studies conducted in poultry with these exogenous enzymes have consistently shown remarkable results (Bedford and Schuzle, 1998; Bedford, 2000). However, the same cannot be said in pigs. Some experiments (e.g. Baidoo et al. 1997; Omogbenigun et al., 2004; Ji et al.,

2008) have reported beneficial effects of enzyme supplementation of pig diets on nutrient digestibilities and performance, but the overall results have been inconsistent (Nyachoti et al., 2006; Woyengo et al., 2008; Zijlstra et al., 2010). The inconsistencies in results may be explained by the differences in experimental design between studies such as; the age of experimental animals, the spectrum of enzyme used, the type and concentration of substrate, possible interaction of exogenous enzymes with other feedstuffs in the diet, particle size, and pH in the digestive tract (Bedford, 2000; Yin et al., 2001; Zijlstra et al., 2010)

The current interest in the use of exogenous enzymes in swine diets is due to the abundance of co-products, particularly, DDGS (Zijlstra et al., 2010). Indeed, several experiments have been conducted recently to evaluate the effect of NSP-degrading enzymes on growth performance (Table 2.4) and nutrient digestibility (Table 2.5) in pigs at various stages of growth. These experiments are based on the premise that DDGS-containing diets have substantial amounts of NSP, particularly, cellulose, xylans, arabinans and (Widyaratne and Zijlstra, 2007; Kim et al., 2008; Zijlstra et al., 2010) which can be hydrolyzed by exogenous enzymes designed to target these substrates and thus, improve nutrient digestibility and utilization for growth as mentioned earlier in this section. For instance, Yoon et al. (2010) supplemented a corn-soybean meal based diet containing corn DDGS fed to growing-finishing pigs with β-mannanase and observed an improvement in nutrient digestibilities and ADG but not ADFI and G:F compared with the control or un-supplemented group. Widyaratne et al. (2009), however, supplemented a wheat based diet containing wheat DDGS fed to growing-finishing pigs with xylanase and observed no effect of xylanase supplementation on nutrient digestibilities and growth

performance compared with the control or un-supplemented group. Feoli (2008) and Emiola et al. (2009) observed improvements in both nutrient digestibilities and growth performance when nursery and/or growing-finishing pigs were fed a corn-soybean and barely based diet containing either corn or sorghum and wheat DDGS, respectively, and supplemented with a multicarbohydrase enzyme blend. It, therefore, appears that supplementing DDGS-containing diets with a multicarbohydrase enzyme blend is more effective in improving both nutrient digestibility and growth performance. However, growth performance in some recent experiments in which a single or multicarbohydrase blends were supplemented to DDGS-containing diets fed to pigs refute this supposition. For instance, in a meta-analysis of four experiments in which the efficacy of several commercial single or multicarbohydrase enzyme preparations designed for DDGS-based diets were evaluated in growing-finishing pigs, Jacela et al. (2009) reported no beneficial effects of the various carbohydrase enzyme preparations on growth performance when compared with the control or un-supplemented pigs. In a recent study, Jones et al. (2010) reported no improvement in the growth performance of nursery pigs fed a corn-soybean based diet containing either corn- or sorghum-based DDGS and supplemented with various multicarbohydrase enzyme blends from different manufacturers compared with the control or un-supplemented pigs. Although the amount of substrate (i.e., various NSP) and concentration of enzymes in the feed are possible reasons for the inconsistencies in the growth performance results as indicated earlier in the discussion, the effects of these single or multi multicarbohydrase enzyme preparations in DDGS-containing diets particularly, on the gastrointestinal tract of pigs and the metabolic adaptation of pigs fed DDGS-containing diet alone or supplemented with carbohydrase enzymes remains to be

Table 2.4. Effect of supplementing DDGS-containing diets with carbohydrase enzymes on growth performance of pigs[1]

Pig	Basal diet[2]	DDGS	Enzyme[3]	ADFI	ADG	G:F	References
Growing	Wheat-barley-RSM-SBM-peas	Wheat	Xylanase	No effect	No effect	No effect	Sigfridson and Haraldsson, 2007
Nursery	Corn-SBM	Corn or	MC	Improved	Improved	Improved	Feoli, 2008
Nursery	Corn-SBM	sorghum	MC	Improved	Improved	Improved	Feoli, 2008
Grow-finish	Corn-SBM	Corn or	MC	Improved	Improved	Improved	Feoli, 2008
Grow-finish	Corn-SBM	Sorghum	MC	Improved	Improved	Improved	Feoli, 2008
Grow-finish	Corn-SBM	Corn	MC	Improved	Improved	Improved	Pierce and Bannerman, 2008
Growing	Barley-corn-SBM	Wheat	MC	No effect	Improved	Improved	Emiola et al., 2009
Grow-finish	Wheat	Wheat	Xylanase	No effect	No effect	No effect	Widyaratne et al., 2009
Nursery	Corn-SBM	Corn	MC	No effect	No effect	No effect	Jones et al., 2010
Grow-Finish	Corn-SBM	Corn	Mannanase	No effect	Improved	No effect	Yoon et al., 2010

[1]Comparison: Supplemented group vs. Un-supplemented groups.

[2] RSM, rape seed meal; SBM, soybean meal.

[3]MC = multi carbohydrase preparation; composed of various NSP degrading enzymes.

Table 2.5. Effect of supplementing DDGS-containing diets with carbohydrase enzymes on total tract nutrient digestibility in pigs[1]

Pig	Basal diet[2]	DDGS	Enzyme[3]	DM	Energy	Nitrogen	References
Growing	Barley	Wheat	MC	Improved	Improved	-	Opapeju et al., 2006
		Corn or	MC	Improved	Improved	Improved	Feoli, 2008
Nursery	Corn-SBM	sorghum	MC	Improved	Improved	Improved	Feoli, 2008
		Corn or	MC	Improved	Improved	Improved	Feoli, 2008
Grow-finish	Corn-SBM	Sorghum	MC	Improved	Improved	Improved	Feoli, 2008
Growing	Barley-corn-SBM	Wheat	MC	Improved	Improved	Improved	Emiola et al., 2009
Grow-finish	Wheat	Wheat	Xylanase	-	No effect	-	Widyaratne et al., 2009
Grow-Finish	Corn-SBM	Corn	Mannanase	Improved	Improved	Improved	Yoon et al., 2010
Growing	Cornstarch	Wheat-corn	Xylanase	No effect	No effect	No effect	Yáñez et al., 2011

[1]Comparison: Supplemented group vs. Un-supplemented groups.

[2] SBM, soybean meal.

[3]MC = multi carbohydrase preparation; composed of various NSP degrading enzyme.

established. Besides, it is not known whether the lack of response in growth rate (based on live weight gain) to supplemental enzyme will still hold if carcass weight gain is used as the performance indicator given the contribution of gut mass and fill in pigs fed high-fiber diets on live weight indicated in section 2.3.6 of this review.

2.4. Effect of high fiber diets on visceral organ mass

Literature contains a plethora of information regarding the influence of high dietary fiber on visceral organ changes in pigs. These studies converge to suggest that feeding pigs high-fiber diets results in individual visceral organ hypertrophy. For instance, Pond et al. (1989) fed finishing barrows (average initial weight, 93 kg) a high-fiber diet (40% alfalfa meal) and observed an increase in the relative weights (i.e., percentage of BW) of the small intestine, large intestine, liver and kidneys compared with pigs on the control diet. Anugwa et al. (1989) also fed growing-finishing pigs (average initial weight, 55.5 ± 2.4 kg) a 40% alfalfa meal-containing diet and observed an increase in the relative weights (a percent of BW) of the stomach, liver and kidneys compared with the control values. In growing pigs, high-fiber diet (286 g DM of dietary fiber) increased the per kg empty body weights of the stomach, caecum and colon compared with those fed a low fiber diet (59 g DM of dietary fiber) (Jørgensen et al., 1996). Nyachoti et al. (2000) observed an increase in the per kg empty body weights of liver, colon and caecum when pigs were fed either a barley-canola meal or a barley-canola meal-alfalfa meal based diet compared with those on a casein-corn starch diet.

Although high-fiber diets increase visceral organ mass as indicated in the above-mentioned studies, the changes in the individual visceral organ mass has not been consistent. This could be due, primarily, to the high crude protein contents in the high fiber diets in some of the experiments. High dietary protein has been reported to increase relative weights of liver and kidney in pigs (Anugwa et al., 1989; Pond et al., 1989; Chen et al., 1999; Kerr et al., 1995, 2003; Frank et al., 2005), ruminants (Wester et al., 1995; Fluharty and McClure, 1997; Marini et al., 2004) and rats (Zhao et al., 1996). This is due to the fact that the liver and kidney are the major sites of AA degradation and nitrogen clearance (Kamin and Handler, 1957; Garcia and Stipanuk, 1992; Westerterp-Plantenga et al., 2009) and thus, excess dietary protein will increase their work load leading to an increase in their mass. Therefore, changes in liver and kidney mass may not be due to the influence of dietary fiber. Not all studies have reported changes in visceral organ mass when pigs are fed high-fiber diets. For instance, Jin et al. (1994) fed nursery pigs (average initial weight, 14.3 kg) a 10% wheat straw-containing diet for 14 days and observed no changes in visceral organ mass compared with the control; feed intake was not different between the control and wheat straw-fed pigs. It is possible that the duration of the experiment was too short for the stimulatory effects of dietary fiber to be observed in the study conducted by Jin et al. (1994).

As indicated earlier in section 2.3.6 of this review, feeding pigs increasing levels of DDGS decreases carcass weight. This is because the weight of the visceral organs increases with increasing amounts of DDGS in the diet and thus, reduces the carcass weight even though live weight gain may not reduce. It will, therefore, be necessary to have a better understanding of the changes in the visceral organ mass associated with

feeding increasing amounts of DDGS to the growing pig in order to design strategies to effectively offset these changes and increase carcass weight.

2.5. Whole-body energy metabolism in the growing pig

Given the importance of energy in animal nutrition, it is not surprising that energy metabolism has been extensively studied in livestock species even though energy is not a nutrient but a characteristic of organic nutrients that are normally present in feedstuffs and the diet of these animals (Baldwin and Bywater, 1984; Noblet and Henry; 1993; Patience et al., 200). In particular, energy expenditure (or heat production) has been a subject of great concern since it represents the amount of the metabolizable energy not retained for body growth but used mostly for maintenance purposes (Ferrell, 1988; NRC, 1998; van Milgen and Noblet, 2003). Several factors influence energy expenditure in the growing pig. Thus, sex, genotype (Noblet et al., 1999), feeding, physical activity (Schrama et al. 1996; van Milgen and Noblet, 2003), nutritional level or previous nutrition (Koong, 1977; Koong et al., 1982), environmental temperature (Verstegen and Close, 1973; Jørgensen et al., 1996; van Milgen et al., 1998) and the diet composition (Yen et al., 2001; Schrama et al., 2003) all affect how energy is expended in the growing pig. Since a larger portion of the energy ingested by the growing pig is expended for maintenance requirements (NRC, 1998; van Milgen and Noblet, 2003), it follows that any strategy designed to reduce energy expenditures for maintenance will increase the amount of energy retained for whole body growth.

Methods such as heart rate (Webster, 1967; Yamamoto et al., 1979; Brosh, 2007), entry rate of carbon dioxide through continuous infusion of $NaH^{13}CO_3$ into the peritoneum (Sahlu et al., 1988; Herselman et al., 1998) and calorimetry (Blaxter, 1966; Johnson, 1986; Rodríguez et al., 2007) are used to study heat production in farm animals. For the purpose of this review and the thesis research, in general, discussions will be limited to the calorimetry method for determining heat production.

2.5.1. Measurement of whole-body heat production using the calorimetry method

The use of calorimetry to measure heat production in farm animals began during the early years of the 20th century (see reviews by Baldwin and Bywater (1984) and Baldwin and Sainz, (1995). Calorimeters designed to measure heat produced by animals are based on the same general principle as the bomb calorimeter, i.e., the heat evolved is used to increase the temperature of the surrounding medium and the change in temperature is used to calculate the amount of heat liberated (Baldwin and Bywater, 1984). The heat produced by animals is a measure of total heat production, which includes the heat used for maintenance functions (as mentioned in section 2.5) and the heat released for digestive and metabolic purposes (termed heat increment) (NRC, 1998). The total heat produced can be quantified by two calorimetry methods: direct and indirect calorimetry. Direct calorimetry involves estimation of the actual heat emitted (lost) by animals. Direct calorimetry is simple in theory – involves only one step to measure heat lost – but has limited practical applications since accurate measurements require considerable time, expense and sophisticated instrumentation (McArdle et al., 2006).

Indirect calorimetry, as the name implies, does not involved a direct determination of heat production of the subject being studied. The indirect calorimetry commonly used in farm animals is the respiration (open-circuit) calorimetry (Brouwer, 1965; Maclean, 1972; Miller and Koes, 1988; Rodríguez et al., 2007). With this approach, the animal is placed in a respiration chamber and measurement of gaseous exchange between the animal and its environment is recorded by a computer programme and the total heat produced is calculated according to Brouwer's (1965) equation:

$H = (3.866 \times O_2) + (1.200 \times CO_2) - (1.431 \times N) - (CH_4 \times 0.518)$, where H is total heat production in kcal; O_2, amount of Oxygen consumed in litres; CO_2, amount of carbon dioxide released in litres; N is amount of nitrogen excreted in grams; and CH_4 is the amount of methane released in litres. The equation is derived using the assumptions of the first and third laws of thermodynamics (Johnson et al., 2003). Methane is usually not included in the equation for pigs since the energy loss due to hindgut fermentation in pigs is small (0.5 – 1.0% of the gross energy, Just, 1982), particularly during the growing phase. Not only is the indirect calorimetry easier to use than the direct calorimetry, but also, if both gases (i.e., O_2 and CO_2) are well monitored (and nitrogen excretion is accurately measured), the indirect calorimetry provides a more precise estimate of the heat produced (McArdle et al., 2006). Most of the estimates of energy expenditure reported in the NRC (1998) for swine were done using the indirect calorimetry method. Because the oxidation of energy substrates in the body generates heat while consuming O_2 (Maclean, 1972; Baldwin and Bywater, 1984; Yen et al., 1989), measurement of whole-body O_2 uptake can used to indicate the energy expenditure by the organism under study.

2.5.2. Influence of visceral organs on whole-body heat production

The contribution of the visceral organs to whole-body energy expenditure is well documented in the scientific literature on animal nutrition. As indicated in the previous discussion, several factors influence energy expenditure in farm animals, but none of them has such a profound impact on total whole-body energy expenditure as the visceral organs although these organs represent 15% or less of the total body mass (Pond et al., 1989; Yen et al., 1989). This is due to the fact that the visceral organs – particularly, the liver and the portal-drained viscera organs (PDVO, consists of the gastrointestinal tract, spleen and pancreas) – are metabolically active tissues and therefore, require a substantial amount of energy for their maintenance; these organs are responsible for digestion, absorption and intermediary metabolism of ingested nutrients (Ferrell, 1988; Huntington, 1990, Yen, 1997). As a percentage of total whole-body oxygen consumption, the liver and PDVO (referred to as splanchnic tissues) are estimated to account for 45 to 50% in cattle (McBride and Kelly, 1990; Reynolds et al., 1991), 45 to 52% in sheep (Burrin et al., 1989), 45% in growing pigs (Yen, 1997) and about 16% in new born lambs (review of Ferrell, 1988). Two processes, Na^+, K^+- ATPase enzyme activities and protein turnover, have been reported to be responsible for the high oxygen consumption by the PDVO and liver (Kelly and McBride, 1989; McBride and Kelly, 1990, Lobley et al., 1980; Lobley et al., 1992). The study by Kelly et al. (1993) provides evidence for implication of these two processes in the high energy demand by the liver and PDVO. In that study Na^+, K^+- ATPase enzyme and protein synthesis activities were estimated to account for about 15 – 41.5% and 27 – 31% as a fraction of oxygen consumption by the visceral organs, respectively.

Since the visceral organs have a profound influence on energy expenditure for maintenance (also known as fasting heat production), it follows that any factor that causes an increase in the mass or activity of these organs would increase whole-body energy utilization. For instance, in growing beef heifers, feeding a high alfalfa containing diet increased the visceral organ mass and fasting whole-body O_2 consumption compared with those fed a concentrate-based control diet (Reynolds et al., 1991). Yen et al. (2001) also observed an increase in visceral organ weight and fasting whole-body O_2 consumption in growing pigs fed a barley-canola meal based diet compared with those fed a casein-cornstarch-based control diet. It can thus, be surmised from the two studies indicated above that increasing the bulkiness of diets fed to growing farm animals (by way of a fibrous feedstuff) would increase fasting O_2 consumption (or maintenance energy requirement); however, in the study by Rompala et al. (1988), when growing ewes were fed a 10% polyethylene based diet (to increase diet bulkiness), fasting O_2 consumption was not affected although total visceral organ weight was heavier compared with those fed the control diet. The authors attributed this to the lack of differences in the relative weight of liver (which accounts for 20-25% of the whole-body O_2 consumption) and the small intestine; these two organs form a greater portion of the splanchnic tissues (i.e. liver and PDVO) and thus whole-body O_2 consumption.

From the foregoing discussions on how visceral organs influence whole-body energy utilization and how any factor which increases visceral organ mass and activity ultimately increase their demand for maintenance energy requirements, the question arises as to whether energy utilization could be manipulated by nutritional strategies to favour body growth given the emphasis placed on efficiency of nutrient utilization in

food animal production. Indeed, reports from studies in which the diets of pigs were supplemented with an antimicrobial growth promoting agents (Yen and Pond, 1990; Yen and Nienaber, 1992, 1993; Yen et al., 1985) provide evidence to support the fact that dietary interventions that reduce the weight of the PDVO may also reduce the oxidative demand of these organs and thus, the maintenance energy requirements making 'more' energy available for whole-body growth. Similar studies with DDGS-containing diets supplemented without or with feed enzymes in growing pigs will be necessary to ascertain the relationship between organ size and energy expenditure given the fact that DDGS-based diets are capable of inducing gut hypertrophy as indicted in Section 2.3.6 and thus, increase energy expenditure for maintenance purposes relative to the amount that will be available for whole-body growth.

2.6 Conclusion

Information captured in this review suggests that DDGS can be included in swine diet to serve as an energy, protein or P source. However, the amount of DDGS that can be included in swine diets resulting in no adverse effects on performance is what remains to be established due to the variations in the concentration and digestibility of the nutrients in DDGS samples indicated in Section 2.3.3 of this review. Supplementing DDGS-containing diets with carbohydrase enzymes have also produced inconsistent results. Additional information must be obtained when growing pigs are fed DDGS-containing diets in order to design effective nutritional strategies such as the use of enzyme technology for efficient utilization of the nutrients in DDGS.

The purpose of this project was to determine the effect of graded levels of DDGS on growth responses and nutrient digestibility and to determine the effect of a multicarbohydrase enzyme supplementation of a DDGS-containing diet on changes in visceral organ mass and whole-body energy expenditure in growing pigs.

3.0 MANUSCRIPT 1

Growth performance and nutrient digestibility in growing pigs fed diets containing graded levels of wheat-corn distillers dried grains with solubles

3.1 ABSTRACT

A growth performance and total tract energy and nutrient digestibilities study was conducted to determine the optimal inclusion rate of a distillers dried grains with solubles (DDGS) derived from cofermentation of wheat and corn in equal proportions (1:1, wt/wt) in the diets of growing pigs. Forty-eight pigs with an initial BW of 25.5 ± 0.14 kg (mean ± SD) were randomly assigned to 24 pens (2 pigs per pen) on the basis of sex and BW, and fed 4 experimental diets in a completely randomised design (6 pens per diet) for 6 wk. The diets were a corn-barley-soybean meal basal fed without or with DDGS at 10, 20, and 30%, and formulated to meet NRC (1998) nutrient requirements for growing pigs. The DDGS was included by changing the levels of the other ingredients in the diets to balance for metabolizable energy (ME) and other nutrients. Average daily gain, ADFI, and G:F were monitored on a weekly basis, and fecal samples were collected during the last 3 d of the experiment to determine apparent total tract digestibilities (ATTD) of energy and nutrients using AIA content of the diet as the indigestible marker. In wk 1, ADG, ADFI and G:F decreased linearly ($P < 0.05$) when DDGS was included in the diets. There were no differences ($P > 0.05$) in ADG, ADFI and G:F among diets during subsequent wk except wk 3 where ADG and G:F decreased in a linear manner ($P < 0.05$)

with dietary DDGS inclusion. Final BW and overall ADG decreased in a linear manner ($P < 0.05$); however, pigs on the 20% DDGS diet had similar overall ADG with pigs on the 0% DDGS diet. There was no effect of diet on the overall ADFI ($P > 0.10$) but a trend was observed for a decrease (linear, $P = 0.067$) in overall G:F as DDGS increased in the diets. A linear decrease in the ATTD of DM and energy ($P < 0.01$) and a cubic decrease in the ATTD of CP ($P < 0.05$) with increasing levels of dietary DDGS were observed. There was no effect ($P > 0.10$) of diet on Ca and P digestibilities; however, ATTD of NDF increased (linear, $P < 0.01$) when DDGS was included in the diet. The results of this study indicate that DDGS derived from cofermentation of wheat and corn (1:1, wt/wt) has an adverse effect on DM, energy and CP but not P, Ca and NDF digestibilities in growing pigs and that for optimum performance during the growing phase, wheat-corn DDGS (1:1, wt/wt) should be limited to 20% of a corn-barley-soybean meal basal diet for growing pigs.

3.2 INTRODUCTION

The production of ethanol from cereal grains produces a co-product, distillers dried grain with solubles (DDGS), which can be used as an alternative feedstuff in swine diet. Although DDGS has higher levels of crude protein, amino acids and fat compared with published values of its corresponding grain, the digestibility coefficients (DC) for the nutrients in DDGS are low due to the high fiber content (Nyachoti et al. 2005; Lan et al. 2007; Widyaratne and Zijlstra 2007) which is predominantly insoluble in nature (Stein and Shurson, 2009). Insoluble fiber enhances dry matter flow and endogenous losses of

nutrients leading to a decrease in ileal and fecal digestibilities of energy and other nutrients (Li et al. 1996; Nyachoti et al. 1997; Souffrant 2001; Wenk 2001) when added to the diets of pigs resulting in reduced BW gain and efficiency of gain (Jørgensen, et al. 1996; Noblet and Le Goff 2001). Distillers dried grains with solubles may also be an ingredient to consider in swine feed formulation to reduce the inclusion of P from inorganic sources and P excretion because of its high P bioavailability (Stein and Shurson 2009).

In North America, corn is the major feedstock for ethanol production but wheat is often used as the main feedstock in western Canada and Europe (Nyachoti et al 2005; Cozannet et al. 2010). It is also a common practice in the ethanol plants in western Canada to use a blend of wheat and corn with the proportions for fermentation dictated by the cost and availability of these cereal grains. Using wheat as a feed stock requires the addition of viscosity reducing enzymes which thus results in a substantial decrease in the total nonstarch polysaccharide (NSP) content in the wheat-derived DDGS (Slominski et al. 2008). It is possible for the same approach to be used when wheat and corn are cofermented for ethanol production depending on the amount of wheat relative to corn.

Various studies have been conducted to determine the amount of corn DDGS that can be included in the diet of the growing pig to support growth performance similar with a corn-soybean meal-based diet (review of Stein and Shurson 2009). With wheat DDGS, Thacker (2006) reported that up to 10% dietary inclusion supports similar growth performance as a control diet without wheat DDGS. Studies (e.g. Nyachoti et al. 2005; Widyaratne and Zijlstra 2007; Yang et al. 2010, Yáñez et al. 2011) have been conducted to characterize the nutritional profile and determine the digestibility of energy and

nutrient in various proportions of wheat-corn DDGS (wcDDGS) for growing pigs. However, there appears to be a lack of information on the amount of wcDDGS that can be included in the diets of growing pigs that will result in no adverse effect on growth performance when compared with a grower diet without wcDDGS. Therefore, the present study was conducted to determine the effect of adding increasing amounts of a wcDDGS (1:1, wt/wt) to the diet of growing pigs on their performance and energy and nutrient digestibilities.

3.3 MATERIALS AND METHODS

3.3.1 DDGS Sample and Experimental Diets

The wheat-corn DDGS (1:1, wt/wt) sample used in this study was obtained from the Husky ethanol plant located in Minnedosa, Manitoba, Canada. The analyzed chemical composition of the DDGS sample is presented in Table 3.1.

The experimental diets were based on corn, barley and soybean meal (SBM) supplemented with 0, 10, 20 and 30% of the wcDDGS. The DDGS was included in the diet by changing the levels of corn, barley, SBM, vegetable oil and monocalcium phosphate to balance for ME and total lysine and available P. The diets were formulated to meet or exceed NRC (1998) nutrient specifications for growing pigs (Table 3.2) and fed in pellet form. The acid insoluble ash (AIA) content of the diets was used as an indigestible marker to determine apparent total tract digestibilities (ATTD) of energy and nutrients.

Table 3.1. Analysed chemical composition (as-is basis) of the DDGS[1]

Item	
DM, %	92.0
CP, %	31.6
GE, kcal/kg	4,707
Crude fat, %	9.6
NDF, %	43.0
ADF, %	15.4
NSP, %	18.6
Ca, %	0.37
P, %	0.92
Non-phytate P, %	0.72
Indispensable AA, %	
Arg	1.15
His	0.77
Ile	1.23
Leu	3.28
Lys	0.76
Met	0.57
Phe	1.68
Thr	1.22
Val	1.51
Dispensable AA, %	
Ala	1.77
Asp	2.03
Cys	0.59
Glu	6.66
Gly	1.43
Pro	3.22
Ser	1.75
Tyr	1.16

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt/wt).

3.3.2 Animals and Housing

The use of animals in this study was reviewed and approved by the Animal Care Committee of the University of Manitoba, and animals were cared for according to the standard guidelines of the Canadian Council on Animal Care (CCAC, 1993). Forty-eight Yorkshire x Landrace pigs (24 barrows and 24 gilts) with an average initial BW of 25.5 ± 0.14 kg (mean ± SD) were obtained from a commercial farm for use in the present study. The pigs were housed in groups of 2 per pen on the basis of sex and BW in an environmentally controlled room with ambient temperature set at 22 ± 2^{o}C. Each pen (1.83 x 1.22 m) was equipped with plastic covered expanded metal flooring, a nipple drinker, and a stainless steel feeder.

3.3.3 Experimental Procedures

The study was conducted over a 6-wk period. Before the commencement of the experiment, pigs were allowed to adapt to their new environment and fed a commercial grower diet (FeedRite, Winnipeg, Manitoba, Canada) for 3 d. After the adaptation period, pigs were randomly assigned to the 4 diets (6 pens per diet) and allowed unlimited access to feed and water throughout the study. Individual pig BW and feed disappearance were monitored weekly to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency on a pen basis.

During the final 3 d of the experiment, fresh feces were collected (once daily) from each pen, placed in Ziploc bags a nd stored at 4^{o}C. At the end of the collection period, fecal samples were pooled per pen and homogenized. Fecal samples were stored

Table 3.2. Composition of experimental diets

Item	Level of DDGS (%)[1]			
	0	10	20	30
Ingredient, %				
Corn	36.60	28.90	32.50	44.80
Barley	34.42	36.59	28.50	11.80
Soybean meal, HP	24.00	18.00	11.97	7.00
Vegetable oil	2.50	4.00	4.50	3.50
DDGS	0	10	20	30
Limestone	0.39	0.55	0.70	1.04
Dicalcium phosphate	0.64	0.38	0.10	0.00
Iodized salt	0.40	0.40	0.40	0.40
Vitamin and mineral premix[2]	1.00	1.00	1.00	1.00
Lysine-HCl	0.05	0.18	0.33	0.46
Calculated chemical composition[3]				
ME, Mcal/kg	3.28	3.29	3.31	3.29
CP, %	18.12	18.26	18.26	18.57
Total lysine, %	0.95	0.95	0.95	0.95
Ca, %	0.60	0.60	0.59	0.69
Total, P	0.52	0.51	0.50	0.51
Available P, %	0.23	0.23	0.23	0.26
Ca:Available P	2.60	2.60	2.57	2.65

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt/wt); included by changing the levels of other feedstuffs to balance the diets for energy and nutrients.

[2]Supplied per kilogram of diet: vitamin A, 8,250 IU; vitamin D3, 825 IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 1 mg; riboflavin, 5 mg; niacin, 35 mg; pantothenic acid, 15 mg; vitamin B12, 25 μg; biotin, 200 μg; folic acid, 2 mg; Cu, 15 mg (copper sulfate);

I, 0.21 mg as Ca(IO3)2; Fe, 100 mg (ferrous sulfate); Mn, 20 mg (manganese oxide); Se, 0.15 mg (sodium selenite); and Zn, 100 mg (zinc oxide).

[3]Calculated based on ingredient composition data from NRC (1998), except the wheat-corn DDGS.

in Ziploc bags at -20^{o}C until used for analysis.

3.3.4 Sample Preparation and Chemical Analyses

Fecal samples were dried in a forced air oven at 60^{o}C for 3 d. Samples of diet, DDGS and feces were finely ground in a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT), and thoroughly mixed for chemical analysis. All samples were analyzed for moisture, GE, CP, NDF, ADF, AA (except feces), Ca and P. Fecal and diet samples were further analyzed for AIA whereas the DDGS sample was further analyzed for NSP, crude fat and phytate P. All chemical analyses were done in duplicate.

Moisture was determined by oven-drying (method 934.01; AOAC 1990) to estimate DM, and CP (N x 6.25) was determined (method 968.06; AOAC 1990) using a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI) with EDTA as a calibration standard. Acid detergent fiber was assayed as described by AOAC (method 973.18; 1990) and NDF according the method of Van Soest et al. (1991) using the Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY), whereas GE was determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) with benzoic acid as a calibration standard. Crude fat analysis was carried out as described by AOAC (method 920.39; 1990). Samples for Ca and P analyses were ashed and digested according to procedures described by AOAC (method 985.01; 1990) and measured using an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Nonstarch polysaccharides were determined using gas-liquid chromatography

(component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was performed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell 1990), whereas uronic acids were determined using the procedure described by Scott (1979). Phytate P was determined by the method described by Haug and Lantzsch (1983) and nonphytate P was calculated as total P minus phytate P. Samples for AA analysis were prepared by acid hydrolysis according to the method of AOAC (method 994.12; 2006). Samples for methionine and cysteine analysis were oxidized with performic acid (method 985.18; AOAC 2006) before acid hydrolysis. Amino acids were analyzed using an amino acid analyzer (SYKAM, Germany). Tryptophan was not determined.

The AIA concentrations in fecal and diet samples were determined following the procedure described by McCarthy et al. (1974) with no addition of celite. Briefly, 100ml of HCl was added to approximately 12 g of each sample, mixed thoroughly and boiled for 30min. After 30 min, the slurry was filtered through an ashless (Whatman 541) filter paper and the residue washed with 500 ml of boiling ionized water until the residue was free of acid. The residue was then ashed at 600^{o}C overnight and the ash weighed. The AIA concentration in each sample was determined as follows:

% AIA = (ash wt. ÷ sample wt.) x 100

3.3.5 Calculations and Statistical Analysis

Apparent total tract digestibility (ATTD) coefficients of energy and nutrients were calculated using the following formula:

$$ATTD\ (\%) = [1 - (N_f \times AIA_d)/ (N_d \times AIA_f)] \times 100,$$

where N_f = nutrient concentration in feces (percentage of DM); N_d = nutrient concentration in diet; AIA_f = AIA concentration in feces; and AIA_d = AIA concentration in diet. Pen was considered as the experimental unit in the statistical analyses. Growth performance data were analyzed as repeated measures using the Proc MIXED of SAS (Statistical Analysis System, SAS Institute, Cary, NC) and dietary treatment was the fixed effect. Nutrient digestibility data were subjected to ANOVA using Proc GLM of SAS with diet as main effect. Orthogonal polynomials were used to determine linear and quadratic effects of graded levels of the DDGS. Data are presented as least square means with respective standard error of the mean (SEM). Significance was defined as $P < 0.05$ and $0.05 < P < 0.10$ was considered a trend.

3.4 RESULTS

3.4.1 Chemical Composition of DDGS and Experimental Diets

The analyzed chemical and nutrient composition of the DDGS and experimental diets are shown in Tables 3.1 and 3.3, respectively. The DDGS contained, on as-is basis, 92% DM, 31.6% CP, 9.6% crude fat, 43% NDF, 0.37% Ca, 0.92% total P, 0.73% non-phytate P, and the GE content was 4,707 kcal/kg. There were similarities between the calculated vs. analyzed CP and lysine (on average, 0.95% vs. 0.95%) values but wide variations between the calculated vs. analyzed Ca (on average, 0.62% vs. 0.95%) and total P (on average, 0.51% vs. 0.73%) values of the experimental diets.

3.4.2 Pig Performance

The effects of inclusion of DDGS on pig performance are summarised in Table 3.4. Final BW decreased (linear, $P < 0.05$) as the level of DDGS increased in the diets. Specifically, at d 42, pigs fed 10, 20 and 30% DDGS diets were 0.6, 2.9 and 4.2 kg, respectively, lighter than the pigs fed the o% DDGS diet. In wk 1, a linear decrease ($P = 0.02$) in ADG, ADFI and G:F was observed with increasing dietary inclusion of DDGS. However, there was no effect ($P > 0.05$) of diet on ADG, ADFI and G:F during subsequent wk except in wk 3 where ADG and G:F linearly decreased ($P < 0.05$) with increasing level of dietary DDGS. Overall, ADG decreased ($P < 0.05$) linearly with an increase in dietary DDGS from 0 to 30%. There was no effect of diet on the overall ADFI ($P > 0.10$) but a trend was observed for a linear decrease ($P = 0.067$) in overall G:F as dietary DDGS increased from 0 to 30%.

3.4.3 Apparent Total Tract Digestibilities

The effects of DDGS inclusion levels on ATTD of energy and nutrients are summarised in Table 3.5. Dry matter and energy digestibilities linearly decreased ($P < 0.001$) with increasing levels of DDGS in the diet. Dry matter and energy digestibilities also tended ($P = 0.08$) to decrease in a quadratic manner. The ATTD of CP decreased ($P = 0.01$) in a cubic manner whereas ATTD of NDF increased (linear, $P < 0.01$) with increasing levels of dietary DDGS. Dietary DDGS inclusion from 0 to 30% had no effect ($P > 0.10$) on Ca and P digestibilities in the present study.

Table 3.3. Analyzed chemical composition of experimental diets (as-is basis)[1]

	Level of wcDDGS (%)			
Item	0	10	20	30
DM, %	87.4	87.8	88.2	88.9
GE, kcal/kg	3,951	4,133	4,207	4,265
CP, %	17.2	17.1	17.6	18.1
Ca, %	1.00	0.88	0.92	0.99
P, %	0.79	0.74	0.69	0.69
NDF, %	13.2	16.7	20.2	22.2
ADF, %	5.4	5.9	6.3	9.0
Indispensable AA, %				
Arg	0.92	0.88	0.75	0.74
His	0.40	0.40	0.36	0.40
Ile	0.52	0.56	0.51	0.52
Leu	1.32	1.43	1.41	1.63
Lys	0.95	0.96	0.92	0.96
Met	0.24	0.25	0.26	0.24
Phe	0.78	0.80	0.75	0.79
Thr	0.58	0.60	0.55	0.62
Val	0.59	0.63	0.60	0.64
Dispensable AA, %				
Ala	0.85	0.89	0.90	1.05
Asp	1.62	1.52	1.29	1.28
Cys	0.25	0.25	0.26	0.25
Glu	3.26	3.68	3.57	4.17
Gly	0.64	0.67	0.63	0.69
Pro	1.16	1.37	1.40	1.68
Ser	0.82	0.85	0.79	0.90
Tyr	0.49	0.53	0.48	0.55

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt/wt).

3.5 DISCUSSION

The DDGS used in the present study had lower CP, GE, ADF and total P but higher crude fat and ash contents compared with the values reported in other studies using DDGS derived from cofermentation of wheat and corn (Widyaratne and Zijlstra 2007; Yang et al. 2010). The nutrient variability between the DDGS sample used in the present study and the studies indicated above may be due to the differences in the proportions of the wheat and corn in the DDGS samples because the nutrient content compared favourably with that of Yáñez et al. (2011) who used the same proportions of wheat and corn DDGS sample as used in the present study (i.e., 1:1; wt/wt). Nonetheless, it is well known that DDGS has variable nutrient contents even when derived from the fermentation of a single cereal source (Spiehs et al. 2002; Stein and Shurson 2009). The differences observed between analyzed and calculated nutrient composition among the experimental diets in the current study were probably due to the fact that NRC (1998) feed composition values (except for the DDGS) were used in diet formulation. The analyzed Ca:P ratio varied among the experimental diets (from 1.19:1 to 1.44:1) used in the present study. A wide dietary Ca:P ratio ($1 \leq 1.3 \leq 2.0$) is known to lower P absorption and thus, reduces the growth of pigs only if the diet has P content less than the pig's P requirement (Reinhart and Mahan 1986; Eeckhout et al. 1995; NRC 1998; Crenshaw 2001). The analyzed P concentration in the diet with the widest analyzed Ca:P ratio was above the amount recommended by NRC (0.69% vs. 0.50%) but within the range (0.4% to 1.0%) recommended by Crenshaw (1986) for growing pigs. Besides, P digestibility was not affected by dietary treatment in the current study (discussed below). We therefore, have no reason to believe that the variation in the analyzed Ca:P ratio

Table 3.4. Performance characteristics of growing pigs fed graded levels of wheat-corn distillers dried grains with solubles

	Level of wcDDGS (%)					*P* value		
Parameter	0	10	20	30	SEM	Trt[1]	linear	Quadratic
Initial BW, kg	25.4	25.4	25.4	25.3	0.067	0.949	0.870	0.902
Final BW, kg	61.1	60.5	58.2	56.9	1.301	0.133	0.022	0.812
Average daily gain, kg/d								
Week 1	0.81	0.70	0.68	0.63	0.036	0.005	0.003	0.520
Week 2	0.72	0.72	0.78	0.68	0.046	0.538	0.749	0.325
Week 3	0.79	0.79	0.63	0.64	0.053	0.040	0.012	0.901
Week 4	0.84	0.91	0.75	0.69	0.071	0.160	0.065	0.398
Week 5	0.84	0.79	1.00	0.93	0.096	0.554	0.309	0.989
Week 6	1.11	1.12	0.87	0.94	0.117	0.450	0.176	0.804
Overall	0.85	0.83	0.78	0.75	0.035	0.072	0.020	0.413
Average daily feed intake, kg/d								
Week 1	1.31	1.24	1.26	1.17	0.038	0.086	0.029	0.783
Week 2	1.55	1.58	1.50	1.39	0.072	0.389	0.087	0.345
Week 3	1.78	1.83	1.68	1.71	0.070	0.380	0.244	0.863
Week 4	1.92	2.02	1.89	1.66	0.115	0.210	0.073	0.134
Week 5	2.06	2.00	2.08	2.06	0.096	0.950	0.853	0.829
Week 6	2.27	2.23	2.24	2.26	0.115	0.890	0.853	0.768
Overall	1.81	1.80	1.78	1.71	0.050	0.270	0.894	0.828
Gain to feed ratio, kg/kg								
Week 1	0.62	0.57	0.54	0.54	0.026	0.110	0.039	0.450
Week 2	0.47	0.46	0.52	0.49	0.022	0.270	0.277	0.740
Week 3	0.44	0.43	0.37	0.37	0.023	0.050	0.012	0.857
Week 4	0.44	0.45	0.39	0.41	0.029	0.508	0.265	0.872
Week 5	0.41	0.38	0.47	0.45	0.036	0.370	0.218	0.967
Week 6	0.49	0.50	0.39	0.42	0.045	0.286	0.090	0.708
Overall	0.47	0.46	0.44	0.44	0.018	0.120	0.067	0.661

Means in the same row with different superscripts differ ($P < 0.05$)

[1]Overall effect of dietary treatment

Table 3.5. Apparent total tract digestibilities (ATTD) of energy and nutrients in growing pigs fed graded levels of wheat-corn distillers dried grains with solubles

	Level of wcDDGS (%)					*P* values		
Parameter	0	10	20	30	SEM	Trt[1]	Linear	Quadratic
Dry matter	85.5	82.4	83.2	82.2	0.544	0.001	0.001	0.082
Energy	86.7	83.5	84.5	83.3	0.519	0.001	0.0007	0.076
Crude protein[2]	83.0	77.2	80.7	78.9	1.181	0.017	0.111	0.113
Calcium	62.7	62.1	66.5	63.1	1.988	0.413	0.542	0.883
Phosphorus	50.6	48.7	51.9	50.5	1.738	0.639	0.702	0.883
NDF	59.1	57.3	64.9	65.2	1.419	0.001	0.0007	0.395

Means in the same row with different superscripts differ ($P < 0.05$)

[1]Overall effect of dietary treatment

[2]Cubic effect

among the experimental diets used in the current study influenced growth performance.

In the present study, the growth rate of pigs fed graded levels of the wheat-corn DDGS up to 30% of diet decreased, linearly. This observation is in agreement with the results of Cromwell et al (1993), Whitney et al (2006) and Linneen et al. (2008) who reported that inclusion of corn DDGS up to 30% of growing-finishing pig diets resulted in a decrease in growth rate. However, Cook et al. (2005), DeDecker et al. (2005) and Widmer et al. (2008) observed no effect when corn DDGS was included up to 30%, even though it is believed that a high-fiber diet adversely affects the growth rate of pigs. The inconsistent effects of DDGS on the growth performance of growing-finishing pigs may be due to the variation in the chemical composition of the DDGS samples used among studies. Moreover, diets used in the different experiments were not formulated on the same basis which may add to the reasons for the inconsistency in results on growth performance. For instance, in some of the studies (Whitney et al. 2006; Linneen et al. 2008), diets containing DDGS had higher CP content than the control which may have confounded the effect of DDGS inclusion on growth performance of pigs due to the energy cost of urea synthesis and excretion of the excess CP or AA. In the study by Widmer et al (2008), where growth performance was not affected by DDGS inclusion, experimental diets were formulated based on SID AA and the CP contents of the diets were slightly below the NRC (1998) specifications for growing pigs to reduce the confounding effect of dietary CP on growth performance. In the present study, the analyzed CP content of the experimental diets were similar with that of Widmer et al. (2008) but a decline in pig performance was observed with increasing levels of DDGS. One possible reason is that the experimental diets used in the present study were not

formulated on SID AA basis as was done by Widmer et al (2008) because formulating diets on total AA basis may not provide an accurate estimate of AA bioavailability for body growth (NRC 1998). The decline in growth rate as DDGS increased in the diets in the current study may also be due to a decrease in energy availability for body growth given the negative effect of dietary fiber on energy utilization (Noblet and Le Goff 2001).

Feed intake, unlike growth rate, was not affected by dietary DDGS inclusion in the current study against the general belief that high-fiber diets decrease voluntary feed intake in growing pigs due to their bulkiness, which may cause early satiety (Kyriazakis and Emmans 1995; Owusu-Aseidu et al. 2006). This observation is in agreement with the results of Whitney et al. (2006) and Yoon et al. (2010) who reported no effect of DDGS on feed intake when corn DDGS was added up to 30% of the diet for growing-finishing pigs. The results of the two studies indicated above and the present study imply that growing pigs will readily consume diets containing up to 30% DDGS without negatively affecting voluntary feed intake. Henry (1985) reported that the daily feed intake of a pig is determined by the energy content and not the bulkiness of the diet and that growing pigs will consume as much of a high-fiber diet as a low-fiber diet if they are allowed time for adaptation. Thus, diets with similar energy but varying fiber contents may not have any adverse effect on feed intake when fed to growing pigs. This may explain why including DDGS up to 30% of the diet had no effect on the overall ADFI in the present study. The decrease in ADFI in wk 1 may be due to the fact that pigs were fed a commercial grower feed for 3 d before introduction to the experiment diets. This might have depressed feed intake in pigs fed DDGS-containing diets on the first wk because they had not adapted fully to these high-fiber diets by then. The tendency for the overall

G:F to decrease in a linear manner with increasing levels of the DDGS in the diets is not surprising given the extra metabolic effort required for digestion and utilization of high-fiber diets which results in increased whole-body energy expenditure (Noblet and Le Goff 2001; Noblet and van Milgen 2004) and therefore decreases the efficiency with which consumed feed is converted to body weight gain.

In the present study, dietary wcDDGS inclusion resulted in a decrease in ATTD of DM, CP and energy. This observation is in agreement with previous studies which reported a reduction in DM, CP and energy digestibility when nursery or growing-finishing pigs were fed diets with increasing levels of wheat, sorghum or corn DDGS (Thacker 2006; Feoli 2008) which is probably due the higher NDF contents of the diets containing DDGS. It is well known that high dietary fiber enhances DM flow in the gut leading to a decrease in ileal and fecal digestibility of energy and nutrients including starch and protein in pigs (Wenk 2001; Montagne et al. 2003). The effect of dietary fiber on feed digestion is highly influenced by its physicochemical properties (Montagne et al. 2003; Owusu-Aseidu et al. 2006). The fiber in DDGS is predominantly water-insoluble meaning DDGS increases nutrient excretion by reducing the transit time (Wenk 2001; Montagne et al. 2003; Owusu-Asiedu et al. 2006). The decrease in DM and energy digestibility when pigs are fed DDGS-containing diets is associated with the reduced digestibility of fiber component (Stein and Shurson 2009). This supposition may not be supported by the present study because ATTD of NDF increased with an increase in dietary wcDDGS. A possible reason for the linear increase in ATTD of NDF with increasing dietary wcDDGS in the current study may be that water solubility of the fiber in wcDDGS may be higher than that in corn DDGS.

Phosphorus digestibility was not affected when wcDDGS was included in the diets in the current experiment, which is in agreement with results from other studies with either wheat or corn based DDGS (Nyachoti et al. 2005; Pedersen et al. 2007; Widmer et al. 2007; Almeida and Stein 2010). This is not surprising given the higher bioavailability of P in DDGS compared with corresponding grains (Stein and Shurson 2009) which is due to the fact that most of the phytate-bound P is hydrolyzed during the fermentation process. In the present study, including DDGS at 30% of the diet eliminated the need to include monocalcium phosphate, however, ATTD of P was similar (50.5% vs. 50.6%) to the diet without DDGS but supplemented with monocalcium phosphate. This observation indicates that there was an increased utilization of the organic P in the wcDDGS compared with the barley, corn and SBM used in the present study and thus, lends support to the significance of using DDGS to reduce P excretion, and the need for supplemental inorganic P (Pedersen et al. 2007; Stein and Shurson 2009) or phytase (Almeida and Stein 2010) in the diets of pigs. Increasing dietary DDGS content had no effect on ATTD of Ca in the present study which may probably be due to the lack of effect of dietary DDGS inclusion on P digestibility. This is because, Ca digestibility and retention is influenced by the level of available P in the body (Stein 2006; Widmer et al. 2007) since these two mineral elements are required for bone tissue synthesis. Therefore, the observation of a lack of dietary treatment on P digestibility may be an explanation for the non significant effect of dietary treatment on ATTD of Ca in the current study.

In conclusion, the wcDDGS (1:1, wt/wt) used in the current study had no effect on feed intake which implies voluntary feed intake may not be negatively affected by dietary inclusion of wcDDGS up to 30%. However, ADG linearly decreased when

wcDDGS was included at 10, 20 and 30% of the diet and up to 20% dietary inclusion results in limited reduction in ADG. Therefore, it appears that for optimum performance during the growing phase, DDGS with equal proportions of wheat and corn should be limited to 20% of grower diets based on corn, barley and soybean meal.

4.0 MANUSCRIPT II

Visceral organ mass, intestinal morphology and fasting oxygen consumption in growing pigs fed diets with distillers dried grains with solubles alone or in combination with a multicarbohydrase enzyme supplement.

4.1 ABSTRACT

The effects of distillers dried grains with solubles derived from cofermentation of wheat and corn (DDGS; 1:1, wt/wt) and a multicarbohydrase enzyme supplement on visceral organ weight, intestinal morphology and fasting whole-body oxygen consumption (FWBOC) were investigated in growing pigs in a 28-d trial. Twenty four pigs (BW = 19.9 ± 0.46 kg, mean ± SD) were individually housed in floor pens and randomly assigned to 3 experimental diets (8 pens per diet). The diets contained corn and soybean meal with 0 (control) or 30% DDGS. The third diet was supplemented with a multicarbohydrase enzyme blend in addition to the 30% DDGS (DDGS+Enzyme). Diets had similar nutrient contents and met the NRC (1998) nutrient specifications for growing pigs. Pigs were fed at 4% of their BW once daily. On d 15, 4 pigs from each dietary treatment were randomly selected for measurement of FWBOC during the 24- to 30-h postprandial period using an open-circuit indirect calorimeter. At the end of the study, pigs were killed to determine visceral organ weights, ileal and cecal digesta viscosity, and intestinal morphology. There was no effect ($P > 0.10$) of diet on final BW, WBFOC and digesta viscosity. Empty BW was heavier ($P = 0.05$) in pigs fed the control and

DDGS+Enzyme diets compared with pigs fed the un-supplemented DDGS diet. There were no differences ($P > 0.10$) in the per kg empty BW of liver, spleen, pancreas, heart, stomach and caecum among treatments. However, small intestine, colon plus rectum and the portal-drained viscera (PDV) were heavier ($P < 0.05$) in DDGS-fed pigs compared with the control; supplementing with the multicarbohydrase enzyme blend substantially decreased the effect of DDGS on the weight of these visceral organs. Although morphological data showed no differences ($P > 0.1$) in the duodenum, jejunum and colon segments among diets, the dietary DDGS tended to decrease ($P < 0.10$) villous height (VH) and VH:CD in the ileum. The results of this study show that including 30% DDGS in grower diets increases PDV mass and reduces dressing percentage but these effects may be overcome by a multicarbohydrase enzyme supplementation.

4.2 INTRODUCTION

There is increasing interest in the use of distillers dried grains with soluble (DDGS) in swine diets due to the current trend in the demand and price of cereals and high quality protein supplements, and the availability of DDGS worldwide. However, the maximum inclusion level of DDGS in growing pig diets remains to be established because of the inconsistencies in growth performance even though numerous corn DDGS studies have been reported in the literature. Variations in the chemical composition of DDGS samples (Spiehs et al., 2002; Nyachoti et. al., 2005) and the basis for diet formulation among studies (Widmer et al., 2008; Stein and Shurson, 2009) contribute to the inconsistencies in growth performance results. Furthermore, supplementing DDGS-

based diets with a single or multi-carbohydrase enzyme improved nutrient digestibilities and/or growth performance in some studies (Feoli 2008; Emiola et al., 2009; Yoon et al., 2010) but not in others (Jacela et al., 2009; Jones et al., 2010; Yáñez et al., 2011). Although the differences may be explained by the age of experimental animals, the spectrum of enzymes used as well as possible interaction of enzymes with other feedstuffs in a DDGS-based diet, the mechanism of action of exogenous enzyme on the gastrointestinal tract (GIT) and how pigs respond metabolically, when fed DDGS-containing diets alone or supplemented with enzymes is not known.

Reduced carcass percentage yield and weight were observed when DDGS was included at more than 20% of the diet even though slaughter weight was not affected (Gaines et al., 2007; Linneen et al., 2008; Xu et al., 2010) which may be due to increased visceral organ mass and gut fill when pigs are fed high-fiber diets (Pond et al., 1988; Jørgensen et al 1996; Wenk, 2001; Nyachoti et al., 2000). This implies that using performance parameters based on live weight gain and the associated feed efficiency may not be the most sensitive indicators for assessing the efficacy of supplemental enzymes in pigs fed DDGS-containing diets due to the confounding effects of gut fill and growth.

The visceral organs, which represent 15% or less of the total body mass, accounts for a high proportion of whole-body energy expenditure in growing pigs (Pond et al., 1989; Yen, 1997). Thus, feeding pigs diets that increase visceral organ mass will consequently lead to an increase in energy expenditure resulting in less energy retained for body growth. The high rate of energy expenditure by the visceral organs has been associated with high rates of cellular turnover (McBride and Kelly, 1994) which could be assessed by alterations in the intestinal morphology (Jin et al., 1994).

The hypothesis tested in the present study was that including 30% DDGS in grower diets will alter intestinal morphology, increase visceral organ mass and whole-body oxygen consumption leading to a decrease in carcass weight and that dietary supplementation with a multicarbohydrase enzyme blend will ameliorate these negative effects. The objective of the present study was to determine the effect of a multicarbohydrase enzyme blend on visceral organ mass and whole-body oxygen consumption in growing pigs fed 30% DDGS-based diets.

4.3 MATERIALS AND METHODS

4.3.1 DDGS Sample and Experimental Diets

The DDGS sample used in this experiment was obtained from the Husky Energy LIoydminster Ethanol Plant in Alberta, Canada and was derived from cofermentation of corn and wheat in a 1:1 ratio (wt/wt). The DDGS sample was analyzed for DM, CP, P, Ca, NDF, ADF, GE (to estimate ME) and AA (Table 4.1) before formulating the experimental diets.

The experimental diets were based on corn and soybean meal with 0 (Control) or 30% DDGS. Supplementing the 30% DDGS diet with a multicarbohydrase enzyme blend (at the expense of a small portion of corn) provided the third experimental diet. The multicarbohydrase enzyme blend used was a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) which

Table 4.1. Analyzed chemical composition of the DDGS[1]

Item	
DM, %	90.9
GE, kcal/kg	4,704
CP, %	28.6
NDF, %	36.5
ADF, %	16.7
Indispensable AA,	
Arg	1.14
His	0.67
Iso	1.08
Leu	2.93
Lys	0.77
Met	0.52
Phe	1.39
Thr	0.96
Val	1.38
Dispensable AA, %	
Ala	1.70
Asp	1.63
Cys	0.57
Glu	5.39
Gly	1.13
Pro	2.44
Tyr	0.93
Ser	1.26

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt:wt).

provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet. The diets were formulated to contain equal amounts of standardized ileal digestible lysine and metabolizable energy contents, and all other nutrients were supplied to correspond with NRC specifications for growing pigs (Table 4.2). The diets were fed in pellet form.

4.3.2 Pigs and Housing

The experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee and pigs were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 1993). Twenty-four Genesus ([Yorkshire x Landrace] x Duroc) pigs (12 barrows and 12 gilts), with an average initial BW of 19.9 ± 0.46 kg (mean ± SD), were obtained from the University of Manitoba Glenlea Swine Research Unit and used in the present study. Pigs were housed individually in pens in an environmentally controlled room with ambient temperature set at 21 ± 2^{o}C. Each pen was equipped with a stainless steel feeder, a nipple-drinker, plastic-covered expanded metal floors, and a metal wall partitioning that allowed visual contact with pigs in adjacent pens. Pigs were assigned at random to the experimental diets such that each diet had 8 pigs (replicate pens) with equal number of barrows and gilts. Pigs were provided with feed equivalent to 4% of their BW once daily at 0800 h but had unlimited access to water throughout the 28-d trial. Pigs were weighed weekly and the data used to adjust daily feed allowance.

Table 4.2. Composition of experimental diets

	Level of DDGS (%)[1,2]	
Item	0	30
Ingredient		
Corn	67.91	54.80
SBM, 45%	27.25	10.00
DDGS	-	30.00
Vegetable oil	1.25	1.70
Salt	0.50	0.50
Biophos	0.96	0.52
Limestone	1.05	1.40
HCl-Lysine	0.06	0.41
DL-Methionine	0.01	0.00
L-Threonine	0.01	0.00
Vitamin and mineral premix[3]	1.00	1.00
Calculated chemical composition		
ME, kcal/kg	3,294	3,265
SID Lys/ME, g/Mcal	2.63	2.63
CP, %	18.18	18.08
SID Lys, %[4]	0.87	0.86
SID Thr, %[4]	0.59	0.62
Ca,%	0.67	0.67
Available P,%	0.27	0.30
Total P, %	0.57	0.56
Ca:Total P	1.18	1.19

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt:wt).

[2] The third diet was a 30% DDGS-based diet supplemented with a multicarbohydrase enzyme consisting of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada): provided 200 units of glucanase, 500 units of xylanase, 1100 units of cellulase, 1800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

[3]Supplied per kilogram of diet: vitamin A, 8,250 IU; vitamin D3, 825 IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 1 mg; riboflavin, 5 mg; niacin, 35 mg; pantothenic acid, 15 mg; vitamin B12, 25 μg; biotin, 200 μg; folic acid, 2 mg; Cu, 15 mg (copper sulfate);

I, 0.21 mg as Ca(IO3)2; Fe, 100 mg (ferrous sulfate); Mn, 20 mg (manganese oxide); Se, 0.15 mg (sodium selenite); and Zn, 100 mg (zinc oxide).

[4]SID = standardized ileal digestible.

4.3.3 Fasting Whole-Body Oxygen Consumption

From d 15 of the study, 4 pigs (2 barrows and 2 gilts) were selected at random from each treatment for measurement of whole-body oxygen consumption during the 24- to 30-h postprandial period (Yen and Nienaber, 1992) using an open-air-circuit indirect calorimeter systems (Columbus Instruments, Columbus, Ohio) designed to measure heat production of animal based on O_2 consumption and CO_2 production. Each chamber (Figure 4.1) has a volume of $11ft^3$, an air-conditioning system for regulating temperature and humidity, and also has the capacity to accommodate a pig with BW ranging from 5 to 50 kg. Each chamber (Figure 4.1) is air-tight and equipped with a feeder, nipple drinker, plastic-covered expanded metal flooring, and a meshed tray for separate urine and fecal collection. Oxygen consumption and CO_2 production is monitored by an integrated instrumentation via a terminal computer installed with Oxymax software. The integrated instrumentation (Figure 4.2a, b) consists of a paramagnetic O_2 sensor (19.3 to 21.5%), a single beam CO_2 sensor (0 to 1.0%), sample pump, gas driers, Oxymax fresh air ventilation blower and a positive mass flow controller (for constant fresh air delivery). The gas-tight blowers draw fresh air into the chambers where it is thoroughly mixed with the air in the chambers. The Oxymax software monitors O_2 and CO_2 gas fractions at both the inlet port (reference air) and output port (air from the chambers) through preset time intervals. The gas fraction and flow measurements (l/min based on BW of the pig) are used to compute O_2 consumption and CO_2 production. The Oxymax gas sensors measure gas concentrations from one chamber at a time. A settling time is required to purge the lines to increase the accuracy of a sample for measurement. The system is validated using the alcohol combustion method described by Aulick et al. (1983). Before an experiment

Figure 4.1. The Oxymax open-circuit indirect calorimeter

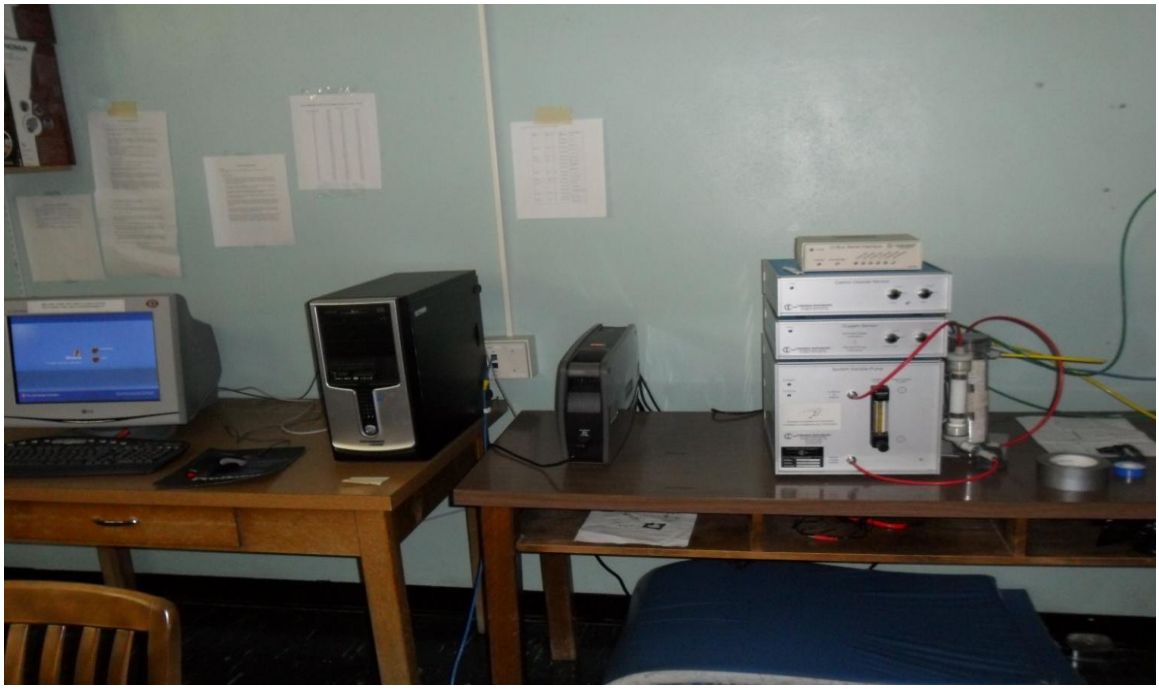

Figure 4.2a. Terminal computer and Oxymax integrated instrumentation with O_2 and CO_2 sensors (extreme right)

Figure 4.2b. From left: Oxymax sample pump, three (3) gas driers (cylindrical system) and three (3) fresh air blowers (one per respiratory chamber)

is conducted, the system is calibrated with a gas of known O_2 and CO_2 concentrations.

Fasting whole-body O_2 consumption was the object of interest in the present study. The system was set up with a measure time of 1 min, a settle time of 2 min, and an airflow rate calculated based on the equation: 1/min = 5.6 x BW (Columbus Instruments, Columbus, OH). Pigs were placed in the chamber after a 24-h fast for 6 h. Assignment of pigs to the chamber was done in a random manner to eliminate possible variations O_2 consumption due to chamber effect.

4.3.4 Slaughter Procedure, Tissue and Digesta Collection

At the end of the 28-d experimental period, pigs were weighed, sedated with an intramuscular injection of ketamine:xylazine (20:2 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and killed by an intravenous injection of sodium pentobarbital (50 mg/kg of BW; Bimeda- MTC Animal Health Inc.). The abdominal cavity was opened along the midline to remove the visceral organs. The liver, spleen, pancreas and heart were obtained immediately after evisceration, blotted with an absorbent paper and then weighed. The empty body weight (EBW, BW-viscera) was also recorded and digesta samples from the ileum and caecum were obtained for viscosity determination. The stomach, intestine (free of mesentery), colon and caecum were completed emptied, blotted with an absorbent paper and weighed. A 1 cm-wide cross section of tissue samples were obtained from the intestinal segments for histological assessments as follows: duodenum, 30 cm from the pyloric-duodenal junction; jejunum, 200 cm from the stomach; ileum, 30 cm from the ieal-cecal junction; and colon, 40 cm

from the caecum. These tissue samples were then immersed in 10% buffered formalin to fix the villi and the crypts.

4.3.5 Histological and Digesta Viscosity Measurements

The formalin fixed intestinal segments were processed for histological examination using the standard hematoxylin and eosin method described by Owusu-Asiedu et al. (2002). The measurement of villous height (VH) and crypt depth (CD) was made on 10 well-oriented villi per segment from each pig using a Nikon YS100 compound light microscope equipped with a Sony DSP 3CCD colour video camera. The images were captured and processed using a Northern Eclipse Image Processing Software version 6.0 (Empix Imaging, Inc., Mississauga, Ontario, Canada). The height of the villous was measured from the tip to the crypt-villous junction and the depth of crypt from the crypt-villous junction to the base.

Thoroughly mixed digesta samples (1 g) were centrifuged at 12,000 x *g* for 8 min. The supernatant fraction was placed in a Brookfield digital viscometer (Model DV-II+ Version 3.0, Brookfield Engineering Laboratories Inc., Stoughton, MA) for measuring viscosity at a shear rate of 60/s at 38^{o}C. The viscometer was rinsed with deionized water and wiped clean between samples. The viscosity values were recorded as apparent viscosity in milliPascal seconds (mPa's).

4.3.6 Chemical Analyses

Diet and DDGS samples were ground finely using a coffee grinder (CBG5Smart Grind; Applica Consumer Products, Inc., Shelton, CT) before chemical analysis. Samples were analyzed for moisture (method 934.01; AOAC, 1990) to estimate DM, and CP (method 968.06; AOAC, 1990) using a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Acid detergent fiber was assayed as described by AOAC (1990; method 973.18) and NDF according to the method of Van Soest et al. (1991) using the Ankom^{200} Fiber Analyzer (Ankom Technology, Fairport, NY) whereas GE was determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) with Benzoic acid as the calibrating standard. The DDGS sample was further analyzed for P, Ca and AA. Samples for Ca and P analyses were ashed and digested according to procedures described by AOAC (1990; method 985.01) and measured using an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Samples for AA analysis were prepared by acid hydrolysis according to AOAC (2006; method 994.12). Samples for methionine and cysteine analysis were oxidised with performic (method 985.18; AOAC, 2006) acid before acid hydrolysis. Amino acids in the samples were analyzed using an amino acid analyzer (SYKAM, Germany). Tryptophan was not determined. All analyses were done in duplicate.

4.3.7 Calculations and Statistical Analyses

Single measurements of VH and CD for each pig were obtained by averaging the 10 measurements of villi and the crypt per specimen for each intestinal segment. Data

were analyzed using the GLM procedure of SAS (Statistical Analysis System, SAS Institute, Cary, NC) with dietary treatment as the main effect in the model and pigs as the experimental unit. Data are presented as least square means with respective standard error of the mean (SEM). Significance was defined as $P < 0.05$ and treatment means were compared using contrasts.

4.5 RESULTS

Pigs remained healthy, readily consumed their daily feed allowance and no technical difficulties were experienced with the indirect calorimeter, throughout the study.

The analyzed chemical composition of DDGS and experimental diets are presented in Table 4.1 and 4.3, respectively. The GE content of the DDGS was 4,704 kcal/kg where as its DM, CP, NDF and ADF contents were 90.9%, 28.6%, 36.5% and 16.7%, respectively. The experimental diets had similar analyzed moisture and CP contents but the NDF content in DDGS-containing diets were twice as high as the Control diet.

Final BW and total visceral weight were not affected *(*$P > 0.10$) by dietary treatment. However, empty BW decreased (28.6 vs. 26.9 kg, $P = 0.02$), and total visceral weight:BW ratio tended to increase (0.23 vs. 0.25 kg, $P = 0.08$) when pigs were fed the 30% DDGS diet (Table 4.4). The empty BW of the pigs fed the 30% DDGS-containing diet supplemented with the multicarbohydrase enzyme blend was similar with those on the control (27.8 vs. 28.6 kg, $P > 0.10$). There were no differences ($P > 0.10$) in the per

kg empty BW of liver, spleen, pancreas, heart, stomach and caecum among dietary treatments. Pigs fed the 30% DDGS-containing diet had heavier small intestines (P = 0.06) (per kg empty BW) compared with those on the control (4.6 vs. 4.25 kg), however, the small intestine weight was not different (P > 0.20) for pigs fed the 30% DDGS-containing diet supplemented with the multicarbohydrase enzyme blend and the control diet (4.47 vs. 4.25 kg). The relative empty weights of the colon plus rectum and the portal-drained viscera (PDV; spleen, pancreas, stomach, caecum, small intestine and colon plus rectum) were also heavier (P = 0.01) in the 30% DDGS fed pigs compared with the control; the relative weights of the colon plus rectum and PDV were not different (P > 0.10) for pigs fed the multicarbohydrase enzyme supplemental 30% DDGS-containing diet and the control diet.

Fasting whole-body O_2 consumption, ileal and caecal digesta viscosity were not affected (P > 0.10) by diet (Table 4.5).

Intestinal morphology of the duodenum, jejunum and colon were not affected (P > 0.10) by dietary treatment as shown in Table 4.6. However, a trend (P < 0.10) for a decrease in VH and the villous height:crypt depth (VH:CD) was observed in the ileal segment when pigs were fed 30% DDGS-containing diet.

4.6 DISCUSSION

Recent experiments evaluating the effects of multicarbohydrase enzyme supplementation of DDGS-containing diets on the growth performance of pigs have not produced consistent results. Some studies (Feoli, 2008; Emiola et al., 2009) observed

Table 4.3. Analysed chemical composition of experimental diets (as-is basis)

Item	Control	30% DDGS	30% DDGS+Enzyme
DM, %	89.5	89.4	89.1
CP, %	17.9	17.7	17.9
GE, kcal/kg	3,960	4,145	4,147
NDF, %	9.3	18.1	18.2
ADF, %	3.9	7.4	7.6

[1]DDGS, distillers dried grains with solubles derived from cofermentation of equal proportions of wheat and corn (1:1, wt:wt).

[2] Multicarbohydrase enzyme blend consisted of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) and provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

improvements whereas others (Jacela et al., 2009; Jones et al., 2010) reported no beneficial effects of various carbohydrase enzyme preparations on growth performance when pigs were fed diets with 30% or more DDGS compared with control diets. It therefore become necessary to obtain additional information on the response of growing pigs to DDGS-containing diets given the reported effects of high-fiber diets on digestive physiology (Pond et al., 1989, Jorgensen et al 1996; Wenk, 2001) to effectively manipulate DDGS-containing diets for maximum nutrient utilization by the growing pig. Besides, it is not known whether the lack of response in growth rate (based on live weight gained) to supplemental enzyme will still hold if carcass weight gain is used as the performance indicator given the confounding effect of gut weight and fill in pigs fed high fiber diets on live weight (Kyriazakis and Emmans, 1995; Whittemore et al., 2003). Thus, it was hypothesized that feeding a 30% wcDDGS containing diet to growing pigs will alter intestinal morphology, increase visceral organ weight and whole-body fasting O_2 consumption and decrease carcass weight, and that a multicarbohydrase enzyme supplementation may overcome these negative effects.

The similarities in CP content among the experimental diets in the current study suggest a possible removal of the confounding effects of dietary CP on individual or total visceral organ weight (Anugwa et al., 1989; Chen et al, 1999; Kerr et al., 2003). Pigs readily consumed diets with up to 30% DDGS in most studies (Wildmer et al., 2008; Jones et al., 2010 Yoon et al., 2010) reported in the literature, which implies voluntary feed intake may not be negatively affected when growing pigs are fed diets containing 30% DDGS. Therefore, pigs in the present study were provided equal amounts of the experimental diets once daily and trained to consume their feed within an h.

Table 4.4. Growth performance and visceral organ weight (% empty BW) of pigs fed diets without or with DDGS and a multicarbohydrase enzyme[1]

					P-values[2]	
Item	Control	30 % DDGS	30% DDGS+Enzyme	SEM	Trt	DDGS	Enzyme
Initial BW, kg	19.9	19.9	19.9	0.17	1.00	1.00	1.00
Final BW, kg	37.3	36.1	36.8	0.59	0.39	0.18	0.41
Eviscerated BW (EBW)[3], kg	28.6	26.9	27.8	0.45	0.05	0.02	0.19
Total visceral wt	8.7	9.2	9.0	0.37	0.63	0.35	0.76
Total visceral wt: final BW	0.23	0.25	0.25	0.01	0.20	0.08	0.45
Organ wt, % EBW							
Liver	3.49	3.62	3.52	0.11	0.69	0.41	0.54
Spleen	0.30	0.29	0.34	0.03	0.31	0.90	0.17
Pancreas	0.20	0.21	0.27	0.03	0.33	0.75	0.26
Heart	0.65	0.69	0.59	0.04	0.23	0.48	0.09
Stomach	0.95	1.02	1.01	0.03	0.34	0.18	0.84
Caecum	0.3	0.28	0.31	0.03	0.83	0.65	0.58
Small intestine	4.25	4.6	4.47	0.13	0.15	0.06	0.47
Colon + rectum	1.75	2.14	1.92	0.10	0.03	0.01	0.11
PDV[4]	7.72	8.52	8.24	0.19	0.02	0.01	0.32

[1] Multicarbohydrase enzyme consisted of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) and provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

[2] *P*-values: Trt= overall treatment effect; DDGS = Control vs. 30% DDGS; Enzyme = Control vs. 30% DDGS+Enzyme. Significance is defined as $P < 0.05$.

[3] BW minus viscera

[4]PDV represents portal drained-viscera and comprise spleen, pancreas, stomach, caecum, small intestine and colon + rectum

Table 4.5. Fasting whole-body oxygen consumption, ileal and caecal digesta viscosity in growing pigs fed diets without or with DDGS and a multicarbohydrase enzyme[1]

Item	Control	30% DDGS	30% DDGS+Enzyme	SEM	*P*-values[2]		
					Trt	DDGS	Enzyme
Number of pigs	4	4	4	-	-	-	-
O_2 consumption, ml/min/kg BW	7.82	7.3	6.88	0.46	0.39	0.45	0.53
Digesta viscosity, mPa.s							
Ileum	1.78	1.67	1.69	0.20	0.91	0.70	0.95
Caecum	1.71	1.92	1.54	0.22	0.50	0.50	0.25

[1] Multicarbohydrase enzyme consisted of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) and provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

[2] *P*-values: Trt= overall treatment effect; DDGS = Control vs. 30% DDGS; Enzyme = Control vs. 30% DDGS+Enzyme. Significance is defined as $P < 0.05$.

Table 4.6. Morphology of the intestinal segments in growing pigs fed diets without or with DDGS and a multicarbohydrase enzyme

					P-values[2]	
Item	Control	30% DDGS	30% DDGS+Enzyme	SEM	Trt	DDGS	Enzyme
Duodenum							
VH, μm	509.9	505.8	492.2	40.90	0.95	0.95	0.82
CD, μm	339.6	334.4	358.0	17.95	0.62	0.84	0.36
VH:CD	1.52	1.52	1.37	0.10	0.49	0.98	0.30
Jejunum							
VH, μm	470.4	453.1	538.4	50.80	0.49	0.81	0.25
CD, μm	273.6	286.2	310.0	20.16	0.45	0.66	0.41
VH:CD	1.75	1.64	1.71	0.16	0.88	0.62	0.74
Ileum							
VH, μm	480.3	408.9	425.9	27.95	0.19	0.09	0.67
CD, μm	307.3	298.4	309.7	16.02	0.88	0.70	0.62
VH:CD	1.58	1.38	1.38	0.08	0.13	0.08	0.08
Colon							
CD, μm	361.0	344.4	353.4	14.02	0.13	0.41	0.65

[1] Multicarbohydrase enzyme consisted of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) and provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

[2] *P*-values: Trt= overall treatment effect; DDGS = Control vs. 30% DDGS; Enzyme = Control vs. 30% DDGS+Enzyme. Significance is defined as $P < 0.05$.

This experimental approach was chosen to reduce variations in feed intake among dietary treatments in order to eliminate possible effects of feed intake on changes in individual visceral organ weight in pigs as reported by some authors (e.g. Rompala et al., 1988).

In the current study, final BW was not affected; however, empty BW decreased in pigs fed the unsupplemented 30% DDGS-containing diet compared with the control. This observation underscores the contribution of gut fill and visceral organ hypertrophy to live BW gain and therefore, implies that carcass weight gain may be a more sensitive indicator for accessing growth performance in pigs fed DDGS-containing diets. Besides, the carcass portion has a higher economic value to the swine producer than the non carcass portion (especially, visceral organs). Dietary DDGS inclusion up to 30% had no effect on liver, spleen, pancreas, heart, stomach and caecum weights relative to empty BW but increased the weights of the small intestine and colon plus rectum compared with those on the control diet.

Feeding high-fiber feedstuffs to pigs has produced variable effects on individual visceral organ mass. Results from the present study suggest no effect of 30% DDGS dietary inclusion on liver, stomach and caecum weight in growing pigs. Jørgensen et al. (1996) and Nyachoti et al. (2000) observed increased liver, stomach and caecum weights but not small intestine weight when growing pigs were fed high alfalfa diets. Pond et al. (1988) also fed high alfalfa diet to pigs and reported an increase in heart and small intestine weights in addition to increases in liver, stomach, caecum weights. Jin et al. (1994) did not observed any change in visceral mass whereas Ma et al. (2002) reported decreases in liver and pancreas weights when growing pigs were fed diets with 10% wheat straw and 5% wheat bran, respectively. The high-fiber feedstuffs used in the

present study and those reported above are all high in insoluble fiber and are thus expected to produce similar results or effects on the visceral organs. The inconsistencies in results among experiments may be due to the source of the insoluble fiber used, level of inclusion and duration of exposure to the high-fiber diets. Moreover, in some of the studies where feeding high-fiber diets to pigs increased the relative weight of the liver (e.g., Pond et al., 1988; Nyachoti et al, 2000), the high-fiber diets also contained higher concentrations of CP which could have caused the increased liver weight because the role of the liver in the deamination process (Chen et al., 1999; Kerr et al., 2003). Nevertheless, PDV (which is directly involved in digestion and absorption) weight increased in pigs fed the unsupplemented 30% DDGS-containing diet compared with the control in the present study which is in agreement with the results of Jørgensen et al. (1996) and Nyachoti et al. (2000).

Empty BW and visceral organ weight of the pigs fed 30% DDGS-containing diet supplemented with the multicarbohydrase enzyme blend in the current study compared favourably with those on the control diet. Thus, the carbohydrase enzyme cocktail was able to substantially reduce the weights (per empty BW) of the small intestines, colon plus rectum and PDV and restore 52.9% of the empty BW lost as a result of feeding the pig unsupplemented 30% DDGS-containing diet. Feeding pigs high-fiber diets increases the secretion of digestive fluids for breaking down the fiber in such diets (Wenk, 2001). The increased secretion of digestive fluid is associated with a higher activity of the secretory organs involved and thus, results in the enlargement of these organs. Therefore, any exogenous factor that aids in the digestion of dietary fiber will reduce the need for the extra effort required by the secretory organs of the GIT to break down these high-

fiber diets. It is therefore, not surprising that the multicarbohydrase enzyme blend was able to reduce the PDV weight when supplemented to the 30% DDGS diet. To the best of our knowledge, similar studies in pigs or other species fed high-fiber or diets able to induce visceral organ hypertrophy and supplemented with a nonstarch polysaccharide degrading enzyme have not be reported.

The whole-body energy expenditure by an organism can be estimated by measuring its whole-body O_2 uptake (McLean, 1970). The energy expended for maintenance requirements relates to fasting metabolism which is closely associated with the mass and activity of the visceral organs in farm animals (Koong et al., 1985; Ferrell, 1988). Therefore, increased visceral organ mass indicates an increase in maintenance energy requirements and as such, any factor that causes visceral organ hypertrophy may have a profound influence on the efficiency of whole-body energy utilization. Thus, in the present study, it was expected that the increased PDV mass in the 30% DDGS fed pigs should increase their fasting whole-body O_2 consumption compared with those fed the control diet. However fasting whole-body O_2 consumption was not affected by dietary treatment which could be due to two possible reasons. Firstly, in the growing pig, lean tissue deposition takes precedence, which is an energy demanding process (Tess et al 1984a, b; Ferrell, 1988; van Milgen and Noblet, 2003). Therefore, an increase in carcass weight of the growing pig in the current study can be speculated to be closely associated with increase in fasting whole-body oxygen consumption. In that sense, it may not be surprising that no differences were detected in the fasting whole-body O_2 consumption among treatments since the pigs on the control diet had a heavier empty BW compared with those fed 30% DDGS diet. Secondly, because the liver alone accounts for about 20-

25% of the whole body O_2 consumption (Huntington and Reynold, 1987; Yen et al., 1989), it could be surmised that hepatic metabolism influences whole-body energy expenditure than PDV. Therefore, any increases in fasting whole-body O_2 consumption due to changes in PDV weight as a result of the 30% DDGS dietary inclusion might have been too small to be detected because diet had no effect on liver weight in the current study. This supposition is supported by the study conducted by Rompala et al. (1988) where sheep were fed diets without or with 10% polyethylene powder (to increase dietary bulk) but no differences were observed in liver weight and fasting whole-body O_2 consumption even though PDV was heavier in the polyethylene powder-fed sheep compared with the control.

Supplementing the DDGS-containing diet with the multicarbohydrase enzyme cocktail also had no effect on fasting whole-body O_2 consumption although the value obtained was the lowest among the dietary treatments. It would therefore, be necessary to determine the O_2 consumption by the PDV, alone, in order to estimate the proportion of whole-body O_2 consumption used by PDV when pigs are fed DDGS-containing diets. This will provide information on energy expenditure by the PDV in DDGS-fed pigs for development of strategies for efficient utilization of energy in DDGS by pigs.

In the current study, ileal and cecal digesta viscosities were not affected by dietary treatment. Digest viscosity is a consequence of NSP dissociating in the GIT to form high molecular weight viscous aggregates (Bedford and Schulze, 1998). Therefore, feedstuffs with high soluble NSP contents will considerably increase digesta viscosity. With regard to DDGS, Stein and Shurson (2009) and Yáñez et al. (2011) have reported that the fiber is predominately water insoluble because the insoluble fraction is almost six (6) times

higher than the soluble fraction. It is, therefore, not surprising that including the DDGS at 30% of the diet did not increase digesta viscosity in the ileum and caecum compared with the multicarbohydrase enzyme supplemented DDGS diet or the corn-soybean meal basal control diet used in the present study. This observation implies that, apart from decreasing nutrient digestibility by way of reducing transit time of ingested feed in the GIT, DDGS does not decrease body growth by reducing nutrient absorption by increasing digesta viscosity. Rather, DDGS increases PDV mass by increasing dietary bulk which increases PDV work load and thus, increase the maintenance energy and nutrient requirements at the expense of body growth.

Studies with several farm animals indicate that intestinal epithelial morphology is greatly altered by dietary fiber (see review by Montagne et al., 2003). For instance, Jin et al. (1994) reported that in growing pigs, high-fiber diets caused an enlargement of villi and deepened the crypts in the jejunum and ileum, and also increased the rate of cell proliferation and crypt depth in the large intestine compared with a low-fiber diet. In the current study, however, morphology of the duodenal, jejunum and colon segments did not change when pigs were fed 30% DDGS-based diets without or with multicarbohydrase supplemental compared with the control diet. It should, however, be noted that the effect of dietary fiber on intestinal morphology is variable and depends on several factors such as the level of incorporation, animal species and age, duration of fiber ingestion and the site in the intestinal tract (Montagne et al., 2003). Pigs used in the current study were older than the ones used by Jin et al. (1994) (initial BW; 19.9 vs. 14.4, respectively). Moreover, pigs in the present study were fed the high-fiber diets for 28 d which was longer than the 14 d period used by Jin et al. (1994). Brunsgaard (1998), however, fed

high-fiber diets (176 to 218 g/kg DM) to approximately 30 kg pigs for 28 d and observed similar alterations in the large intestine as reported by Jin et al. (1994). Reasons why similar results were not observed in the current study are not clear. Nevertheless, a tendency for VH (P = 0.09) and VH:CD (P = 0.08) to decrease in the ileum were observed in the present study when pigs were fed the DDGS-containing diets. A decrease in VH:CD is associated with an increase in the rate of crypt cell proliferation and the number of cells exhibiting DNA fragmentation (Jin et al., 1994), and these processes lead to cell turnover (an energy demanding process) resulting in the growth of the specific intestinal segment. It is therefore, of no surprise that the weight of the small intestine tended to increase in the DDGS fed pigs. Research on the effect of DDGS at the intestinal cellular level is required to elucidate the dynamics of the intestinal cells which is vital to metabolic adaptation and thus, digestive physiology of the DDGS fed pig.

In conclusion, the results of the present study indicate that including 30% DDGS in grower diets based on corn and SBM and formulated on ME, SID AA and available P basis appears to have no adverse effect on final BW. However, empty BW decreases and gut mass increases. Supplementing the DDGS-containing diet with a multicarbohydrase enzyme blend was able to overcome these adverse effects.

4.7 Implication

The results of the present study indicate that gut mass contributes to live BW gain and that carcass weight gain provides a more sensitive interpretation of data on growth performance in pigs fed DDGS containing diets. The reduction in empty BW and

increase in visceral organ mass as a result of feeding growing pigs a 30% DDGS-containing diet represents financial loss to swine producers because the visceral organ has less economic importance compared with the carcass. However, this negative effect of dietary DDGS may be overcome by supplementing with an appropriate carbohydrase enzyme combination. As portal-vein drained organs are metabolically active, increase in their mass are likely to exert additional maintenance energy and nutrient requirements. It will therefore, be necessary to determine the effect of DDGS inclusion in grower diets on nutrient absorption and energy consumption by these organs for development of strategies for efficient utilization of the nutrients in DDGS by the growing pig.

5.0 GENERAL DISCUSSION

As indicated in the literature review section (Chapter 2) of this thesis, DDGS is not a new feedstuff to swine nutritionists and producers since research on its feeding value dates back to the 1950s. The current interest in the use of DDGS in swine diets, however, is due in part to its wide spread availability (due to the increase in biofuel production) and improved quality, and partly to the increasing cost of conventional feedstuffs. As clearly demonstrated by some recent experiments captured in Chapter 2, DDGS can serve as a cheaper source of energy, protein and P when considered in swine diets. However, the high-fiber content in DDGS limits its inclusion level, particularly, in the growing pig diets due to the well established adverse effects of dietary fiber on nutrient digestibility and thus, growth performance in non-ruminants. The question that arises is how much of the DDGS can be included in the diets for growing pigs before the negative effects start to show and what can be done to overcome these adverse effects for more of the DDGS to be used or included in grower diets? With respect to the latter question, exogenous enzymes capable of degrading nonstarch polysaccharides (NSP) are often considered. However, results from recent experiments (Chapter 2) evaluating effects of DDGS alone or in combination with NSP-degrading enzymes on growth performance and/or nutrient digestibility in pigs have not produced consistent results. It therefore, becomes necessary for additional information to be obtained when growing pigs are fed DDGS-containing diets to design effective strategies such as the use of enzyme technology for efficient utilization of the nutrients in DDGS by the growing pig.

In this thesis, two experiments were conducted, primarily to: 1) determine growth responses and nutrient digestibility in growing pigs fed graded levels of DDGS and 2) to determine the effect of a multicarbohydrase enzyme supplement in a DDGS-based diet on visceral organ mass, intestinal morphology and whole-body energy expenditure. A DDGS sample resulting from cofermentation of wheat and corn in equal proportions (1:1, wt/wt) was use to achieve the objectives of this thesis.

In Manuscript 1, growing pigs were fed a corn-barley-SBM basal diet with 0, 10, 20 and 30% of the DDGS to determine the optimal inclusion rate of DDGS. Because the current version of NRC (1998) has no data on the SID of AA for wheat-corn DDGS and such information was also not available in our laboratory (or elsewhere) when the experimental diets were being formulated for this particular study, the AA content of these diets were formulated on the basis of total AA. The energy and P contents were, however, formulated on ME and available P basis. Thus, the experimental diets were formulated to contain similar amounts of ME, total AA and available P recommended for growing pigs (NRC, 1998). Including DDGS in the grower diet resulted in a linear decrease in the overall ADG. Including DDGS up to 30% of grower-finisher diets had no effect on BW gain in some experiments (Cook et al., 2005; Xu et al., 2010) whereas a decrease in BW gain was observed in other experiments reported in Chapter 2 (e.g., Whitney et al. 2006; Linneen et al., 2008). Possible reasons for these discrepancies include the nutrient composition and digestibility of the DDGS samples used, contributing effects of other nutrients and the basis upon which the various experimental diets were formulated. For instance, in some experiments where BW was reduced (e.g. Whitney et al. 2006) with increasing amounts of DDGS in the diet, the DDGS-containing

diets also had higher CP contents which might have contributed to the growth depression since excess CP must undergo deamination and ultimately excreted. This process, however, requires the use of energy as indicated in the literature review and therefore, the amount of energy retained for body growth is reduced. In Manuscript I, the experimental diets were formulated to have equal amounts of CP and the analyzed chemical composition also showed similarities in CP contents among the experimental diet. Therefore, the linear decrease in growth rate cannot be attributed to excess dietary CP in the DDGS-containing diet. However, the diets were formulated on total AA which may not be a good indicator of the availability of AA for body protein synthesis compared with formulating diets on SID AA (NRC, 1998). Thus, it is possible that formulating the diets on total AA instead of SID AA might have contributed to the decrease in growth rate of pigs as DDGS increased in the diets because DDGS has lower AA digestibility even though it has higher AA content when compared with its corresponding grain (Nyachoti et al., 2005).

The analyzed chemical composition of the experimental diets used in Manuscript I suggests that the wide Ca:P ratio among these diets might have also contributed to the decline in BW gain in the pigs, particularly, those on the 30% DDGS diet (Ca: P ratio, 1.44:1). It must, however, be noted that a wide variation between these two minerals (more than 1.3 to 2.0) depresses the growth of pigs only when the diet is marginal in P (Reinhart and Mahan, 1986; NRC, 1998), and since the analyzed P content in the experimental diets was above the recommendation amount (NRC, 1998), it is not possible for the wide Ca:P ratio to have confounded the growth rate of pigs in the study reported

in Manuscript I. Moreover, P digestibility and thus, P utilization was not affected by dietary treatment in the experiment reported in Manuscript I.

Feed intake, however, was not affected by dietary treatment as reported in Manuscript I. This observation – which agrees with some studies reported in the literature review section of this thesis – implies that including 30% DDGS in grower diets may not negatively affect voluntary feed intake. However, in a feed preference study, Hastad et al. (2005) observed that including 30% DDGS in diets for growing pigs significantly depressed feed intake and also, the pigs preferred the corn-soy diets to the DDGS-containing diets. It is, however, not known from that study whether providing growing pigs choices between only DDGS-containing diets (up to 30% dietary inclusion) will influence preferences and feed consumption.

Increasing the inclusion levels of DDGS decreases the digestibilities of DM, energy and CP which is not surprising given the adverse effect of high dietary fiber on the digestibilities of these nutrients as indicated in the literature review. However, P and Ca digestibilities were not affected by dietary treatments in the experiment reported in Manuscript I. This is because the P in DDGS has a higher bioavailability since most of the phytate-bound P in the grain used as feedstock is liberated during the fermentation process. The diet with 30% DDGS in the experiment reported in Manuscript I was not supplemented with inorganic P but the ATTD coefficient for P was similar with the control diet supplemented with inorganic P (50.5% vs. 50.6%). This observation implies that including 30% of DDGS in grower diets based on corn, barley and SBM may completely eliminate the need for supplementing such diets with inorganic P or phytase – enzyme responsible for hydrolyzing phytate-bound P in feedstuffs for non-ruminants –

which are not cheap. It also implies that substituting substantial amounts of corn, barley and SBM with DDGS in grower diet formulas may help in decreasing the amount of P excreted in the manure.

In Manuscript II, the experiment was designed based on the results of Manuscript I. Thus, 30% DDGS dietary inclusion rate was chosen because it was observed from the experiment reported in Manuscript I that 20% dietary DDGS inclusion of grower diets was the level that support BW gain similar with the control diet and that, the adverse effects of DDGS on growth performance starts to show above this inclusion rate. The 30% dietary DDGS inclusion level was used in Manuscript II to determine the effect of DDGS alone or in combination with supplemental enzyme on visceral organ mass, intestinal morphology, carcass weight and fasting whole-body oxygen consumption. Three diets were used in this study: a corn-SBM basal control diet, a corn-SBM+30% DDGS and a corn-SBM+30% DDGS supplemented with a multicarbohydrase enzyme cocktail. The experimental diets were formulated on ME, SID AA and available P basis. Since pigs in the experiment reported in Manuscript I consumed as much of the 30% DDGS-containing diet as the control diet, pigs used for the experiment in Manuscript II were fed equal amounts, i.e., 4% of their BW (close to ad libitum feeding) of the experimental diets once daily and trained to consume their feed within an hour. This experimental approach was to eliminate the possibility of variations in feed intake contributing to changes in individual visceral organ weight as reports by some authors (e.g. Rompala et al., 1988). It also ensured that pigs do not come into contact with feed for 24 hours before they are placed in the indirect calorimeter to measure fasting whole-body O_2 consumption. The calculated and analyzed CP contents were similar among the

experimental diets in Manuscript II to eliminate possible effects of dietary CP on visceral organ mass as reported in some studies (e.g. Anugwa et al., 1989; Chen et al., 1999) and the in literature review presented in Chapter 2.

In the experiment reported in Manuscript II, pigs fed the 30% DDGS-containing diet had similar live weight as those on the control diet and the enzyme supplemented 30% DDGS-containing diet. The similarity in BW of pigs fed the control and 30% DDGS-containing diets in Manuscript II is contrary to the results in Manuscript I where more than 20% dietary DDGS inclusion did not support similar BW of pigs as those on the control diet. The 30% DDGS-containing diet in Manuscript I had higher NDF content (22.7% higher) than the one in Manuscript II (22.2% vs. 18.1%). This is probably due to the differences in the number of the major feedstuffs used in formulating the 30% DDGS-containing diets in the experiment reported in Manuscripts I and II (corn, SBM and barley vs. corn and SBM) which might have resulted in a higher NDF content in the former experiment. This may probably explain why feeding pigs the 30% DDGS-containing diet in Manuscript I did not produce similar results with the control diet as the 30% DDGS containing diet used in Manuscript II. This supposition may explain, in part, why some of the studies reported in Chapter 2 and Stein and Shurson (2009) concluded that including 30% corn-based DDGS in grower diets based on corn and SBM supports similar growth performance as a corn-SBM basal control diet. Moreover, the 30% DDGS-containing diet used for the study in Manuscript I was formulated on total AA basis where as the 30% DDGS-based diet in Manuscript II was based on SID AA. It is, therefore, also possible that AA bioavailability in the 30% DDGS-containing diet in Manuscript I was under (or

inaccurately) estimated which might have adversely affected live weight gain as opposed to the 30% DDGS-based diet used in Manuscript II.

Even though live weight gain in the 30% DDGS-fed pigs was similar with those on the control and enzyme supplemented DDGS-base diet in the experiment reported in Manuscript II, the empty BW (final BW minus visceral organs) of the pigs fed the 30% DDGS-based diet were lighter than those fed the control and enzyme supplemented 30% DDGS-based diets. This observation implies that in some of the performance studies where including DDGS in diets for pigs had no effect on BW gain, the gut mass might have contributed to the live weight gained of the DDGS-fed pigs. Similar observations have been reported by Kyriazakis and Emmans (1995) and Whittemore et al. (2003) in pigs fed high-fiber diets. Thus, using live weight gain may not be a sensitive parameter for growth performance when evaluating effect of DDGS inclusion in diets alone or in combination with a carbohydrase enzyme. Since the primary objective of pork production is to efficiently produce lean meat, it will be imperative to base performance in DDGS-fed pigs on carcass weight rather than live weight gain. Besides, the non-carcass portion (offal) has less economic value when compared with the carcass.

It was, however, observed that supplementing the 30% DDGS-based diet with the enzyme cocktail was able to restore the empty BW lost due to dietary DDGS inclusion. This implies that using appropriate carbohydrase enzyme combination should be able to offset the adverse effect of high dietary DDGS inclusion on carcass weight. In that sense, in some of the DDGS experiments reported in Chapter 2 where supplementing the DDGS-containing diet with enzymes did not improve weight gain compared the unsupplemented diets, gut mass might have masked the positive effect of carbohydrase

enzyme on BW gain. This is clearly demonstrated by the visceral organ weight data reported in Manuscript II where pigs fed the 30% DDGS diet had heavier gut mass compared with the control or enzyme supplemented DDGS diets.

In table 5.1, economic estimates of the experiment conducted in Manuscript 2 are provided. It can be observed from this analysis that substituting considerable amounts of corn (13.11 %), SBM (17.25%) and Biophos (0.44%) with 30% DDGS in the Control diet reduced feed cost per ton considerably (i.e., by C$ 37.44) which implies that partial replacement of corn, SBM and inorganic P with DDGS in swine diet ultimately reduces feed cost as indicated in section 2.3.7 of the literature review. However, the cost of feed required to produce a kg of BW was not significantly different ($P > 0.05$) between pigs fed the Control and the 30% DDGS-containing diet which disagrees with results of the study conducted by Lee et al. (2011) where feed cost required to produce a kg BW was significantly lower ($P > 0.05$) for finishing pigs fed 20% DDGS-containing diet compared with the Control diet (won/kg 821 vs. 1251, respectively). Nevertheless, the cost of feed to produce a kg EBW was significantly lower ($P = 0.05$) for pigs fed the 30% DDGS-containing diet compared with the control. When the enzyme supplemented 30% DDGS-containing diet is compared with the Control, the drop in feed cost per ton was C$ 33.94 but there was no significant difference ($P > 0.05$) in the cost/kg BW between the enzyme supplemented DDGS-containing and the Control diets. There was, however, a tendency for the cost/kg EBW to decrease for the pigs fed the enzyme supplemented DDGS-containing diet compared with those on the Control diet

Table 5.1. Economics of production on pigs fed diets without or with DDGS and a multicarbohydrase enzyme[1]

					P-values[2]		
Item	Control	30 % DDGS	30% DDGS+Enzyme	SEM	Trt	DDGS	Enzyme
Feed cost/ton, C$/ton[3]	460.22	422.78	426.28	-	-	-	-
Cost/kg BW, C$/kg	0.86	0.81	0.81	0.033	0.49	0.30	0.30
Cost/kg EBW, C$/kg	0.52	0.48	0.49	0.010	0.07	0.03	0.09
Carcass wt. at slaughter, kg [4]	88.26	85.73	86.77	0.957	0.20	0.08	0.28
Carcass value, C$[5]	151.56	147.21	149.00	1.643	0.20	0.08	0.28

[1] Multicarbohydrase enzyme consisted of a combination of Superzyme W and Superzyme OM (2:1 wt/wt, Canadian Bio-Systems Inc., Calgary, Alberta, Canada) and provided 200 units of glucanase, 500 units of xylanase, 1,100 units of cellulase, 1,800 units of amylase, 50 units of invertase, 70 units of mannanase, 10 units of galactanase, and 35 units of protease per kg of diet.

[2] *P*-values: Trt= overall treatment effect; DDGS = Control vs. 30% DDGS; Enzyme = Control vs. 30% DDGS+Enzyme. Significance is defined as $P < 0.05$.

[3]Estimated using the current unit prices for feed ingredients purchased by the Glenlea Feed Mill, University of Manitoba, Winnipeg, Canada.

[4]Carcass wt. at slaughter was extrapolated assuming that all pigs will reach a target market of 115 kg around the same time and that dressing percentage is a reflection of the EBW as a percentage of the final BW after the 28-d study.

[5]Carcass value was estimated using a carcass index of 108 (i.e., assuming an average carcass wt. of 85 kg at slaughter and a lean yield of 51.5%) and a bid price of C$ 1.59/kg (derived from June 2011 price for Manitoba Pork Co-op Contracts on the MAFRI website)

When the data for the 28-day study reported in Manuscript 2 are extrapolated to pigs at a slaughter weight of 115 kg using the assumption that the EBW, as a percentage of the final BW at day 28 of study is a good parameter for calculating dressing percentage at slaughter, it can be observed that carcass weight tended to decrease ($P = 0.08$) when 30% DDGS was used to replace some amounts of corn, SBM and Biophos in the pigs' diet. However, the carcass weight of the pigs fed the 30% DDGS-based diet supplemented with the multi enzyme blend was not different ($P > 0.05$) from those fed the Control diet. Likewise, the carcass value tended to be lower ($P = 0.08$) for pigs fed the 30% DDGS-based diet compared with the Control but pigs fed the enzyme supplemented DDGS-containing diet had a carcass value which was not different ($P > 0.05$) from the Control. It is rather difficult to provide a casual oversimplification of feed cost estimates for pigs at slaughter because it is not known how much the pigs would have consumed by the time they reach the slaughter weight adapted for the economic analysis reported in Table 5.1.

In the experiment reported in Manuscript 2, pigs fed the 30% DDGS diet had heavier colon + rectum, small intestine and PDV (per kg empty BW) compared with pigs fed the control diet, and supplementing the 30% DDGS diet with the carbohydrase enzyme cocktail was able to reduce the weight of these organs attributed to the inclusion of DDGS in the diet. The organs drained by the portal vein (PDV; spleen, pancreas, stomach, intestines and caecum) are directly involved in digestion, absorption and intermediary metabolism of ingested nutrients (Yen and Killefer, 1987; Ferrell, 1988;

Burrin 1989). Thus, any factor that increases the work load of these organs will cause an increase in their mass and any factor that facilitates their work load, will ultimately lessen the increase in their mass. It is therefore, not surprising that feeding the growing pigs the 30% DDGS (high-fiber) diet increased the PDV mass whereas supplementing the 30% DDGS diet with the carbohydrase enzyme cocktail was able to reduce the PDV mass (which compared favourably with the pigs fed the corn-SBM control diet). The increase in PDV mass of the pigs due to dietary DDGS inclusion implies that more energy and nutrients was used for their maintenance at the expense of edible pork production.

As indicated in Chapter 2, energy expenditure for maintenance purpose (i.e., fasting heat production) is of great concern in farm animals because it represents a greater part of the metabolizable energy not utilized for body growth. As the name implies, fasting heat production is determined when the subject is "fasting" or in the post-absorptive state. The most widely used approach for determining this fasting heat production in swine is the indirect calorimetry with an open-circuit respiration chamber. These respiration chambers are designed to measure heat production based on the O_2 consumption and CO_2 production of the test subject. As discussed in Chapter 2, it is possible to use only the O_2 consumed by the test subject to indicate heat production because oxygen is consumed for substrate oxidation in the body to generate heat. Thus, in the experiment reported in Manuscript II, the fasting whole-body O_2 uptake of the pigs was used to represent whole-body energy expenditure for maintenance requirements.

As indicated in Chapter 2, fasting whole-body O_2 consumption is closely associated with the mass and activity of the visceral organs since these organs are metabolically active and requires more energy for their maintenance. This energy is

expended by the visceral organs for tissue repairs through the process of cellular turnover (McBride and Kelly, 1994). From the morphological data reported in Manuscript II, 30% DDGS inclusion in the grower diets had a tendency to decrease villous height (VH) and villous heigh:crypt depth (VH:CD) ratio in the ileum. A decrease in VH:CD implies an increase in the rate of crypt cell proliferation and thus, increase in the rate of gastrointestinal mucosal cell turnover with a resultant increase in the gut and energy expenditure (Jin et al., 1994). This suggests that including 30% DDGS in pig diets will increase the gut mass as well as whole-body O_2 consumption. In Manuscript II, however, the fasting whole-body O_2 consumption was not affected by diet even though pigs on the 30% DDGS-containing diet had heavier PDV mass compared with the control. It is possible that the lack of effect of diet on liver weight might have cancelled out any increases in the fasting O_2 consumption due the changes in the PDV weight attributed to the 30% DDGS inclusion in the diet because of the significant role played by hepatic metabolism on whole-body energy expenditure (Huntington and Reynolds, 1987; Ferrell 1988; Yen et al., 1989). It is also possible that the increased empty BW observed in the pigs fed the control diet compared with those on the 30% DDGS-based diet might have contributed to the lack of difference in the whole-body O_2 consumption due to dietary treatment since an increase in empty BW implies an increase in tissue protein synthesis which is an energy demanding process (Ferrell, 1988; van Milgen and Noblet, 2003). The pigs fed the enzyme supplemented DDGS-based diet had the lowest fasting whole-body O_2 consumption even though not significantly different from those on the control and unsupplemented DDGS-containing diets.

6.0 SUMMARY AND CONCLUSIONS

1. Growing pigs can consume diets containing 30% DDGS derived from cofermentation of wheat and corn (wcDDGS, 1:1, wt/wt) which implies that, voluntary feed intake may not be adversely affected at such dietary inclusion rate.

2. Including 30% wcDDGS in grower diets decreases the apparent total tract digestibility of dry matter, energy and nitrogen but not P and Ca.

3. Substituting grower diets based on corn, barley and SBM with 30% wcDDGS and formulated on available P basis completely eliminates the need for supplemental inorganic P. The 30% wcDDGS-containing diet also had similar P digestibility when compared with corn-barley-SBM based diet supplemented with inorganic P.

4. Including 20% wcDDGS in grower diets based on corn, barely and SBM, and formulated on ME, total AA and available P basis support similar growth performance as a corn- barely-SBM based diet.

5. Including 30% wcDDGS in grower diets based on corn and SBM and formulated on ME, SID AA and available P has no adverse effect on final BW; however, carcass weight decreases implying that gut mass and fill contributes to the live weight gain in pigs fed DDGS-containing diets.

6. Using live weight gain is not the most sensitive parameter when evaluating the effects DDGS on growth performance of pigs because the non-carcass portion has less economic value compared with the carcass.

7. Supplementing grower diets containing high levels of DDGS with appropriate carbohydrase enzyme combination is able to overcome the decline in carcass weight and increase in gut mass due to dietary DDGS inclusion.

7.0 FUTURE STUDIES

The digestibility method was used in this thesis and the other experiments reported in Chapter 2 to evaluate the effect of DDGS on nutrient availability and utilization for the growth of pigs. Even though the digestibility method – which allows measurement of the disappearance of nutrients from the gastrointestinal tract (GIT) – provides an estimate of the amount of nutrients available for utilization by the animal, it does not quantify the net absorption of nutrients from the GIT (Rerat et al., 1984a, b; Yen and Killefer, 1987) and may therefore, not correlate well with other nutritional indices such as retention (Low, 1976). This is because the gut lumen is external to the animal's systemic environment and as such the entry of a substance into the systemic environment from the intestinal lumen may be a more critical measurement of ultilization than the disappearance from the GIT environment (Bajjalieh et al., 1981). It would thus, be necessary to determine the net nutrient absorption (or portal appearance) in pigs fed DDGS-containing diets to provide a better knowledge on how the ingested nutrients from such high-fiber diets are utilized by pigs.

Furthermore, based on the results obtained from this thesis, it was concluded that feeding growing pigs diets containing 30% DDGS results in an increase in the organs drained by the portal vein (PDVO). Although the PDVO forms about 15% or less of the body mass, they use a substantial amount of the body's energy and nutrients for their maintenance requirements. Thus, the increase in their mass due to the 30% dietary DDGS inclusion means that more energy and nutrients will be used for their upkeep at the expense of body growth. It would therefore, be important to estimate the amount of energy and nutrients, particularly AA, used by the PDVO when pigs are fed 30% or more

DDGS-containing diets to design strategies for efficient utilization of the nutrients in DDGS-based diets by pigs.

On the other hand, the hepatic portal vein is responsible for transporting blood which contains absorbed nutrients from the intestinal capillaries to the liver. Therefore, by placing catheters in the portal vein and carotid artery for continuous blood sampling, net nutrient absorption can be quantified by the difference in nutrient concentration between blood in the portal vein and carotid artery; multiplying this value by the simultaneously determined blood flow rate through the portal vein provides information on nutrient absorption kinetics. Blood flow rate can be measured using electromagnetic blood flow probes (Bajjalieh et al., 1981; Rerat et al., 1984a, b), ultrasonic blood flow probes (Hooda et al., 2009) or indicator dilution method (Yen and Killefer, 1987). With respect to the latter approach, an additional catheter is placed in the ileal vein for constant infusion of a marker and blood flow rate is determined using the Fick principle as outlined by Katz and Bergman (1969). Choosing any of the approaches indicated above to determine blood flow rate is a matter of convenience since they all have shortcomings (Eisemann et al. 1987).

We have been able to develop the chronic vessel cannulation model using the procedures described by Yen and Killefer (1987) with some modifications to ensure its application in our laboratory. This model should be used to design experiments in future studies that will provide answers to the following questions:

1. What are the effects of dietary DDGS inclusion (up to 30% or more) on the net influx of portal glucose and AA, and oxygen consumption by the

whole-body and PDVO in growing pigs.

2. What proportion of the whole-body oxygen consumption is accounted for by the PDVO in growing pigs fed diets with DDGS up to 30% or more?
3. What is the effect of dietary manipulation such as enzyme technology on nutrient flux and oxygen consumption by PDVO when growing pigs are fed 30% or more DDGS-containing diets?

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