GREENHOUSE GAS PRODUCTION AND PERFORMANCE OF GROWING PIGS FED HIGH WHEAT MILLRUN DIETS SUPPLEMENTED WITH A MULTI-CARBOHYDRASE ENZYME

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ABSTRACT

The inclusion of wheat millrun, a low-cost milling co-product, into swine rations is limited by high non-starch polysaccharide (**NSP**) content. The high NSP content may decrease digestibility and performance and increase fermentation, perhaps leading to increased greenhouse gas (**GHG**) production. The high NSP content also suggests that carbohydrase enzymes may improve digestibility. This thesis, therefore, evaluated the impact of wheat millrun and enzyme (carbohydrase) supplementation in diets of growing-finishing pigs on the environment (GHG production) and performance of pigs. A partial life cycle analysis (**LCA**) was performed to assess GHG output when feed production and manure management was considered. A performance study carried out in the first experiment, showed no interactive effects on growth performance and nutrient digestibility when wheat millrun was supplemented with multi-carbohydrase enzymes in diets of growing-finishing pigs. Increasing wheat millrun linearly reduced energy, nitrogen (**N**) and phosphorus (**P**) apparent total-tract digestibility (**ATTD**) and net energy (**NE**) content whiles enzyme reduced energy digestibility but not N or P ATTD. Overall average daily gain (**ADG**) and gain to feed ratio (**G:F**) were reduced, and average daily feed intake (**ADFI**) was unaffected in pigs fed the wheat millrun diets. Pigs on the enzyme supplemented diets experienced reduced G:F in the first 14 d. Neither wheat millrun nor multi-carbohydrase supplementation affected days to market. In the second experiment, wheat millrun inclusion in swine diets failed to increase GHG (CO_2, N_2O, CH_4) emissions from growing pigs housed in environmental chambers. Multi-carbohydrase supplementation also had no effect on GHG production. There was an increase in CO_2 flux and a decrease in CH_4 flux in manure gas measurements with time. In the final experiment, data from the Holos model used in an LCA framework indicated that when feed production was considered, feeding pigs up to 30 % wheat millrun inclusion in the diet could result in about a 25 % reduction in GHG emission in terms of global warming potential (**GWP**). In summary, the results indicate that feeding growing-finishing pigs up to 30 % wheat millrun diets may be beneficial to the farmer and the environment as it reduces GHG emissions when feed production is also considered.

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DEDICATION

This thesis is dedicated to my parents, Godwin Yaw Kpogo and Wilhemina Mensah, for allowing me to pursue my dreams and aspirations. My siblings, Priscilla and Richard, for the constant support and prayers. To my undergraduate supervisor, Dr. Eric Timpong-Jones, for believing in me.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 3: PERFORMANCE OF GROWING PIGS FED WHEAT-BASED DIETS CONTAINING WHEAT MILLRUN AND A MULTI-CARBOHYDRASE ENZYME Error! Bookmark not defined.

CHAPTER 4. GREENHOUSE GAS EMISSIONS FROM PIGS FED WHEAT MILLRUN SUPPLEMENTED WITH A MULTI CARBOHYDRASE ENZYMEError! Bookmark not defined.**3**

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAFCO	Association of American Feed Control Officials
ADF	Acid detergent fibre
NH_3	Ammonia
ADFI	Average daily feed intake
ADG	Average daily gain
AIA	Acid insoluble ash
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists
ATTD	Apparent total tract digestibility
BW	Bodyweight
Ca	Calcium
CCAC	Canadian Council of Animal Care
CO_2	Carbon dioxide gas
CP	Crude protein
DDGS	Distiller dried grains and solubles
° C	Degree celsius
DM	Dry matter
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
G: F	Gain to feed
GWP	Global warming potential
GHG	Greenhouse gases
GE	Gross energy
IPCC	Intergovernmental Panel on Climate Change
IDF	Insoluble dietary fibre
kg	Kilogram
L	Litre
LCA	Life cycle assessment/analyses
m	Metre
ME	Metabolizable energy

CH_4	Methane gas
µg	Microgram
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NRC	National Research Council
NSP	Non-starch polysaccharide
N_2O	Nitrous oxide gas
P	Phosphorus
PSCI	Prairie Swine Centre, Inc.
PPM	Parts per million
RCBD	Randomized complete block design
SAS	Statistical Analysis System
SID	Standard ileal digestibility
SDF	Soluble dietary fibre
SBP	Sugar beet pulp
TDF	Total dietary fibre
VFA	Volatile fatty acids

CHAPTER 1: INTRODUCTION

Increased global population and standards of living have led to an increased demand for livestock products (Crist et al. 2017). Globally, pork is the most widely consumed meat product (OECD and FAO 2019), and according to the Food and Agriculture Organization (**FAO**) (Mcleod 2011), pork production is expected to increase by 38 % between 2010 and 2050.

Sustainability in pork production requires that the environmental impact of commercial pig production be critically examined (Philippe and Nicks 2015). The current challenge is to produce pork, which will meet the increase in demand at an affordable cost while ensuring that the environment is not compromised. There is an increasing awareness of the contribution of livestock to the increase in greenhouse gas (**GHG**) production and, thus, climate change (Herrero et al. 2011). There are two main sources of GHG emissions in pig houses; the pig and the manure (Philippe and Nicks 2015). The pig contributes to enteric GHG (especially CH_4) production mainly through the dietary fibre and fermentative capacity of the pig's digestive tract (Le Goff et al. 2002a). The low digestibility of high fibre diets leads to an increase in the production of GHG from both the pig and manure (Philippe and Nicks 2015) and reduces pig performance (Stewart et al. 2013).To achieve "sustainable" pork production with low GHG emissions, improved nutritional and manure management strategies are therefore required (Monteiro et al. 2017).

Wheat millrun, a by-product of wheat milling, is relatively cheap, readily available in North America, and used as an alternative feedstuff for swine (Nortey et al. 2007). Wheat millrun contains a greater proportion of crude protein (**CP**), calcium (**Ca**), and phosphorus (**P**) than wheat (Slominski et al. 2004). It also contains a higher content of fibre, and thus it could be expected that substituting wheat millrun for wheat in pig diets would increase enteric GHG emissions.

However, there is some evidence that supplementation of diets containing wheat millrun with fibre degrading enzymes such as xylanase (a carbohydrase) and phytase will improve nutrient and fibre digestibility and growth performance (Nortey et al., 2008; Zijlstra et al., 2010). The effect of these enzymes on GHG output has not been examined.

In summary, the expansion of the livestock industry will depend on the ability to produce pork while reducing GHG production. As swine producers are being encouraged to increase the use of co-products in their diets, it is imperative to understand that this will not be detrimental to pig production since the co-products are high in fibre and may increase fermentation and hence, GHG output. Pork is a major protein worldwide with low GHG output compared to ruminants, and demand is expected to increase further. Therefore, it is important not to increase the GHG output with the increased demand as the low output may be advantageous to pork producers to produce pork with a low environmental footprint.

The experiment, therefore, seeks to provide data required to determine if the expansion of the pork industry in Western Canada can be accomplished while mitigating the overall environmental impact of pork production.

1.1 General objectives and research hypotheses

1.1.1 General objectives

The overall objective of this project was to determine if increasing the wheat millrun content of swine rations will change the overall GHG emissions when the life cycle analysis considers just emission inside the barn or includes feed production.

1. To determine nutrient digestibility, nutrient output in the manure, growth performance and GHG production when increasing concentration of wheat millrun diets are fed to growing-finishing pigs.
2. To determine if multi-carbohydrase enzyme supplementation will improve nutrient digestibility and mitigate GHG emission. This information is required to determine if improvements in digestibility mitigate GHG emissions.

1.1.2 Research hypotheses

The hypotheses of this thesis are:

1. High inclusion of wheat millrun in swine diets will decrease nutrient digestibility, growth performance and increase GHG emissions from growing-finishing pigs.
2. Supplementing high wheat millrun diets with a multi-carbohydrase enzyme will improve nutrient digestibility, growth performance and reduce GHG emissions from growing-finishing pigs.
3. Wheat millrun inclusion in diets of growing pigs supplemented with a multi-carbohydrase will reduce GHG (CO_2, N_2O, CH_4) output when the production cycle, includes feed production.

1.2 Organization of thesis

This thesis is written in a manuscript format. Chapter two presents a literature review of GHG production, the effect GHG's have on the environment, and GHG contribution from livestock (especially pigs) and feed, and some GHG mitigation strategies. In chapter three, a study is presented on the performance of growing-finishing pigs fed wheat millrun diets with or without multi-carbohydrase enzyme supplementation. Chapter four describes a study measuring GHG output when growing pigs were fed wheat millrun diets supplemented with a multi-carbohydrase enzyme. In this chapter, actual GHG measurements are taken from pigs housed in specialized environmental chambers. In chapter five, a lifecycle assessment (**LCA**) framework study on the impact of feeding growing-finishing pigs wheat millrun diets supplemented with multi-carbohydrase enzymes on the environment when considering feed production and manure management is presented. In chapter six, all the major findings from the three different studies (Chapters 3,4,5) are synthesized, followed by a conclusion. The list of references is in the final chapter.

CHAPTER 2: LITERATURE REVIEW

2.1 General background

The human population is expected to increase from 7.7 billion to 9.7 billion between 2017 and 2050 (FAOSTAT 2018). Similarly, the livestock sector is predicted to grow. For example, animal protein intake increased by 62 % between 1961 and 2011 (McGuire 2015) and according to Alexandratos and Bruinsma (2012), unless significant changes occur, the global demand for meat will increase by 57 % between 2005 and 2050.

Consumption of animal protein varies across the world. Per capita consumption is higher in the Americas, Europe, and Oceania than in Africa or Asia (FAO 2015). The quantity of animal protein consumed correlates with economic growth and evolution in animal production practices (Thornton 2010; Alexandratos and Bruinsma 2012).

Meat is a source of high-quality protein and an excellent source of vitamins and minerals such as vitamin A, vitamin B_{12}, Ca, iron, zinc, amongst others (Mottet et al. 2017). Approximately 40 % of the protein consumed worldwide is derived from animal sources, and it contributes to 15 % of the calories consumed globally (Daniel et al. 2011; Sans and Combris 2015). Livestock provides economic benefits such as employment, labour and manure (Thornton 2010; Mottet et al. 2017).

The livestock sector, however, has negative impacts on the environment, including the air, water, and soil (Vergé et al. 2016). Unless mitigation steps are successfully implemented, this increase in population and protein demand will lead to an increase in carbon (GHG) production/emissions, exacerbating environmental problems, including climate change (Jacobsen et al. 2014; Oonincx 2015).

Effects of climate change on the production of some major crops such as maize, wheat, and rice are already evident in various parts of the world, and this is expected to worsen if current agricultural production systems or the current trends in climatic conditions continue [Intergovernmental Panel on Climate Change (**IPCC**) 2014]. Approximately 80 % of the total damage and losses in the livestock and crop production sector comes from drought (FAO et al. 2018). Therefore, adverse effects of climate change will affect ecosystems and livestock production systems, cause hunger and even threaten the survival of certain species (Ingale et al. 2013).

Thus, the challenge to modern agriculture is to increase agricultural production to meet the increased human population through more sustainable and climate-resilient methods (FAO et al. 2018). Two main environmental impact reduction strategies have been proposed; “production-side strategies and consumption-side strategies” (van Zanten 2016). The production-side strategies focus on increasing productivity through increased efficiency in the livestock sector, whiles the consumption-side strategies focus on changing human consumption patterns (van Zanten 2016). While the consumption-side strategies typically entail a reduction in animal protein intake, there is a debate on how the shift from the consumption of animal protein to plant protein will reduce the impact caused by livestock on the environment while meeting the required quantities of minerals and vitamins protein provided by animal protein (White and Hall 2017).

A shift from ruminants to monogastrics derived products has also been proposed since monogastrics cause less stress to the environment relative to ruminants (Nijdam et al. 2012).

This thesis focuses on how to reduce GHG emissions from pork production without negatively impacting efficiency, productivity, or economic sustainability.

2.2 Greenhouse gas and swine production

Greenhouse gases are atmospheric gases that trap the heat leaving the earth’s surface [Environmental Protection Agency (**EPA**) 2017]. Anthropogenic (caused by human activities) GHG have been linked to climate change (IPCC 2014). Agricultural production systems influence climate change, and conversely, climate affects agricultural production systems. Greenhouse gas accumulation results in global warming, which causes changes in seasonality, affecting rainfall patterns and temperatures as well as causing an increase in extreme climatic occurrences (flood and drought). This affects crop growth and pasture availability for livestock resulting in significant negative implications for food security (FAO et al. 2018).

Various types of GHGs exist, but principal amongst them are carbon dioxide (**CO_2**), methane (**CH_4**), nitrous oxide (**N_2O**), ozone, and chlorofluorocarbons (Lashof and Ahuja 1990; Cloy and Smith 2017). These gases are produced from direct and indirect anthropogenic emissions from different industrial sectors, such as energy production, buildings, and transportation (IPCC 2014). Methane comprises about 44 % of the total GHG emissions in the livestock sector, followed by N_2O and CO_2 at 29 % and 27 %, respectively (Gerber et al. 2013). However, their global warming potentials are different. Global warming potential (**GWP**) is a way of comparing different gases and their impacts on global warming (EPA 2017). The GWP of CH_4 is 28, N_2O is 265, and CO_2 is 1 (IPCC 2014). The GWP of gases is usually compared to CO_2 over a period of 100 years. For instance, the GWP of CH_4 by IPCC (2014) means that it will take 28 kg of CO_2 to have the same impact on climate change as 1 kg of CH_4.

Gerber et al. (2013), in a report for the FAO, concluded that about 14.5 % of anthropogenic global GHG emissions are derived from the livestock sector. Cattle production accounts for 61 % and small ruminants, 22 % (Gerber et al. 2013). Only 9 % of the emissions from the livestock sector comes from swine production (Figure 2.1) (Dyer et al. 2010; Gerber et al. 2013).

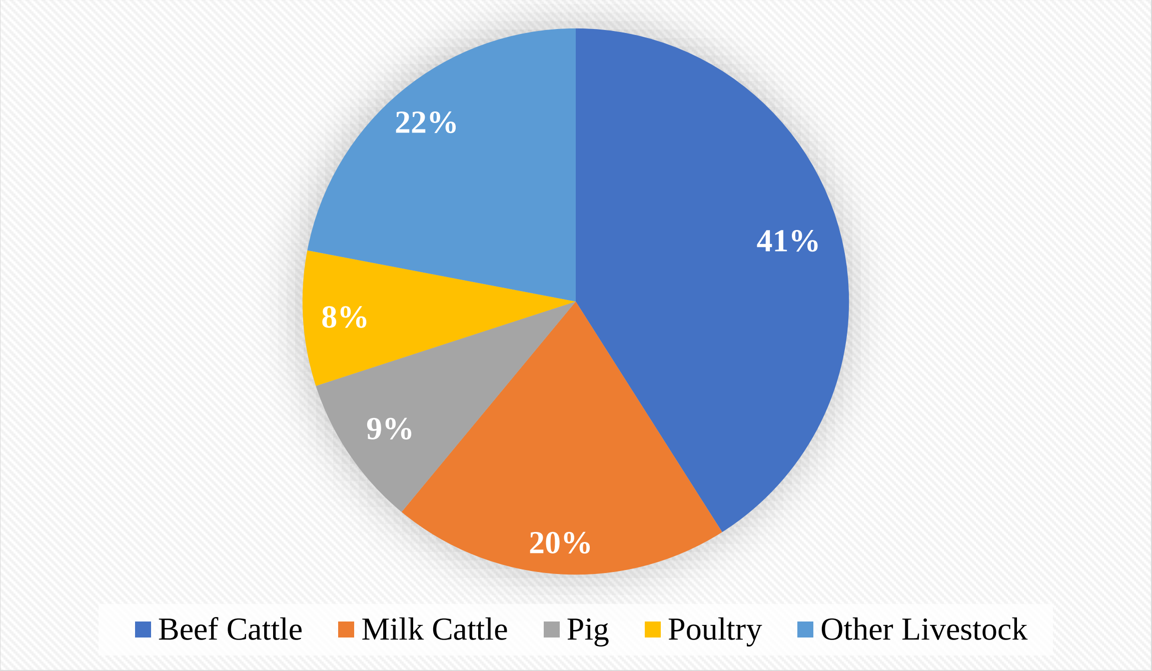

Figure 2. 1 GHG emissions from the livestock sector. Adapted and modified from FAO (Gerber et al. 2013).

Although pig production accounts for a relatively small contribution, if the expected growth in worldwide pork production is considered, GHG emissions could increase considerably, and mitigation efforts should be considered. It is predicted that pork production will increase from about 102.3 to 140.7 million tonnes between 2010 and 2050 (FAOSTAT 2018). Even though pig production systems are considered efficient (in terms of feed efficiency, land use, and reproduction rate) when compared to ruminants, the large numbers in terms of production have the potential to increase the environmental burden. Improvement in feed and energy efficiency is required to reduce the carbon footprint from pig production (Merks et al. 2017).

The most common GHG found in pig production facilities; CO_2, CH_4, N_2O, are mainly from the pig and the manure (Philippe and Nicks 2015).

Methane arises from enteric fermentation, which is mainly determined by the fibre content of the diet and the physiological stage of the pig (Le Goff et al. 2002). A linear relationship between energy loss from CH_4 and digestible dietary fibre intake was shown in a study by Le Goff et al. (2002b). Methane emissions also originate from manure (Kebreab et al. 2006). An anaerobic environment combined with highly degradable organic matter and high temperatures provide a suitable environment for CH_4 production by methanogenic bacteria (Philippe and Nicks 2015).

Output from the manure and animal respiration are the primary source of CO_2 from swine facilities (Philippe and Nicks 2015). Carbon dioxide output is a function of body weight (**BW**), as discussed in equation 2.1 used in the prediction of CO_2 exhalation (Philippe and Nicks 2015).

$$CO_2 \text{ emissions} = 0.136\ BW^{0.573}\ (R^2 = 0.91) \qquad [2.1]$$

This also demonstrates how important it is to characterize emissions on the basis of kg of pork produced. Atakora et al. (2011) reported a reduction in CO_2 production with feed restriction; however, overall emissions per kg of pork produced increased due to reduced growth and delayed marketing of the pig. In the manure, CO_2 is produced from aerobic and anaerobic decomposition of the organic matter (Møller et al. 2013; Philippe and Nicks 2015).

In contrast, N_2O primarily arises from the manure as a by-product of nitrification (usually under anaerobic conditions) or denitrification by microorganisms from the conversion of ammonia (**NH_3**) to molecular nitrogen (**N**) (Oenema et al. 2005; Philippe and Nicks 2015). It can also be produced through other microbial pathways under either aerobic or anaerobic conditions during the oxidation of ammonium. The amount of N_2O emissions produced is linked to the amount of N excreted by the animal (Oenema et al. 2005). Decreasing the quantity of N in the excreta will reduce the amount of N_2O emitted into the environment (Liu et al. 2017).

Therefore, in order to achieve improved sustainability of pork production with low GHG emissions, improved nutritional and manure management strategies are required (Monteiro et al. 2017).

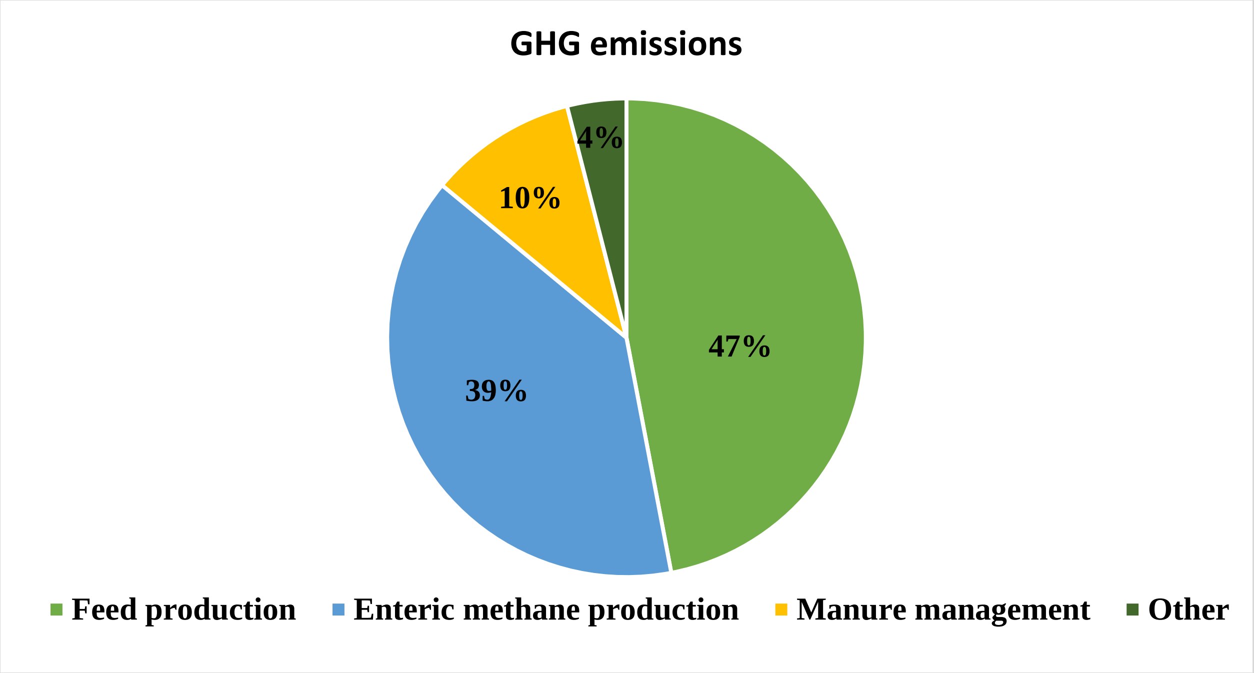

Figure 2. 2 GHG emissions from various processes in the livestock sector (Adapted from van Zanten 2016).

2.3 Inclusion of co-products (wheat millrun) in swine diets

Several factors have been attributed to increased GHG from livestock production. For example, types of flooring and manure management practices, as well as climatic conditions, affect the amount of GHG emitted from the swine production facility (Philippe and Nicks 2015). Nutrition (feed) is among the significant contributors to the production of GHG from livestock production. According to the FAO report prepared by Gerber et al. (2013) and van Zanten (2016), approximately 48 % of GHG emissions associated with pork production is from feed production. These include the use of machinery in feed preparation and transportation.

For many years, cereals (corn and wheat) have been the primary source of energy in swine diets. Wheat remains the primary source of energy for swine diets in western Canada. Feeding pigs with cereals, however, increase competition for food and land with humans (Mottet et al. 2017) and therefore, the cost of the cereals is usually high. The use of co-products derived from processing grains has increased, as this can reduce diet cost (Zijlstra and Beltranena 2013). Since feed represents the highest cost of production in pork production, averaging about 70 % of the overall cost of production (Zijlstra and Beltranena 2013; Woyengo et al. 2014), co-product usage is expected to increase. The use of these co-products in animal production may also reduce the environmental stress caused by the livestock sector (Godfray et al. 2010). For example, swine can convert the co-products from the food production industries into high-quality protein, reducing food wastage and reliance on grains (Zijlstra and Beltranena 2013). This minimizes the destruction of vegetation to produce grains and other feedstuffs to feed the livestock.

Co-products are primarily obtained from the harvesting of crops for humans, the milling industries, food processing industries, and biofuel industries (Cooper and Weber 2012; Popp et al. 2016). Co-products have several benefits in swine production. The dietary fibre found in co-products helps improve the gut health of weaned piglets, supports the growth of beneficial microbiota, reduces fatness and stereotypical behaviour in sows (Jha and Berrocoso 2015; Philippe et al. 2015). The use of co-products, however, presents other challenges, such as reduced or varied nutrient profiles (Woyengo et al. 2014). They are a potential source of mycotoxins and may contain non-nutritious residues (Zijlstra and Beltranena 2013).

"Millrun" is the co-product obtained after the production of flour, which involves the removal of most of the starch fraction in the grain by dry milling (Holden and Zimmerman, 1991). Wheat millrun usually include individual wheat by-products such as bran, shorts, screenings, and middlings (AAFCO,1988). Wheat millrun is readily available in North America and commonly used as an alternative feedstuff for swine (Nortey et al. 2007a). Due to the removal of the endosperm during the milling, wheat millrun typically contains less starch, a greater proportion of fibre, CP, ether extract, Ca and P than wheat (Slominski et al. 2004; Woyengo et al. 2014).

Fibre is not well hydrolyzed by the digestive enzymes of mammals, including pigs, and also "traps" nutrients in plant cells, thus limiting the availability of nutrients for pigs (Barrera et al. 2004; Woyengo and Nyachoti 2011,). The decrease in nutrient digestibility is due to both the high levels of fibre (quantity) and also the fibre content (quality) of the co-product (Adeola and Cowieson 2011; Zeng et al. 2018). Jarret et al. (2011) reported that 20 % inclusion of wheat distiller dried grains and solubles (**DDGS**) significantly reduced the digestibility of nutrient and energy content compared to control diets based on wheat and soybean meal. The NE content of wheat by-products is low for swine compared to wheat (NRC, 2012). Replacing wheat with 30 % wheat millrun reduced the DE content of the diet by 10 % for 32 kg pigs (Nortey et al. 2008).

Nutrient digestibility influences growth performance in pigs. Average daily gain (**ADG**), gain to feed ratio (**G:F**), and dressing percentage were reduced when corn was replaced with wheat millrun or soybean hulls in corn-soybean based diets fed to growing pigs as a result of reduced nutrient digestibility (Stewart et al. 2013). Nortey et al. (2007a) also reported that reduced nutrient digestibility caused a reduction in ADG, G:F and dressing percentage of grow-finish pigs when 40 % wheat millrun was included in a corn-soybean meal-based diet.

2.4 Carbohydrases in swine diets

Non-starch polysaccharides (NSP), also known as fibre, are found in plant cell walls and trap and reduce the availability of nutrients found within the plant cells (Woyengo and Nyachoti 2011). They include arabinoxylans, β-glucans, cellulose, pectin, mucilage, and gums (Choct 1997; Selvendran and Robertson 2010). These have adverse effects on the nutritional quality and energy content of the diet, however, some benefit may be achieved through the addition of "NSP enzymes" (carbohydrases). Carbohydrases break down the cell wall barrier to release unavailable nutrients for use by the pigs (Bedford 2000). Carbohydrases such as β-glucanase and xylanase can release nutrients from the endosperm and aleurone layers of cereals by degrading the plant cell walls (Paloheimo et al. 2010). Research has also suggested that carbohydrases hydrolyze NSP, enhancing the availability of low molecular weight oligosaccharides (Pedersen et al. 2015), which are then available for fermentation.

Carbohydrases can increase the energy value of the feed, reduce the undigested organic matter in the feces, and makes it possible for the inclusion of low-quality raw materials (Paloheimo et al. 2010). Supplementing diets containing wheat millrun with fibre degrading enzymes such as xylanase (carbohydrase) and phytase improved nutrient and fibre digestibility and growth performance (Nortey et al., 2008; Zijlstra et al., 2010).

Carbohydrases are usually combined to provide additive or synergistic responses (Adeola and Cowieson 2011; Zeng et al. 2018). These carbohydrase combinations usually include two or more of the following; xylanase, mannanase, amylase, galactase, and cellulase (Tsai et al. 2012; Zeng et al. 2018; Li et al. 2019). Piglets fed diets supplemented with an enzyme blend of xylanase and other carbohydrases had improved growth performance relative to piglets fed diets supplemented with only xylanase (Li et al. 2018). Similarly, a combination of carbohydrases improved the growth performance of piglets fed diets containing 20 % wheat bran replacing corn despite decreased apparent ileal digestibility amino acid content with the wheat bran inclusion (Zeng et al. 2018).

The degradation of fibre shifts from predominantly occurring in the hindgut to the small intestine when diets are supplemented with carbohydrases (Zeng et al. 2018). Li et al. (2019) hypothesized that a reduction in colonic Lactobacillus and total volatile fatty acids (**VFA**) observed in the caecum when a blend of carbohydrases was added to diets of piglets, result from improvements in the fibre and starch degradation prior to entry into the large intestine; thus reducing the availability of substrate to the large intestine microbes. The blend of carbohydrases resulted in an improvement in the expression of markers indicative of improved intestinal integrity such as claudin-3 mRNA abundance and a decreased urinary lactulose-mannitol ratio when newly weaned piglets were fed high fibre diets (Li et al. 2018).

2.5 Co-products in swine diets and effects on GHG

Swine producers are increasing the use of grain co-products in their diets. This is mainly because they are relatively cheap and hence will reduce overall feed cost (Woyengo et al. 2014) but also results in a decrease in the dependence on human-grade foods (Mottet et al. 2017). Increased use of co-products in swine diets presents a GHG "cost-sharing" opportunity between the primary users of the grain and livestock producers. For example, in a modelling exercise by Bremer et al. (2010), the GHG credits associated with bioethanol production from corn increased by more than two-fold when the by-products were used to feed livestock.

However, enteric CH_4 from pigs mainly depends on the fibre content in diet (Jørgensen 2007), and high fibre diets have been shown to increase the production of GHG from both the pigs and the manure (Jarret et al. 2012; Philippe and Nicks 2015).

Strategies to reduce the impact of these diets on the environment need to be investigated. Interventions allowing a reduction in GHG emissions while including co-products into swine diets will be very beneficial to the pork production industry.

Greenhouse gas emissions from high fibre diets are influenced by the fermentability and solubility of the dietary fibre (Philippe and Nicks 2015). In a study by Le Goff et al. (2002b), it was reported that more energy from CH_4 was lost when sows were fed diets that contained wheat bran than diets that contained sugar beet pulp (**SBP**). This was attributed to the higher energy digestibility of the SBP diet than the wheat bran diet.

This effect also extends to the manure. For example, Jarret et al. (2012) showed that, when fattening pigs were fed conventional or high fibre diets, the CH_4 gas produced from the slurries of pigs on the high fibre diets was 66 % higher than that of the conventional diet. They explained that this was due to low dry matter (**DM**) digestibility, resulting in the excretion of large quantities of organic matter. Organic matter content (partial or undigested ingredients) of the manure contributes towards N_2O emissions as well (Broucek 2018).

Conversely, high fibre diets do not always result in increased production of GHG. In an experiment conducted by Pepple et al. (2011), CH_4, CO_2 and N_2O emissions were not significantly affected when wean-to-finish pigs (5.5 to 118 kg body weight, BW) were fed diets containing 22 % DDGS. Dietary composition may play an important role in the lack of an increase in GHG production with the inclusion of high fibre diets. The physico-chemical characteristics of the dietary fibre ingredient in the diet influence the fermentability of fibre (Jha and Berrocoso 2016). Soluble fibre is more readily fermentable than insoluble fibre leading to higher production of CH_4 (Philippe and Nicks 2015). Le Goff et al. (2002b) reported higher production of CH_4 in an experiment where sows were fed diets containing maize bran compared to wheat bran. Additionally, animal variation, methodology, instrumentation, and seasonal fluctuations could all affect measurements and calculations of GHG (Pepple et al. 2011; Trabue and Kerr 2014)

Evidence has shown that N excretion is shifted from urea in urine (which is more volatile) to microbial protein in feces (which is a less accessible form) when high fibre feedstuffs are added to the diets of pigs (Galassi et al. 2010). Canh et al. (1997) reported a high fecal N excretion and a reduced urinary N excretion when fattening pigs were fed a "by-product" (pea, tapioca and cane molasses) and SBP based diets compared to a grain-based diet.

2.6 Life cycle analyses and GHG emissions

Several factors contribute to GHG production in the livestock sector, including grain production, use of machinery, transportation of grains and feed, and electricity. Therefore, to achieve sustainability in swine production and reduce GHG emissions in the swine industry, it is important to consider or view this process in a comprehensive way.

A scientific approach to this is by performing an LCA. Thoma et al. (2013) define LCA as "quantitative analyses of complex systems for evaluation of impacts and risks associated with management decisions." This is usually an assessment that incorporates emissions throughout the production system and involves the use of assessment models. Different LCA models have been used to quantify GHG emissions in the livestock sector around the world; GLEAM (Macleod et al. 2013), Lesschen et al. (2011) used MITERRA-Europe, (Lesschen et al. (2011) and ULICEES (Vergé et al. 2016).

The LCA depends heavily on data compiled from statistical and international organizations. Parameters for GLEAM are obtained from the FAO's statistics division (FAOSTAT) and the United Nations Framework Convention on Climate (UNFCCC) (Macleod et al. 2013), CAPRI utilizes data from the European Union Statistics (EUROSTAT) and ULICEES from Canadian agricultural statistics (Vergé et al. 2016; Zhou et al. 2018).

Life cycle assessment studies measuring environmental impact on pork production have been widely studied in Europe and China (Reckmann et al. 2013; Zhou et al. 2018). These studies targeted how different sectors of the pork production cycle, including feed production and manure management, affect the environmental profile (Luo et al. 2015). Also, different management practices such as the use of immuno-castration, removal of antimicrobials, group housing in gestation, and use of different anesthesia methods and their effect on the environment have been studied using LCA (Thoma et al. 2013; Bandekar et al. 2019).

There is little information on comprehensive studies about the environmental impacts of using non-conventional feed ingredients such as wheat millrun in pig production in Canada, especially Western Canada. The Canada-US Global Feed LCA Institute database project was designed to create a database of ingredients on GHG production from different ingredients. However, values for the initial ingredients will be based on the "Environmental Performance for Pig Supply Chains" FAO document (FAO 2016). The advisory group for the FAO document included representatives from major pork-producing regions around the world, such as China, the USA, and Europe, but not Canada.

It is essential to have accurate information and determine the applicability of this database to Western Canadian Feeds; therefore, research is required to fill the gap. This will ensure that the data on Western Canadian feeds are based on accurate data and not extrapolations when feed formulators are considering GHG emissions from ingredients in their least-cost diet formulations.

In summary, the expansion of the livestock industry will depend on the ability to produce protein while reducing the carbon footprint. As swine producers are being encouraged to increase the use of co-products in their diets, it is imperative to mitigate the potential increase in fermentation and GHG output. Swine may contribute to future global protein needs, and while they are not currently major contributors to GHG production, dietary strategies must not reduce this advantage.

CHAPTER 3: PERFORMANCE OF GROWING PIGS FED WHEAT-BASED DIETS CONTAINING WHEAT MILLRUN AND A MULTI-CARBOHYDRASE ENZYME

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3.1 Abstract

The objective of this study was to determine the response of growing-finishing pigs to wheat millrun inclusion in diets, with or without a multi-carbohydrase enzyme. In experiment 1, 48 individually housed barrows (60.2 ± 2.2 kg) were fed one of 6 dietary treatments (0 %, 15 % or 30 % millrun; with or without enzyme); feces and urine samples were collected on d 8-11. In experiment 2, 180 pigs (60.2 ± 2.2 kg) housed in groups of 5 were fed 0 % or 30 % millrun with or without enzyme until they attained market weight (about 128 kg BW). In experiment 1, energy, N and P apparent total-tract digestibility (**ATTD**) and net energy (**NE**) content declined linearly ($P < 0.01$) with increasing wheat millrun. Enzymes reduced ATTD of energy ($P = 0.05$) but did not affect N or P ATTD ($P > 0.10$). In Exp 2, overall ADG and G:F were reduced in pigs fed wheat millrun ($P < 0.05$) while pigs fed enzyme supplemented diets had reduced G:F during the initial 14 d. Backfat depth was reduced, and carcass yield was increased, with wheat millrun ($P < 0.05$). Pigs fed 30 % wheat millrun had reduced growth and nutrient digestibility while the enzyme had minimal effect.

Keywords: Swine, multi-carbohydrase enzyme, wheat millrun, growing-finishing pigs

3.2 Introduction

In typical Western Canadian swine diets, wheat is the major ingredient used to supply dietary energy (Kiarie and Nyachoti 2009). This creates competition with humans for food and land (Mottet et al. 2017). Current efforts to reduce this competition while decreasing the cost of production has resulted in increased usage of co-products from wheat processing, such as wheat millrun and wheat bran (Zijlstra and Beltranena 2013b). Low nutritional values, high fibre content, and varied nutrient profiles are some of the challenges co-products present to nutritionists (Kiarie and Nyachoti 2009; Zijlstra and Beltranena 2013). There are also benefits associated with the use of high fibre co-products, such as serving as substrates for microbial fermentation in the hindgut, which yields metabolites for enhanced animal health, reducing dietary energy density and thus improving satiety and decreasing stereotypical behavior of gestating sows, and reducing post-weaning diarrhea caused by bacterial infections in piglets (Jha and Berrocoso 2015; Philippe et al. 2015).

Wheat millrun (IFN 4-05-206) is a co-product readily available and widely used in North America, especially in Western Canada (Nortey et al. 2007a). It is the portion remaining after the extraction of starch from wheat for the production of wheat flour by dry milling and usually includes products such as bran, shorts, screenings, and middlings (AAFCO 1988; Holden and Zimmerman 1991). Due to the removal of the endosperm during the milling process, wheat millrun typically contains less starch, a higher proportion of NSP, CP, Ca and P, than wheat (Slominski et al. 2004; Woyengo and Nyachoti 2011).

The NSP content of cereals include cellulose, arabinoxylans and β-glucans (Choct 1997; Selvendran and Robertson 2010). These NSP are not well hydrolyzed by the digestive enzymes of monogastrics, including pigs, limiting the availability of nutrients (Barrera et al. 2004; Woyengo and Nyachoti 2011; Agyekum and Nyachoti, 2017). However, some improvement in nutritional quality may be achieved through the addition of enzymes (carbohydrases) which break down the cell wall barrier to release unavailable or bound nutrients (Bedford 2000).

Carbohydrases may increase the energy value of an ingredient, reduce the undigested organic matter in the feces and make the inclusion of low-quality raw materials into swine diets possible (Paloheimo et al. 2010). Additive or synergistic responses when two or more carbohydrases are combined have been observed (Adeola and Cowieson 2011; Zeng et al. 2018). For example, Li et al. (2018) reported improved BW and ADG when piglets were fed diets supplemented with an enzyme blend of xylanase and other carbohydrases compared to piglets fed diets supplemented with only the xylanase.

The objective of the present study was to determine the response of growing pigs to wheat millrun inclusion in diets, with or without a multi-carbohydrase enzyme.

We hypothesized that the positive effects of a multi-carbohydrase enzyme on nutrient digestibility and growth performance would be greater in a high wheat millrun diet than in a wheat diet.

3.3 Materials and methods

Animal protocols were reviewed and approved by the Animal Research Ethics Board of the University of Saskatchewan and followed principles established by the Canadian Council on Animal Care Guidelines for Humane Animal Use [Canadian Council on Animal Care (**CCAC**) 2009].

3.3.1 Experimental diets

The experimental diets (Table 3.1 and 3.2) were formulated to be typical Western Canadian diets (wheat and barley-based) for growing pigs using analyzed total amino acid (AA) content and published SID and NE values [National Academy of Sciences – National Research Council (**NRC**) 2012]. Diets were formulated to meet or exceed the requirements for growing pigs in each phase of growth (NRC 2012). Wheat, wheat millrun and complete diets were sampled and subjected to analysis using wet chemistry (Central Testing Laboratory Ltd, Winnipeg, MB). The wheat contained 15.2 % crude protein (**CP**), 3.8 % acid detergent fibre (**ADF**) and 12.1 % neutral detergent fibre (**NDF**); the wheat millrun contained 17.7 % CP, 15.6 % ADF and 38.9 % NDF, all on DM basis. The wheat and wheat millrun used for the study were obtained from a local company (P & H Milling Group, Saskatoon, SK). In the performance study (Exp 2), two-phase feeding was employed (60 to 90 and 90 to 128 kg BW) to meet the nutritional requirements of pigs in those specific weight ranges.

The multi-carbohydrase enzyme, Superzyme-W (glucanase, 300 GLU units g^{-1}; xylanase, 1,000 XLU units g^{-1}; cellulase, 1,900 CMC units g^{-1}; amylase, 4,200 FAA units g^{-1}; invertase, 150 INV units g^{-1}; Canadian Bio-systems Inc. Calgary, AB) was included at 1 kg $tonne^{-1}$ of finished feed (manufacturer recommendation). Celite (Celite 545, Celite Corporation, Lompoc CA, USA), a source of acid-insoluble ash (**AIA**), was added to the diet as an indigestible marker for the estimation of nutrient digestibility. Diets were mixed at the Canadian Feed Research Centre, North Battleford, SK. Pigs were fed animal protein and antibiotic-free diets, and pigs requiring treatment with an antibiotic were removed from the experiment.

Table 3. 1 Ingredient and nutrient composition (as-fed basis) of the 0 %, 15 % and 30 % wheat millrun diets for the digestibility study and Phase 1 (60-90 kg)[a,b]

Item	**0 %**	**15 %**	**30 %**
Ingredients (%)			
Wheat millrun (IFN 4-05-206)	**-**	**15.00**	**30.00**
Wheat (IFN 4-05-211)	61.85	47.78	33.70
Barley (IFN 4-00-549)	24.80	24.96	25.11
Canola oil	1.50	1.60	1.70
Soybean meal (IFN 5-04-604)	9.00	8.00	7.00
Limestone	1.20	1.20	1.20
Monocalcium phosphate	0.30	0.15	-
Salt	0.30	0.30	0.30
L-Lysine HCl (78 %)	0.34	0.31	0.28
DL-Methionine (99 %)	0.03	0.03	0.03
L-Threonine (98.5 %)	0.08	0.08	0.08
Vitamin and mineral premix[c]	0.20	0.20	0.20
Celite	0.40	0.40	0.40
Calculated nutrient composition			
Dry matter, %	88.08	87.55	87.00
Crude protein, %	16.45	16.45	16.49
Calcium, %	0.49	0.53	0.52
Phosphorus, %	0.45	0.52	0.54
SID Lysine, %	0.78	0.76	0.75
SID Methionine, %	0.24	0.24	0.24
SID Threonine, %	0.50	0.50	0.50
SID Tryptophan, %	0.17	0.17	0.17
Net energy, Mcal kg^{-1}	2.43	2.39	2.35
LYS/NE g Mcal^{-1}	3.19	3.20	3.20
Analyzed nutrient composition			
Dry matter, %	92.28	92.15	92.00
Crude protein, %	17.11	18.00	18.64
Gross energy, Mcal kg^{-1}	3.84	3.88	3.86
Ether extract, %	2.60	3.97	4.04
Calcium, %	0.63	0.67	0.72
Phosphorus, %	0.46	0.50	0.56
Soluble dietary fibre, %	3.18	2.78	4.28
Insoluble dietary fibre, %	12.94	14.80	17.51
Total dietary fibre, %	16.12	17.57	21.80

Note: ***HCl, hydrochloric acid; SID, standard ileal digestibility.***

[a] Same diet (without celite) was used as Phase 1 diets in the performance study.

[b] Each diet was divided into two portions and supplemented with a multi-carbohydrase enzyme (0.1 %) to create 3 additional diets. The enzyme contained 300 units of glucanase, 1000 units of xylanase, 1900 units of cellulase, 4200 units of amylase and 150 units of invertase per g of enzyme (Superzyme W; Canadian Bio-Systems Inc., Calgary, Alberta, Canada).

[c] Supplied vitamins and minerals per kg of complete diet : vitamin A, 8,000 IU; vitamin D, 1,500 IU; vitamin E, 30 IU; vitamin B12, 0.02 mg; menadione, 2 mg; thiamine, 1 mg; biotin, 0.1 mg; niacin, 20 mg; riboflavin, 12 mg; pantothenate, 12 mg; folic acid, 0.50 mg; and pyridoxine, 2mg. iron, 100 mg; zinc, 100 mg; manganese, 40 mg; copper, 15 mg; selenium, 0.30 mg; and iodine, 1 mg.

Table 3. 2 Ingredient and nutrient composition (as-fed basis) of the 0 % and 30 % wheat millrun diets for Phase 2 (90-128 kg)[b]

Item	**0 %**	**30 %**
Ingredients (%)		
Wheat Millrun (IFN 4-05-206)	**-**	**30.00**
Wheat (IFN 4-05-211)	45.72	18.40
Barley (IFN 4-00-549)	43.00	42.32
Canola oil	1.20	1.40
Soybean meal (IFN 5-04-604)	8.00	6.00
Limestone	1.00	1.20
Monocalcium phosphate	0.30	-
Salt	0.30	0.30
L-Lysine. HCl (78 %)	0.20	0.15
DL-Methionine (99 %)	0.03	0.03
L-Threonine (98.5 %)	0.05	0.00
Vitamin and mineral premix [c]	0.20	0.20
Calculated nutrient composition		
Dry matter, %	88.75	87.00
Crude protein, %	15.34	16.49
Calcium, %	0.49	0.52
Phosphorus, %	0.45	0.54
SID Lysine, %	0.64	0.62
SID Methionine, %	0.23	0.24
SID Threonine, %	0.44	0.40
SID Tryptophan, %	0.16	0.16
Net energy, Mcal kg^{-1}	2.38	2.31
LYS/NE g Mcal^{-1}	2.70	2.70
Analyzed nutrient composition		
Gross energy, Mcal kg^{-1}	3.73	3.81
Dry matter, %	88.58	89.69
Crude protein, %	15.19	15.80
Ether extract, %	3.12	4.32
Calcium, %	0.53	0.57
Phosphorus, %	0.47	0.60
Acid detergent fibre, %	3.88	6.80
Neutral detergent fibre, %	13.35	19.72

Note: ***HCl, hydrochloric acid; SID, standard ileal digestibility.***

[b] Each diet was divided into two portions and supplemented with a multi-carbohydrase enzyme (0.1 %) to create 2 additional diets. The enzyme contained 300 units of glucanase, 1000 units of xylanase, 1900 units of cellulase, 4200 units of amylase and 150 units of invertase per g of enzyme (Superzyme W; Canadian Bio-Systems Inc., Calgary, Alberta, Canada).

[c] Supplied vitamins and minerals per kg of complete diet : vitamin A, 8,000 IU; vitamin D, 1,500 IU; vitamin E, 30 IU; vitamin B12, 0.02 mg; menadione, 2 mg; thiamine, 1 mg; biotin, 0.1 mg; niacin, 20 mg; riboflavin, 12 mg; pantothenate, 12 mg; folic acid, 0.50 mg; and pyridoxine, 2mg. iron, 100 mg; zinc, 100 mg; manganese, 40 mg; copper, 15 mg; selenium, 0.30 mg; and iodine, 1 mg.

3.3.2 Experiment 1: Nutrient digestibility

3.3.2.1 Animals, experimental design and housing

Barrows (n = 48; Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, MB, Canada) with an initial body weight of 60 ± 2.2 kg were randomly assigned to 1 of 6 dietary treatments in four blocks (8 pigs per treatment). Dietary treatments were arranged as a 3 × 2 factorial in a randomized complete block design (**RCBD**) with the following main factors; wheat millrun; (0 %, 15 % or 30 %), and enzyme (0 or 1 mg kg^{-1} multi-carbohydrase enzyme).

Pigs were housed individually in raised crates (1.5 x 1.5 m) in an environmentally controlled room with temperature set at 15 °C, humidity at ~ 40 % and a 12h-12h light-dark photoperiod. Each crate had polyvinyl chloride walls (0.9 m high) with plexiglass windows between crates (0.3 x 0.3 m), plastic-coated metal floors, a single space dry feeder and a bowl drinker. Each crate had a sloped urine collection tray (1.5 x 1.5 m) below the penning floor.

Pigs were fed 3 times their maintenance energy requirements for ME (197 kcal ME/kg $BW^{0.60}$; NRC 2012). The feed was divided into 2 equal meals and presented at 0830 and 1500. Pigs were allowed free access to water throughout the experiment. Feed intake was recorded throughout the adaptation and collection periods, and individual body weight was recorded before the adaptation period and the start and end of the collection period.

3.3.2.2 Sample collection

The experiment consisted of a 7 d adaptation to the experimental diets, followed by a 4 d collection period for urine and fecal samples. Fresh fecal grab samples collected twice per day at 0900 and 1530 were immediately frozen (-20 °C) and pooled for the duration of the collection period. Urine was collected into a storage vessel containing an adequate amount of 12 N HCl to maintain urine pH below 3 to limit microbial growth and prevent the volatilization of urinary N (Nortey et al. 2007a). Funnels leading into the collection vessels contained glass wool to remove any solid particles and limit contamination. Pens and urine collection trays were cleaned daily to limit fecal contamination of the urine. Collected urine was weighed daily, and a 10 % (by weight) subsample was retained. These subsamples were pooled at the end of the collection period, and an aliquot was taken and stored at -20 °C until analysis.

3.3.2.3 Chemical analyses

Fecal samples were dried in a forced-air draft oven at 55 °C for 72 h (Jacobs et al. 2011). The feed and oven-dried feces were then finely ground in a centrifugal mill (Model ZM 100, RETSCH GmBH & Co. Rheinsche Straβe, Germany) through a 1 mm screen. The dry matter (DM) content of the feed and feces was measured in duplicate by drying at 135 °C in an airflow-type oven for 2 h [method 930.15; Association of analytical chemists (**AOAC**) 2007].

The AIA content was analyzed in both feed and feces in duplicate according to the methods of van Keulen and Young (1977). The gross energy (**GE**) content of the feed and feces was analyzed by using an adiabatic bomb calorimeter (6400 automatic Isoperibol system, Parr Instruments Company, IL, USA) with benzoic acid as the standard. The NDF and ADF content of the feed and feces were determined according to AOAC 991.43 (AOAC, 2007) using an ANKOM 200 fibre analyzer (ANKOM Technology, Macedon NY). Crude protein (N × 6.25) content of the feed, feces, and urine samples was analyzed by combustion with an automatic analyzer (LECO FP 528; MI; Method 990.3; AOAC 2007). Phosphorus in feed and feces was analyzed by a colorimetric method using a spectrophotometer (method 965.17; AOAC, 2007).

3.3.2.4 Calculations

The results from the chemical analyses were used to calculate the ATTD of GE, N, P, and DM based on the indicator method using the following equation (Adeola 2000).

$$\text{ATTD, \%} = [1 - (N_F \times M_D) / (N_D \times M_F)] \times 100 \qquad [3.1]$$

Where N_F is the nutrient concentration in feces; N_D is the nutrient concentration in the diet; M_D is the marker concentration in the diet; and M_F is the marker concentration in feces

NE was calculated as (NRC 2012);

$$\text{NE} = (0.700 \times DE) + (1.61 \times EE) + (0.48 \times Starch) - (0.91 \times CP) - (0.87 \times ADF) \quad [3.2]$$

Where *DE* is digestible energy, *EE* is ether extract, *CP* is crude protein, *ADF* is acid detergent fibre.

3.3.3 Experiment 2: Growth performance

3.3.3.1 Animals, experimental design and housing

A total of 180 pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) with an initial BW of 60.2 ± 2.2 kg were used in a growth performance experiment at the Prairie Swine Centre Inc. (Saskatoon, SK. Canada). Pigs were housed in pens (2.4 × 1.7 m) in groups of 5 in an environmentally controlled room. Pigs were selected such that BW and standard deviations were similar among pens, and pens were randomly assigned to 1 of the 4 experimental diets. This ensured that there were 3 pens per treatment in each of the 3 blocks (*n* = 9 pens per treatment). Within each block, an equal number of barrows and gilts of equal BW were used and equalized between treatments. After pigs were selected and placed in appropriate pens, pigs were fed a commercial diet for at least 5 d prior to the start of the experiment.

The flooring of the pens was fully slatted concrete over a shallow manure pit, polyvinyl chloride (PVC) gate in front and concrete partitions between pens. Each pen had a single-space dry feeder located at the front of the pen and a nipple drinker at the back. A proportional environment controller (Phason, Winnipeg, MB) controlling the ventilation and temperature was installed in each room with room temperature set at 15 °C and relative humidity at ~ 40 %. Pigs were kept on a 10 h light (0700 to 1700), 14 h dark cycle set on an automatic timer. Rooms were washed, sanitized, and manure pits emptied prior to each block. Diets were provided ad libitum as a pelleted feed, and pigs had free access to water. Pens were checked daily to monitor animal health and feed availability.

3.3.3.2 Sample collection

Pigs were weighed individually on experimental d 1 and every 14 d thereafter till the first group of pigs reached 128 kg BW (d 14, 28, 42 and 56). Feed disappearance was determined on each weigh day by subtracting the amount of feed left in the feeder from the amount of feed offered. Feed disappearance divided by the number of days between weigh days (14 d) provides the average daily feed intake (**ADFI**). The gain to feed ratio (G: F) was calculated by dividing the ADG by the ADFI.

3.3.3.3 *Market weight and carcass characteristics*

Pigs were marketed weekly and were sent to a commercial abattoir when they reached a minimum BW of 128 kg. Before loading, pigs were weighed and tattooed by pen. Pigs were not fasted prior to shipping. The journey from the barn to the abattoir was 8 h. Pigs were slaughtered at the abattoir under standard commercial procedures. Slaughter weight, fat, loin depth, and carcass yield results were collected on each pig at or after slaughter.

3.3.3.4 *Statistical Analyses*

Statistical analyses for both the digestibility and performance study was carried out as an analysis of variance (**ANOVA**) for a RCBD in a factorial arrangement using the mixed model procedure of the Statistical Analysis System (**SAS**) (version 9.4; SAS Institute, Inc. Cary, NC). The univariate procedure of SAS was used to verify the normality of the data. All data analyzed in this study were normally distributed (Shapiro-Wilk test; $P > 0.05$). The model for the nutrient digestibility data included wheat millrun inclusion and multi-carbohydrase enzyme and their interaction as fixed effects and block as a random effect. Pig was the experimental unit. The digestibility data were further analyzed for linear effects of wheat millrun addition using the GLM procedure of SAS. The model for growth performance and carcass characteristics data included wheat millrun, multi-carbohydrase enzyme and their interaction as fixed effects and block as random effect and pen as the experimental unit.

The statistical model used for the digestibility and performance study was:

$Y_{ijk} = \mu + \rho_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$: where Y was the measured parameter; μ, the overall mean, ρ_i, the random effect of the i^{th} block; α_j, β_k, the fixed effect of the j^{th} and k^{th} treatment; $(\alpha\beta)_{jk}$, the interaction between the j^{th} and k^{th} treatment; ε_{ijk} is the error term associated to the j^{th} and k^{th} treatment and i^{th} block. Significant differences between means were declared at $P \leq 0.05$, and tendency towards significance was considered at $0.05 < P \leq 0.10$. The tukey-kramer mean separation test was used to separate means that were declared significant.

3.4 Results

3.4.1 Apparent total tract digestibility

Data for the ATTD of DM, N, energy, NE, DE, and P are presented in Table 3.3. There were no significant interactions between wheat millrun inclusion and enzyme supplementation for any of the digestibility parameters. The ATTD of DM, energy, N, and P were linearly reduced ($P < 0.01$) with increasing wheat millrun inclusion in the diets. ($P < 0.01$) Enzyme supplementation reduced ($P < 0.05$) the ATTD of DM and energy ($P < 0.05$). Further, increasing the inclusion of wheat millrun in the diets linearly reduced the DE and NE contents of the diets ($P < 0.05$) while enzyme supplementation tended to reduce dietary DE and NE contents ($P < 0.10$; Table 3.3). Enzyme supplementation, however, had no effect on the ATTD of N or P ($P > 0.10$; Table 3.3).

Table 3. 3 Effect of wheat millrun inclusion and multi-carbohydrase supplementation on apparent total tract digestibility of energy and minerals of diets fed to grower pigs (60 kg) in Exp 1[a]

	Millrun, %				**Enzyme**			***P*-value**	
Item [b]	0	15	30	SEM	No	Yes	**SEM**	Enzyme	Linear (Millrun)
Total tract digestibility									
DM, %	86.5	84.7	83.9	0.28	87.3	82.8	0.22	<0.01	<0.01
Energy,%	87.3	84.9	81.5	0.01	85.0	84.2	0.01	0.05	<0.01
DE, Mcal kg^{-1}	3.50	3.47	3.34	0.01	3.45	3.42	0.01	0.08	<0.01
NE, Mcal kg^{-1}	2.46	2.44	2.34	0.01	2.43	2.40	0.01	0.06	<0.01
N, %	86.4	85.3	83.5	0.53	85.1	85.0	0.44	0.89	<0.01
P, %	52.6	43.5	41.1	0.60	46.2	45.2	0.52	0.33	0.02

[a] Values are treatment means of 8 individually housed pigs and are reported as least square means.

[b] No significant interaction between millrun and enzyme (*P* > 0.10).

SEM; standard error of means.

3.4.2 Nitrogen intake, excretion and retention

There was a significant interaction between wheat millrun inclusion and multi-carbohydrase supplementation on N intake ($P < 0.01$; Table 3.4). Nitrogen intake increased with enzyme supplementation in both the 0 % and 30 % wheat millrun diets. The 30 % wheat millrun diets increased ($P < 0.01$) fecal N but failed to show any significant difference in urine N, total N excretion and N retention. Multi-carbohydrase supplementation showed no significant difference in urine, fecal and total N excretion. However, there was an increase in N retention with multi-carbohydrase supplementation.

Table 3. 4 Effect of wheat millrun inclusion and multi-carbohydrase supplementation on N intake, excretion and retention of diets fed to grower pigs (60 kg) in Exp 1[a]

	Millrun, %				**Enzyme**			***P*-value**		
Item	0	15	30	**SEM**	No	Yes	**SEM**	Enzyme	Millrun	Enzyme × Millrun
N, g d^{-1}										
Intake	59.41	60.71	63.96	0.40	60.12	62.60	0.33	<0.01	<0.01	<0.01
Urine	13.21	13.06	12.78	1.79	13.59	12.44	1.66	0.40	0.96	0.65
Fecal	8.10	8.91	10.54	0.30	8.97	9.39	0.24	0.24	<0.01	0.79
Excretion	21.31	21.97	23.32	1.79	22.57	21.83	1.65	0.59	0.48	0.74
Retention	38.10	38.74	40.64	1.71	37.55	40.77	1.56	0.03	0.31	0.14

[a] Values are treatment means of 8 individually housed pigs and are reported as least square means.

SEM; standard error of means.

3.4.3 Growth performance

Growth performance data are presented in Table 3.5. One pig was removed from the experiment due to ill health. There were no significant interactions between wheat millrun inclusion and enzyme supplementation for any of the parameters determined. Including wheat millrun in the diets tended to reduce the d 42 and the d 56 BW ($P = 0.10$), a result of reduced ADG on d 29 to 42 ($P = 0.03$) and overall ($P = 0.05$). Similarly, from d 29 to 42, G:F was lower in the wheat millrun diets compared to the no wheat millrun diets (0.35 vs 0.38; $P < 0.01$) and overall (d 0 to 56) G:F was lower (0.37 vs 0.39; $P < 0.05$) when pigs were fed diets with wheat millrun. Wheat millrun inclusion had no significant effects on ADFI. Enzyme supplementation resulted in a lower G:F during the initial 14 d of the experimental period but had no effect on the overall (d 0 to 56) G:F, BW, ADG or ADFI.

Table 3. 5 Effect of dietary wheat millrun and multi-carbohydrase supplementation on performance of growing pigs in Exp 2 (60 kg) [a]

Item [b]	Millrun, %		Enzyme		Pooled SEM	*P*-value	
	0	30	No	Yes		Millrun	Enzyme
BW, kg							
Initial	60.1	60.3	59.8	60.6	1.27	0.63	**0.10**
d 14	76.0	75.8	76.1	75.6	1.12	0.92	0.62
d 28	92.1	91.6	91.9	91.8	0.71	0.50	0.88
d 42	107.3	105.8	106.6	106.4	1.03	**0.10**	0.84
d 56	120.6	118.9	119.6	120.0	0.72	**0.10**	0.69
ADG, kg d^{-1}							
d 1 to 14	1.13	1.10	1.16	1.08	0.07	0.68	0.14
d 15 to 28	1.16	1.13	1.13	1.15	0.04	0.28	0.42
d 29 to 42	1.09	1.02	1.05	1.05	0.04	**0.03**	0.90
d 43 to 56	0.94	0.95	0.91	0.98	0.04	0.91	0.11
d 0 to 56	1.10	1.07	1.09	1.08	0.02	**<0.05**	0.65
ADFI, kg d^{-1}							
d 1 to 14	2.47	2.47	2.47	2.47	0.12	0.98	0.98
d 15 to 28	2.84	2.88	2.87	2.86	0.06	0.60	0.91
d 29 to 42	2.88	2.94	2.87	2.95	0.07	0.58	0.42
d 43 to 56	3.19	3.31	3.20	3.30	0.26	0.24	0.32
d 0 to 56	2.85	2.90	2.85	2.90	0.05	0.41	0.51
G:F							
d 1 to 14	0.45	0.45	0.47	0.43	0.02	0.74	**<0.05**
d 15 to 28	0.41	0.39	0.40	0.40	0.02	0.19	0.68
d 29 to 42	0.38	0.35	0.37	0.36	0.01	**<0.01**	0.38
d 43 to 56	0.32	0.30	0.31	0.30	0.01	0.34	0.91
d 0 to 56	0.39	0.37	0.38	0.37	0.01	**0.01**	0.20

[a] Data are presented as least-square means of 9 replicate pens with 5 pigs per pen.

[b] No significant interaction between millrun and enzyme ($P > 0.10$)

SEM; standard error of means.

3.4.4 Carcass traits

Wheat millrun inclusion or multi-carbohydrase supplementation did not affect days to market, market weight, slaughter weight, loin depth or dressing percentage (Table 3.6). However, backfat depth was reduced by 7 % from 16.71 to 15.54 mm ($P < 0.01$), while carcass yield increased from 61.8 to 62.4 % with the inclusion of 30 % wheat millrun ($P < 0.05$). Backfat depth tended to be increased when the diets were supplemented with the multi-carbohydrase enzyme ($P = 0.07$), but the enzyme had no effect ($P > 0.10$) on days to market or other measured carcass parameters.

Table 3. 6 Effect of wheat millrun inclusion and multi-carbohydrase supplementation on carcass traits in Exp 2

	Millrun, %		**Enzyme**		**Pooled**	**P-value**	
Item [b]	0	30	No	Yes	**SEM**	Millrun	Enzyme
Days to market	68	69	69	68	2.57	0.46	0.31
Final BW, kg	132.25	131.81	132.05	132.01	0.41	0.42	0.94
Slaughter weight, kg	105.84	105.10	105.38	105.56	0.54	0.19	0.75
Backfat depth, mm	16.71	15.54	15.74	16.50	0.75	**<0.01**	0.07
Loin depth, mm	66.82	68.41	67.97	67.27	1.05	0.28	0.63
Carcass yield, %	61.80	62.42	62.29	61.94	0.36	**0.01**	0.13
Dressing, %	80.03	79.74	79.80	79.96	0.28	0.35	0.60

[b] No significant interaction between millrun and enzyme ($P > 0.10$).

SEM; standard error of means.

3.5 Discussion

Similar to other co-products, the inclusion of wheat millrun in swine diets is increasing, primarily due to economic factors and availability (Nortey et al. 2007a; Woyengo et al. 2014). There is also interest in the use of co-products to reduce carbon footprint and improve the sustainability of animal agriculture (Philippe et al. 2015). However, the high fibre content of the milling co-products, including wheat millrun, limits energy and nutrient availability in non-ruminants. Enzyme inclusion to improve digestion and utilization of nutrients is therefore suggested. Due to synergistic or additive actions (Adeola and Cowieson 2011), multi-carbohydrase enzymes are believed to improve digestion and performance more than individual carbohydrases.

In the current study, we hypothesized that the effect of a multi-carbohydrase enzyme on improving nutrient digestibility and growth performance for growing pigs would be greater in a high wheat millrun diet than in a wheat diet.

Diets for the experiments were formulated to meet the dietary requirements of the pigs at a cheaper cost, and as a result, the energy in the diets was reduced as the wheat millrun inclusion in the diet increased (from 2.46 to 2.34 Mcal NE kg^{-1}). Pigs were expected to compensate for the reduction in energy with increased consumption (Beaulieu et al. 2009; Quiniou and Noblet 2012). Moreover, this ensured that any improvement in ADG or G:F would be from the enzyme addition and not from the addition of fat (Jacela et al. 2010) or other energy-dense ingredients.

Enzymes are substrate specific, and combinations of enzymes improve efficacy in mixed diets (Hu et al. 2013; Kiarie et al. 2013). The NSP in wheat millrun are primarily insoluble xylose and arabinose (Garcia et al. 2015). These NSPs comprise the cell wall, acting as a physical barrier that trap nutrients, preventing utilization (Grieshop et al. 2000). The function of carbohydrases, therefore, is to break down or destroy the barrier and release these nutrients (Jacela et al. 2010). The multi-carbohydrase enzyme used in the current study contained glucanase, xylanase, cellulase, amylase and invertase.

The quantity of fibre and type of dietary fibre in the diets affects enzyme efficacy. Jaworski and Stein (2017) reported a 20 % greater digestibility of soluble dietary fibre (**SDF**) compared to insoluble dietary fibre (**IDF**) when pigs were fed corn-soybean meal basal diets containing either DDGS, wheat middlings or soybean hulls. Soluble dietary fibre is more fermentable than IDF in the hindgut (Zeng et al. 2018).

Digestibility improvements from multi-carbohydrase supplementation studies in swine diets have been very inconsistent. A study by Ao et al. (2010) in growing pigs (29.7±0.69 kg) fed corn-soybean meal-based diets showed an increase in DM and N digestibility but not energy, whereas a study by Olukosi et al. (2007) showed no increase in either N or energy digestibility with enzyme supplementation when 24 kg pigs were fed wheat-soybean based diets. Nortey et al. (2007b) reported an improvement in energy digestibility and DE content in wheat by-products when supplemented with 4000 xylanase units per kg of feed. Even though a multi-carbohydrase enzyme was used in our study, it only supplied 1000 xylanase units, which could have caused the lack of effect in DE digestibility with enzyme supplementation in our study. Also, the lack of an effect on DE digestibility with enzyme supplementation could be a result of a higher IDF in the wheat millrun diets. The IDF in the 30 % wheat millrun diets were 35 % more than that of the 0 % wheat millrun diets in our study. In the current study, N and P digestibility were not improved with enzyme supplementation and energy and DM digestibility were reduced. This was contrary to our expectations and to the results of Emiola et al. (2009) and Omogbenigun et al. (2004), who reported improved DM and energy digestibility from multi-carbohydrase supplementation. However, the reduction in DM digestibility and a tendency to reduce energy digestibility observed in our study may explain the reduced G:F in the first 14 d of the performance study with enzyme supplementation. Lu et al. (2019) reported reduced growth when xylanase was fed to weanling pigs. The authors suggested that the negative impact on nutrient digestibility and growth was due to the digestive enzyme secretion or activity being affected by the supplementation. This is supported by an earlier study by Fan et al. (2009), who observed a significant reduction in amylase and lipase activities in the digesta of piglets when xylanase was supplemented in the diet.

Consistent with other studies (Galassi et al. 2010), fecal N output increased with the increase in the wheat millrun diets (due to the high fibre content). However, the lack of difference in the urine N output did not support the claim that N excretion is shifted from urine to feces when pigs are fed high fibre diets. The increase in fecal N output could be attributed to the excess N intake by pigs fed the wheat millrun diets.

Inconsistent performance results have also been observed with the use of multi-carbohydrase enzyme supplementation in swine diets. The fibre-degrading capacity and microbial population in the small intestine of the pig increases with age (Lindemann et al. 1986); therefore, weaned piglets may show a greater response to multi-enzyme supplementation than growing or finishing pigs. Indeed, Omogbenigun et al. (2004) reported improvements in ADG and G:F in newly weaned piglets (18 ± 1 d of age) when wheat-based diets were supplemented with xylanase, glucanase, amylase, protease, invertase and phytase enzymes. This contrasts with earlier work by Officer (1995), who did not see any improvement in growth, feed intake or feed efficiency when newly weaned piglets were fed wheat-based diets supplemented with β-glucanase, pentosanase, and hemicellulase. However, since the majority of feed is used for older pigs, we focused on the potential benefits of carbohydrase enzymes when included in the diet of grow-finish pigs, 60 to 128 kg BW.

The reduction in ADG and G:F in pigs fed the high wheat millrun diets in the current work is similar to what was shown in a study by Nortey et al. (2007b). Although their work (Nortey et al. 2007b) utilized diets formulated to be isonitrogenous and isoenergetic, the bulkiness of the wheat millrun leading to gut fill could have been a limiting factor in maintaining energy intake. Stewart et al. (2013) also reported a reduction in ADG, G:F and dressing percentage when corn was replaced with wheat millrun or soybean hulls in a corn-soybean based diet fed to growing pigs. Conceivably, comparable to the present work, these results are due to the higher NSP content in the wheat millrun diets compared to the diets containing no wheat millrun. The efficiency of the use of NE, determined by dividing the ADG by the NE (caloric) intake, estimates the efficiency of energy use by the pigs on these diets. The efficiency of the use of NE (0.16) was similar regardless of wheat millrun inclusion and the higher fibre content of these diets.

The increase in the total empty weight of the gastrointestinal tract and volume of undigested feed associated with the consumption of high fibre diets has been shown to decrease the dressing percentage (Kerr and Shurson 2013). However, this was not observed in our study. We also did not see treatment effects on loin depth. According to Smit et al. (2018), pigs fed different energy level diets, but balanced for SID Lys to NE ratio should have similar loin depth. Conversely, the lower NE in the wheat millrun diets might have been responsible for the 7 % reduction observed in backfat depth as the backfat depth is mainly influenced by the energy intake in the finishing phase. This could explain the tendency for the increase in backfat depth in the diets supplemented with enzymes, proving that the enzyme provided surplus energy from the diet, which was deposited in the backfat. Carcass yield is mainly influenced by backfat depth, and pigs with excessive backfat depth are penalized, and thus it is not surprising that there was an increase in carcass yield with the wheat millrun diets.

3.6 Summary and Conclusion

The results of the present study failed to demonstrate an interaction between the multi-carbohydrase supplementation and wheat millrun inclusion. The effect of the multi-carbohydrase enzyme on nutrient digestibility and growth performance was not markedly higher in the wheat millrun diets than in the wheat diets. Our study suggests multi-carbohydrase supplementation reduced energy digestibility but had no effect on the growth performance of growing-finishing pigs, whereas up to 30 % dietary wheat millrun inclusion reduced nutrient digestibility and growth performance. Days to market, however, was not affected by 30 % wheat millrun inclusion in diets. There was also a decrease in the backfat depth and an increase in carcass yield with wheat millrun inclusion. There were, however, no interactions observed between wheat millrun inclusion and multi-carbohydrase supplementation on nutrient digestibility, growth performance and carcass quality characteristics. Further studies should investigate if reducing the dietary energy further will improve the efficacy of the multi-carbohydrase enzyme in the high wheat millrun diets.

3.7 Acknowledgments

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CHAPTER 4. GREENHOUSE GAS EMISSIONS FROM PIGS FED WHEAT MILLRUN SUPPLEMENTED WITH A MULTI CARBOHYDRASE ENZYME

A modified version of this material will be submitted to the Journal of Animal Science for publication.

Citation

Kpogo, L.A., J. Jose, J. C. Panisson, B. Predicala, J. Agnew and A.D. Beaulieu. 20xx. Greenhouse gas emissions from growing pigs fed wheat-based diets containing wheat millrun and a multi-carbohydrase enzyme. *J. Anim. Sci.*

4.1 Abstract

Wheat millrun, a co-product of wheat milling, is commonly used as an ingredient in swine diets in Western Canada. The high fibre content of wheat millrun may cause an increase in fermentation in the large intestine of pigs and lead to an increase in GHG, especially CH_4. The high fibre content of wheat millrun suggests that carbohydrase enzymes may improve digestibility. It is important to understand if the high fibre components in co-products in swine diets negatively affect the environment in terms of manure output and GHG emissions. The objective of this study was to evaluate the impact of feeding growing pigs high wheat millrun diets on GHG (CO_2, N_2O, CH_4) output. Four treatments, arranged as a 2 × 2 factorial, included millrun (0 % or 30 %, replacing wheat) and enzyme (0 or 1 mg kg^{-1} of a multi-carbohydrase enzyme) as main effects. The enzyme contained amylase, cellulase, glucanase, xylanase, and invertase activities. For each of 16 reps, 6 pigs (60.2 ± 2.2 kg) were housed in environmental chambers for 14 d. Air samples were then collected and analyzed for CO_2, N_2O, and CH_4. Next, the slurry was collected and stored in 65 L barrels. Gas samples were collected in month 1 and 3 of storage. GHG emission rates from the pigs, as well as GHG fluxes from manure, were calculated. Total manure output from pigs fed 30 % wheat millrun diets was > 30 % ($P > 0.05$) more than pigs on the 0 % wheat millrun diets. Results indicated that feeding diets with 30 % millrun did not significantly affect GHG production (CH_4, 4.7, 4.9; N_2O, 0.45, 0.42; CO_2, 1610, 1711 μg m^{-2} s; without or with millrun inclusion, respectively; $P > 0.15$). Moreover, enzyme supplementation had no effect on GHG production

(CH_4, 4.5, 5.1; N_2O, 0.45, 0.42; CO_2, 1808, 1513; mg s^{-1} without or with enzyme supplementation, respectively; $P > 0.15$). Mean CO_2 flux (μg m^{-2} s) from the barrels increased from month 1 to month 3, while mean CH_4 flux (μg m^{-2} s) decreased during this time, N_2O flux was negligible from the barrels. In conclusion, the inclusion of 30 % wheat millrun in diets of growing pigs did not increase GHG emissions.

Keywords: Swine, environmental chambers, wheat millrun, multi-carbohydrase enzyme, growing pigs

4.2 Introduction

Increased demand for animal protein will increase livestock GHG emissions, exacerbating environmental problems, including climate change, unless mitigation steps are implemented (Oonincx and de Boer 2012; Jacobsen et al. 2014). Although pork production accounts for just 9 % of emissions from the livestock sector, the predicted growth in global pork consumption makes mitigation efforts imperative (Dyer et al. 2010; Gerber et al. 2013). The most prevalent gases emitted from the pig and the manure in swine facilities, CH_4, CO_2, and N_2O (Philippe and Nicks 2015), are of concern because of their long residence time and thus, GWP (IPCC 2006). The GWP for CO_2, CH_4, and N_2O is 1, 28, and 265, respectively (IPCC 2014).

Swine producers are encouraged to use cereal co-products in their diets to reduce competition with humans for cereals and because they are relatively inexpensive (Woyengo et al. 2014; Mottet et al. 2017). Wheat millrun is a co-product that is readily available and commonly used in North America, especially western Canada (Nortey et al. 2007a). It is obtained after the removal of the endosperm during wheat milling; hence it is high in NSP (Slominski et al. 2004; Woyengo et al. 2014). Greenhouse gas production, especially CH_4, is increased by the NSP content in diets of growing pigs and adult sows (Jørgensen 2007). However, the NSP in swine diets shifts N excretion from urine to feces, potentially reducing the amount of N_2O gas emitted into the environment (Canh et al. 1997; Liu et al. 2017).

Pigs do not have the digestive enzymes required to hydrolyze NSP, and exogenous enzymes are often added to high NSP diets to improve the energy value of the feed and reduce the undigested organic matter in the feces (Paloheimo et al. 2010; Woyengo et al. 2014). For example, an improvement in nutrient and fibre digestibility was observed when wheat millrun diets were supplemented with xylanase (Nortey et al. 2008).

The overall objective of this study was to determine the impact of wheat millrun inclusion supplemented with a multi-carbohydrase in swine diets fed to growing-finishing pigs on GHG production from the pigs and manure. We hypothesized that feeding wheat millrun diets to growing pigs will increase GHG production, however, this would be mitigated by using a multi-carbohydrase enzyme.

4.3 Materials and methods

Animal protocols were reviewed and approved by the Animal Research Ethics Board at the University of Saskatchewan and followed principles established by the Canadian Council on Animal Care Guidelines for Humane Animal Use (CCAC 2009).

4.3.1 Animals, Experimental design and Housing

The study utilized 96 pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada), with an initial BW of 60 ± 2.2 kg assigned to 1 of 4 dietary treatments. Treatments were arranged as a 2 × 2 factorial in a randomized complete block design, comprised of a 0 % and a 30 % wheat millrun diet with and without carbohydrase enzymes.

Pigs were housed in two identical environmental chambers (Figure 4.1) at the Prairie Swine Centre Inc. (Saskatoon, SK. Canada). Each chamber is a distinct room with a separate manure pit (collection tub) and ventilation. The interior of each chamber measures 4.2 × 3.6 × 2.7 m (L×W×H). The floor of the chamber is 2/3 solid and 1/3 slatted concrete. A plastic matrix around the pen allows easy access to the collection tub below the slatted portion of the pen, facilitating manure collection. The ceilings and internal walls are sealed with stainless steel sheets to eliminate emissions from these surfaces. The chambers are equipped with commercial feeders and nipple water drinkers.

Each chamber operates on a negative pressure ventilation system. Outdoor fresh air entered the airspace through ceiling inlets and was conditioned to desired settings using an air-conditioning unit (Raka-060 CAZ, Setra Systems, Boxborough, MA, USA) or a 10 kW electric heater (Chromalox, Dimplex North America Ltd., Cambridge, ON, Canada). The pre-conditioned room air passed through a HEPA filter in a filtration unit (Circul-Aire USA-H204-B, Dectron International, Roswell, GA, USA) with a 0.6-m-diameter centrifugal fan (Delhi BIDI-20, Delhi Industries Inc., Delhi, ON, Canada) and entered the chambers through an actuated inlet located on the ceiling of each chamber. Air from each chamber was exhausted through one sidewall fan (H18, Del-Air Systems Inc., Humboldt, SK, Canada).

A CO_2 sensor (CM-0016, www.CO2meter.com, FL, USA), and a relative humidity/ temperature sensor (Hobo U12-013, Onset Computer Corp., MA, USA) were installed in each chamber. Air velocity measurements were obtained with an ALNOR 501 anemometer (RVA501 Data Logging Vane Anemometer, TSI Incorporated, Shoreview, MA, USA). Water consumption in each chamber was measured at each drinker by water meters (C700 polymer ABB, Atlantic Liquid Meters, Ontario, Canada).

A preliminary trial was undertaken to ensure the chambers and equipment operated properly, and all sensors were calibrated. The length of time required for the stabilization of gases when pigs were housed in the chambers was also determined from the preliminary studies.

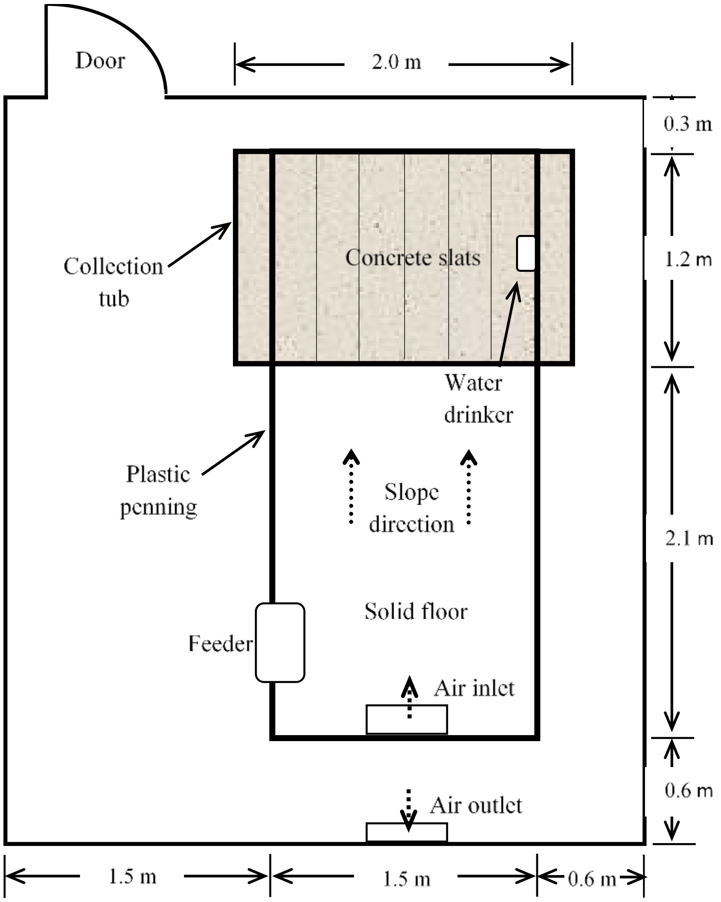

Figure 4. 1 A schematic diagram of the chambers at the PSCI where animals were housed (Adapted from Alvarado et al. 2015).

4.3.2 Diets

Diets (Table 4.1), same as diets used in the digestibility and growth performance study, were formulated using published SID and NE values to meet or exceed nutrient requirements for growing pigs (NRC 2012). Diets were designed to be typical of Western Canadian diets. The NE was allowed to decrease with the increase in wheat millrun inclusion (from 2.46 to 2.34 Mcal kg^{-1}). The multi-carbohydrase enzyme was comprised of 5 different enzymes; glucanase, 300 GLU units g^{-1}; xylanase, 1,000 XLU units g^{-1}; cellulase, 1,900 CMC units g^{-1}; amylase, 4,200 FAA units g^{-1}; invertase, 150 INV units g^{-1}. (Superzyme-W®; Canadian Bio-systems Inc., Calgary, AB), and used at a recommended dose of 1 kg tonne^{-1} of finished feed.

4.3.3 Experimental approach

At the beginning of each replicate, 6 grower pigs of the same sex were brought into each chamber. The sexes were alternated between chambers and treatments. These pigs had been receiving their assigned diets for 1 wk prior to their introduction into the chambers. Feed and water were supplied *ad libitum*. The pigs were housed in the chambers for 14 d to allow for stabilization of gas prior to measurements on d 15. The room and manure collection tub were cleaned, sanitized, and allowed to dry for 3 d before the next group of pigs were admitted to the chamber. Temperature, relative humidity, and manure depth were recorded every day throughout the experiment.

4.3.4 Gas emissions

On d 15, air velocity measurements were taken before and after gas sampling to aid in the determination of the airflow rate. Before the gas collections, the Tedlar® bags and Teflon tubes were flushed with clean air (Air ultra-zero, Praxair, Saskatoon, Canada). Air samples were pumped through Teflon tubes (approximately 0.4 cm internal diameter) from the chamber (close to the exhaust fans, ensuring that the air collected was representative of the air leaving the chamber and the barn) into Tedlar® bags with the aid of a vacuum apparatus (Figure 4.2). To transfer a sample to an Exetainer® tube, a 20 ml syringe with a needle was inserted into the septum in the Tedlar® bag, purged three times before withdrawing a 20 ml sample, which was then injected through the septum of a 12 ml evacuated Exetainer® tube.

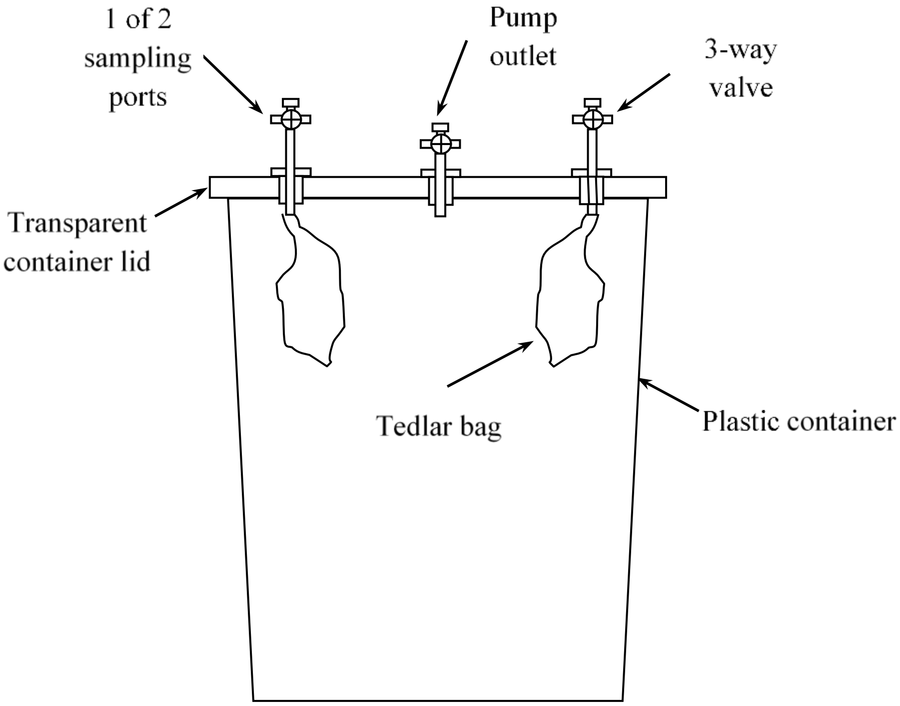

Figure 4. 2 Schematic diagram of the vacuum apparatus for gas sampling (Adapted from Predicala et al. 2011).

The following equations were used to calculate gas emissions:

$$E = C \times Q \qquad [4.1]$$

$$Q = V \times A \qquad [4.2]$$

Where E = gas emissions (mg s^{-1}); C = gas concentration in room measured close to the exhaust duct (mg m^{-3}); Q = volumetric airflow rate (m^3 s^{-1}); V = airspeed (m s^{-1}); A = exhaust fan duct area (m^2).

4.3.5 Manure gas sampling

Manure samples were taken after the chamber gas sampling to ensure the manure agitation did not affect the concentration of room gases. The slurry in the collection tub was stirred with a rake to ensure homogeneity. The slurry was then collected using a submersible utility pump into a barrel to mimic an open (uncovered) lagoon. This ensured 1 barrel per chamber and an *n* of 4 per treatment. The diameter and height of the barrels' headspace were measured, and the barrels were stored open at room temperature. The stored manure was then left undisturbed for 7 d before gas sampling (month 1). A second gas sample was taken 3 months later (month 3).

On sampling day, the barrel was covered, and samples were taken at 0, 5, and 10 min using a 20 ml syringe attached to a sampling port in the lid. The samples were transferred into a 12 ml evacuated Exetainer® tube for gas chromatography analysis.

The GHG flux from the barrels was calculated using regression analysis because the 15 min enclosure time was considered inadequate to affect the concentration gradient between the surface of the slurry and the headspace air, and was calculated as follows (Agnew et al. 2010);

$$F = \rho\ (V / A) \times (\Delta C/\Delta t) \qquad [4.3]$$

Where F = surface gas flux (µg m^{-2} s^{-1}); ρ = density of gas (kg m^{-3}); V = volume of headspace (m^3); A = area of headspace (m^2); $\Delta C/\Delta t$ = rate of change of gas concentration (ppm min^{-1})

4.3.6 Gas chromatography

Gases (CO_2, CH_4, and N_2O) were analyzed and quantified by gas chromatography (**GC**) at the GHG laboratory in the Department of Soil Science, University of Saskatchewan. Two ml of each sample from the Exetainer® tube was injected into the GC (Scion 456-GC, Scion Instruments, Livingston, UK). Gases were separated on a column packed with Hayesep N and Haysep D polymers; CO_2 was detected using a thermal conductivity detector (**TCD** at 120 ^oC) CH_4 by a flame ionization detector (**FID** at 120 ^oC), and N_2O was detected with an electron capture detector (**ECD** at 330 ^oC). Each analysis took 2 to 6 min, depending on the gas being analyzed. The columns and injectors were at a temperature of 85 ^oC.

4.3.7 Statistical analyses

Gas emission, room temperatures, relative humidity, total water consumption, and total manure collection were evaluated with ANOVA using the mixed model procedure of SAS (version 9.4; SAS Institute, Inc. Cary, NC) with wheat millrun and multi-carbohydrase enzyme supplementation, and their interactions as fixed effects and chamber (block) as the random effect. Each pen of 6 pigs was considered the experimental unit. Significant differences between means were declared at $P \leq 0.05$. Due to the high variability in the GHG flux calculations, similar to analyses published elsewhere (Dong et al. 2007; Blunden and Aneja 2008), descriptive statistics; mean, standard deviation, minimum and maximum values were used to describe the results obtained.

4.4 Results

Pigs remained healthy throughout the experiment. Temperature and humidity were consistent throughout the trial.

The effect of wheat millrun and carbohydrase on total manure output, temperature, relative humidity, and water consumption from the chambers is described in Table 4.1. Total manure output was increased by about 31 % when pigs consumed the 30 % wheat millrun diets ($P < 0.05$). Pigs fed 30 % wheat millrun diets consumed about 22 % more water than pigs fed the zero wheat millrun diets ($P < 0.05$).

Greenhouse gas emission rates are presented in Table 4.2. Feeding pigs with 30 % wheat millrun had no effect on the GHG measured in this experiment. The highest concentration of gas emitted was CO_2 at 1610 and 1711 mg s^{-1}, without or with wheat millrun inclusion, respectively ($P = 0.57$), followed by CH_4 (4.7 and 4.9 mg s^{-1}; $P = 0.78$) and N_2O (0.45 and 0.42 mg s^{-1}; $P = 0.79$). There was no significant effect of enzyme supplementation on GHG concentrations ($P > 0.15$).

The CO_2 flux from the barrels is presented in Table 4.3. The average flux in month 1 for the 30 % wheat millrun diets was 369 (± 433 **SD**, standard deviation) and 375 (± 197) µg m^{-2}s with and without multi-carbohydrase supplementation, respectively. Mean CO_2 flux was 82 (± 89) and 235 (± 42) µg m^{-2}s for the 0 % wheat millrun diets, with and without multi-carbohydrase supplementation, respectively. The mean CO_2 flux increased in the month 3 measurements to 627 (± 431) (with enzyme) and 355 (± 299) µg m^{-2} s (without enzyme) for the 0 % wheat millrun diets, 514 (± 338) (with enzyme) and 357 (± 113) µg m^{-2} s (without enzyme) for the 30 % wheat millrun diets.

Data for the CH_4 flux are shown in Table 4.4. Methane flux rates were highly variable in month 1 with a mean flux of 72 (± 120) µg m^{-2} s (with enzyme) and 40 (± 44) µg m^{-2} s (without enzyme) for the 30 % wheat millrun diets, and 224 (± 381) µg m^{-2} s (with enzyme) and 135 (± 128) µg m^{-2} s (without enzyme) for the no wheat millrun diets. The CH_4 flux, however, reduced after month 3 calculations for an average of 29 (± 16) and 25 (± 16) µg m^{-2} s with or without wheat millrun inclusion, respectively.

Table 4. 1 Environmental (temperature and relative humidity) and room (water disappearance and total manure output) parameter measurements of growing pigs fed wheat millrun and a multi-carbohydrase supplementation [a]

Item [b,c]	**Millrun, %**		**Enzyme**		**Pooled SEM**	***P*-value**	
	0	30	No	Yes		Millrun	Enzyme
Temperature, °C	17.6	16.7	18.0	16.3	1.1	0.47	0.19
Relative humidity, %	50.2	51.8	49.3	52.7	1.3	0.50	0.15
Water, L pig^{-1} day^{-1}	8.3	10.1	9.6	8.8	0.43	0.05	0.29
Manure [d], L pig^{-1} day^{-1}	3.9	5.1	4.5	4.5	0.25	<0.01	0.90

[a] Pigs were kept in chambers for 14 days.

[b] Values are means of 4 replicates (chambers) with 6 pigs per chamber.

[c] No interaction between millrun and enzyme (*P* > 0.10).

[d] Manure refers to a combination of feces and urine.

SEM; standard error of mean.

Table 4. 2 Effect of wheat millrun and multi-carbohydrase supplementation on greenhouse gas emission rate in grower pigs (60kg) housed in an environmental chamber [a]

Item [b,c]	Millrun, %		Enzyme		Pooled SEM	*P*-value	
	0	30	No	Yes		Millrun	Enzyme
Gas, mg s^{-1}							
Methane	4.7	4.9	4.5	5.1	3.40	0.78	0.51
Nitrous oxide	0.45	0.42	0.46	0.42	0.06	0.79	0.65
Carbon dioxide	1610	1711	1808	1513	214	0.57	0.15

[a] Pigs were kept in chambers for 14 days. Gas measurements taken after 14 days with 6 pigs housed in chambers, with manure stored below the pens.

[b] Values are means of 4 replicates (chambers) with 6 pigs per chamber.

[c] No interaction between millrun and enzyme ($P > 0.05$).

SEM; standard error of mean.

Table 4. 3 Effects of wheat millrun inclusion and multi-carbohydrase supplementation on CO_2 flux[a]

Item [a]	0 % Wheat millrun		30 % Wheat millrun	
	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No
Month 1, CO_2, μg $m^{-2}s$				
Mean	82	235	369	375
SD	89	42	433	197
Max	185	265	867	499
Min	30	205	93	148
N	3	2	3	3
Month 3, CO_2, μg $m^{-2}s$				
Mean	627	355	514	357
SD	431	299	338	113
Max	1094	802	753	519
Min	65	173	275	260
N	4	4	2	4

[a] Headspace analysis, measured from stored 65L open barrels.

SD: standard deviation; Max: maximum; Min: Minimum; n: number of observations; CO_2: carbon dioxide. μg: micrograms; m: meter; s: second; Enzy +, with enzyme; Enzy -, without enzyme.

Table 4. 4 Effects of wheat millrun inclusion and multi-carbohydrase supplementation on CH_4 flux[a]

	0 % Wheat millrun		**30 % Wheat millrun**	
Item	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No
Month 1, CH_4, µg m^{-2}s				
Mean	224	135	72	40
SD	381	128	120	44
Max	663	259	211	71
Min	0	4	1	9
N	3	3	3	2
Month 3, CH_4, µg m^{-2}s				
Mean	31	19	35	23
SD	11	18	19	12
Max	43	39	52	40
Min	21	5	14	14
N	3	3	3	4

[a] Headspace analysis, measured from stored 65L open barrels.

SD: standard deviation; Max: maximum; Min: Minimum; n: number of observations; CO_2: carbon dioxide. µg: micrograms; m: meter; s: second.

4.5 Discussion

Strategies to reduce the impact of the livestock sector on the environment are gaining prominence. One potential feeding strategy for the swine industry is to rely more on co-products and reduce “human” grade food fed to pigs since these pigs can convert these co-products, which is otherwise a waste product, into quality protein (Zijlstra and Beltranena 2013a; Makkar 2018). However, the high fibre content of many co-products, which serve as a substrate for bacterial fermentation, especially in the large intestine (Jha and Berrocoso 2015), suggests that GHG output might increase. Therefore, this experiment was carried out to determine if the increase in the use of co-product (wheat millrun) will increase the quantity of GHG produced from the pig and stored manure.

In the current study, 30 % wheat millrun was added to swine diets at the expense of wheat. Diets were formulated on a least-cost basis, assuming daily caloric intake would be similar, regardless of the energy content of the diet. The 30 % wheat millrun diets had 40 % more ADF, 53 % more NDF, and 26 % more total dietary fibre (**TDF**) (Table 3.2) than the no wheat millrun diets.

The higher manure (slurry) output could be as a result of the high fibre content and low digestibility of the 30 % wheat millrun diet. Also, high water consumption coupled with water wastage could be a contributing factor to the high manure output. The results were similar to work by Anderson et al. (2012) and Trabue and Kerr (2014), who reported an increase in DM manure output when pigs were fed diets containing DDGS. Due to the limited activity of endogenous enzymes in the large intestine, most of the fibre in high fibre diets are not completely fermented and therefore are excreted in the feces (Kiarie et al. 2013). In the current study, however, enzyme supplementation had no effect on manure output.

Similar to what has been shown by others (Atakora et al. 2011; Trabue and Kerr 2014; Philippe et al. 2015), CO_2 was the highest GHG emitted among the 3 gases determined in this study. CO_2 is mainly from animal respiration. It has been reported that CO_2 from the pig is more likely to be influenced by pig activity and less from diet composition (Atakora et al. 2011; Philippe et al. 2015). However, Schrama et al. (1998) discovered that high fibre diets reduced physical activities in pigs. They related the reduction to the gradual release of energy and the “bulkiness” of the high fibre diets. In contrast, Laitat et al. (2015) reported an increase in pig activity due to higher feeding time required by fattening pigs consuming high fibre diets. Pig activity was not measured in our study, but perhaps, the confined spaces of the chambers would have reduced pig activity.

Methane emissions in swine production are primarily from methanogens breaking down organic matter in the pig's digestive tract and in the slurry (Philippe and Nicks 2015). These bacteria, mainly from the family, *Methanobacteriaceae*, are abundant in the hindgut of pigs (Mi et al. 2019). Therefore, CH_4 production may be increased by high dietary fibre as the fibre increases the activity of the methanogenic community (Le Goff et al. 2002). For example, Jarret et al. (2011) reported an increase in CH_4 production when high fibre diets containing DDGS, SBP, and high-fat rapeseed meal were fed to growing pigs. In contrast, our study showed no significant difference among the dietary treatments for CH_4 output. Perhaps, the type of fibre in the diets may be responsible for the different results. Sugar beet pulp is a highly fermentable fibre source with 25 % of its TDF being soluble whereas only about 6 % of TDF in wheat millrun is soluble (Garcia et al. 2015; Wang et al. 2016). However, similar to results from the current study, others have found no significant difference in CH_4 emissions with increase in fibre content. Pepple et al. (2011), in a study designed to measure GHG from a DDGS and a non-DDGS barn, discovered no significant differences in CH_4 production. Two-thirds of CH_4 emissions from the pig barn is produced from manure (Monteny 2004). Methane emissions from the manure are affected by several factors, including temperature, manure composition, and pH (Philippe and Nicks 2015). A decrease in the manure production of CH_4 in pigs fed DDGS diets coupled with high variability in the CH_4 emissions could have been the cause of the outcome. Differences in the manure accumulation, room temperature, and organic matter content, even within treatment in the current study may have resulted in high variability observed in CH_4 production. A study by Jørgensen (2007) also cited animal variation as part of the reason why they failed to show an effect of high fibre feedstuffs on CH_4 production.

The 30 % wheat millrun diets had no effect on N_2O emissions. Research has shown that N_2O emissions are mainly influenced by the age of the manure (Laguë et al. 2013). The N_2O from manure primarily arises through incomplete nitrification or denitrification by microorganisms (Oenema et al. 2005). Also, N_2O emissions are related to the excretion of N; therefore, a reduction of N in the manure could result in a potential reduction of N_2O during the storage of manure (Atakora et al. 2011). The reduction of the CP in the diet fed to pigs is, therefore, another potential diet manipulation to reduce GHG (Liu et al. 2017). Interestingly, although the N intake was higher in the 30 % wheat millrun diets, there was no difference in the total N output among the treatments.

The inclusion of high fibre feedstuffs in the diets of pigs may shift the N in urine to microbial protein in feces, which is a less accessible form of N (Galassi et al. 2010). Therefore, the wheat millrun diets could potentially reduce the N_2O emissions, hence resulting in no overall difference in N_2O emission between the diets. Pepple et al. (2011) and Montalvo et al. (2013) also showed no difference in N_2O emissions when co-products were added to the diets of pigs.

There was high variability in the data from the manure storage data barrels. Several reports have indicated that stored manure is influenced by several factors, including manure composition and accumulation, temperature, and season (Liu et al. 2013; Trabue et al. 2016). Temperature and season probably did not affect the barrels since the barrels were maintained in a room where the temperature was monitored and was not affected by the external environmental conditions. The manure composition and accumulation (fraction of solids and liquids), however, could have affected the barrels as this varied among replicates.

Comparable to other studies (Pelletier et al. 2004; Laguë et al. 2013), the N_2O emissions from stored liquid swine manure was negligible.

4.6 Conclusion

The 30 % inclusion of wheat millrun in swine diets had no effect on GHG emissions from swine barns. It can be concluded that even though wheat millrun inclusion increases the fibre content in swine diets, the addition of up to 30 % of wheat millrun will not significantly increase the GHG produced by the pigs in swine barns. Further studies should investigate the long-term effect of manure storage and other storage techniques on GHG emissions.

4.7 Implications

This information will assist livestock producers, especially swine producers, grain farmers and policymakers in Western Canada, with accurate data required for the expansion of the pork industry while mitigating the impact of pork production on GHG output.

4.8 Acknowledgements

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CHAPTER 5: IMPACT OF FEEDING GROWING PIGS WITH WHEAT MILLRUN SUPPLEMENTED WITH A MULTI-CARBOHYDRASE ENZYME ON GLOBAL WARMING POTENTIAL – A LIFE CYCLE ASSESSMENT FRAMEWORK

A modified version of this material will be submitted to the Journal of Animal Science for publication.

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5.1 Abstract

This study aimed to determine the effect of wheat millrun inclusion in the diets of growing-finishing pigs on overall GHG emissions production using a life cycle assessment framework. Emission data were obtained from a previous experiment where growing pigs (60 ± 2.2 kg) were maintained in environmental chambers and fed treatment diets with and without 30 % wheat millrun supplemented with or without a multi-carbohydrase enzyme (6 pigs/chamber; 4 dietary treatment; replicated four times) while performance data was obtained from a feeding trial with the same treatments. The Holos farm model (Agriculture and Agri-Food Canada, Lethbridge. AB) was used to estimate emissions from feed production. Emissions from inputs such as fertilizer and herbicide applications were also included in the emissions from crop production. Environmental impact was calculated as GWP with CH_4, N_2O, and CO_2 as the gases. Production and feeding of wheat millrun in diets resulted in an approximately 25 % reduction in GWP when compared to the no wheat millrun diets. CO_2 from pig respiration was the largest contributing GHG (79 %) from the pig chambers. Overall, enteric GHG production from pigs was not affected by dietary treatment. The inclusion of wheat millrun in diets of growing pigs can reduce the GWP of pork production.

5.2 Introduction

Pork production accounts for a relatively small GHG emission contribution, but due to the expected global growth in pork production, mitigation is imperative (Dyer et al. 2010; Gerber et al. 2013; Philippe and Nicks 2015). There are several factors in the livestock production sector, such as machinery use, production and transportation of grains and feed, and fuel and electricity usage, amongst others, that lead to the production of GHG such as CH_4, CO_2, and N_2O (de Vries and de Boer 2010). As a result, feed ingredients and diet compositions are considered critical when considering the impact of pork production on the environment. Co-product usage in swine diets is increasing, primarily to reduce the overall cost of feed, but also to reduce pressure on human food systems (Zijlstra and Beltranena 2013; Woyengo et al. 2014). It has been shown, however, that the use of co-products increases GHG emissions from the barn due to the increase in fermentation in the large intestine of pigs and hence CH_4 production (Philippe and Nicks 2015). However, there is limited information on the impact of co-products in swine diets on overall GHG output.

Production processes must be viewed comprehensively to reduce overall GHG emissions and improve the sustainability of pork production. One such approach is by performing an LCA. An LCA incorporates emissions throughout the various production stages and uses assessment models. Life cycle assessments in swine production have been widely utilized in Europe and China (Reckmann et al. 2013; Zhou et al. 2018) and typically rely on previously obtained data from various organizations (Macleod et al. 2013; Vergé et al. 2016).

The objective of this study was to determine the effect of wheat millrun inclusion in the diets of growing-finishing pigs on the overall GHG emissions, considering the entire production cycle, including feed production and manure management.

It was hypothesized that wheat millrun inclusion in swine diets would generate lower production emissions and reduce overall GHG emissions when processes such as feed production are considered.

5.3 Materials and methods

For this study, an LCA framework was developed with the incorporation of the Holos model (developed by Agriculture and Agri-Food Canada, Lethbridge, AB) to compare GHG emissions amongst the diets. The Holos model is primarily based on the methodology from IPCC (2006), modified for Canadian condition and uses data from several studies. All significant emissions from the farm, including emissions from the manufacture of inputs such as fertilizers and herbicides, are considered in the model (Beauchemin et al., 2010). Holos estimates emissions from N_2O (soil and manure), CH_4 (enteric and manure) and CO_2 (energy use and changes in carbon storage). The Holos model allows adjustments in farm management practices such as a change in feed composition, amongst others (Agriculture and Agri-Food Canada, 2020). It also allows for the input of actual data from other experiments. This framework followed an LCA format that includes a goal and scope, inventory analysis, impact assessment, and interpretation (Wolf et al. 2012). The life cycle emissions inventory categories employed for this study were feed production, livestock growth/production, and manure management. The Holos farm emissions estimating model software (Agriculture and Agri-Food Canada, Lethbridge, AB) was used for acquiring crop production data. The impact assessment compared emission differences on a per animal-day basis. Data from two previous experiments (a performance study and a GHG emission study) where growing pigs with initial BW of 60 ± 2.2 kg were fed treatment diets, with and without 30 % wheat millrun supplemented with or without a multi-carbohydrase enzyme, was utilized in the analysis.

5.3.1 Climatic and geographical location of the crop field

The eco-district and soil type parameters used were representative of the agricultural region in East-Central Saskatchewan. A black/gray chernozem medium-textured soil with reduced-tillage practice, commonly practiced in the region, was used.

5.3.2 Feed production

Crop production emissions were calculated based on the land area used to produce a particular commodity. The total feed amount, number of pigs, and number of days specific to each diet attained from the performance study were included in the calculations. The diet ingredients were included on a percent basis. Ingredients that comprised less than 0.5 % of the total diet and non-organic ingredients (minerals and vitamins) were not included. Soybean meal, canola oil, and wheat millrun represented 79 %, 44 %, and 15 % of whole kernel soybean, canola, and wheat, respectively (U.S. Soybean Export Council 2015; Canola Council of Canada 2017). The yield values were obtained from averages reported by the 2018 Saskatchewan Crop Planning Guide as well as N and P fertilizer application rates (Government of Saskatchewan, 2018). The analysis in Holos followed the conventional agricultural practices in Saskatchewan, which was that the crops were not irrigated and that an appropriate herbicide management plan was followed.

Data from the performance study in the same series of experiments provided information on the number of pigs, the number of days the pigs were on a particular diet, and the average consumption per pig. The 180 pigs (60.2 ± 2.2 kg) used in the performance study were housed in groups of 5 and were fed the same diets (Table 3.2) as that of the current study until market weight (about 125 kg). This information was used to calculate the average GHG produced from pigs consuming a particular diet.

5.3.3 Diets

The impact of 30 % wheat millrun inclusion in grow-finish pig diets with and without enzyme supplementation was compared to a control diet containing no wheat millrun (Table 3.2). The control diet was formulated to emulate a typical western Canadian diet based on wheat and barley and complied with nutrient requirements for pigs of this age (NRC 2012). The NE content of the diet was allowed to decrease with the addition of the wheat millrun, as this would reduce diet cost. Holos was used to estimate GHG emissions associated with the preparation of each diet.

5.3.4 Pig GHG production

Pigs were fed the treatment diets described above in a GHG emission experiment. Each treatment had 4 replicates with 6 pigs (60.2 ± 2.2 kg) housed in specialized environmental chambers (with interior measurements of 4.2 × 3.6 × 2.7 m) for 14 d at the Prairie Swine Centre Inc., Saskatoon, SK. On d 15, air samples were collected and analyzed by gas chromatography for CH_4, CO_2 and N_2O, and total emissions were determined based on the air exchange rate. There was a baseline value of the ambient CO_2 levels recorded at the beginning of each trial. The total emissions were attained with the following equations used by Montalvo et al. (2013);

$$E_{gas} = \frac{(C_{gas\,out} - C_{gas\,in})}{10^6} \times \left(\frac{\beta_{gas} \times Q \times 86400}{M_{pig}}\right) \times 10^6 \qquad [5.1]$$

$$\beta_{gas} = D_{gas} \times \rho_{\mathrm{air}} \qquad [5.2]$$

Where E_{gas} (mg $_{\mathrm{gas}}$ $\mathrm{kg_{pig}}^{-1}\mathrm{d}^{-1}$) = gas emissions; $C_{gas\ out}$ (mg m^{-3}) = gas concentration in the chamber collected on sampling day; $C_{gas\ in}$ (mg m^{-3})= baseline gas collected at the beginning of the trial; β_{gas} (kg $_{\mathrm{gas}}$ m^{-3})= mass of gas by air volume; Q ($\mathrm{m}^3\ \mathrm{s}^{-1}$)= room ventilation rate; M_{pig} (kg) = total mass of the pigs in the chamber; D_{gas} (kg $_{\mathrm{gas}}$ $\mathrm{kg_{air}}^{-1}$) = specific gravity; ρ_{air} (kg $_{\mathrm{air}}$ m^{-3}) = air density. 86400 s d^{-1} = number of seconds in a day.

The model also used data from a performance study that utilized the same treatment diets to determine the effect on ADFI, ADG, G:F ratio, and days to market.

Assumptions used in the model for manure management were from a modelling experiment by Mackenzie et al. (2016). In this experiment, the diets compared were diets containing wheat shorts and a corn-soybean control diet. The assumptions for the model were that the manure was collected, stored, and applied to land as fertilizer. It was also assumed that the manure applied replaced synthetic fertilizers at a rate of 0.75, 0.97, and 1 for N, P, and K, respectively.

5.3.5 Impact assessment

The emissions for each diet case study were converted into a kg CO_2 eq/pig-day basis to compare the diets. The total emissions from all the inventory sources allowed a comparison of the differences in LCA emissions. The impact category used in the study was the GWP. The GWP was quantified with a 100-year timescale with CH_4: 28, N_2O: 265, and CO_2: 1 (IPCC, 2014).

5.4 Results and discussion

The objective of the current study was to consider factors other than just enteric production from the pigs that contribute to GHG in swine production. This included factors such as crop production and feed production that contribute significantly to GHG production from swine production. Although the GHG production from swine production is relatively small compared to ruminants, efforts to understand contributing factors and potential for mitigation will become even more critical as global pork consumption increases.

An LCA framework was used to conduct a partial LCA. The framework was, therefore, not a “cradle-to-grave” assessment of pork production as most LCA. The framework used in this study considered emissions from growing pigs (60 to 128 kg BW), feed production, and manure management to allow an estimation of the effect of including wheat millrun in the diet on GWP of pork production.

The average crop yield and fertilizer application data were obtained from the 2018 Saskatchewan Crop Planning Guide (Government of Saskatchewan 2018) (Table 5.1). Nitrogen and P fertilizer application contributes to emissions. One meaningful way to reduce the emissions from crop production is by reducing the N fertilizer application (Jianyi et al. 2015). The N fertilizer application used in Saskatchewan falls within the recommended rate by the European Union’s recommended class II values (EU 2014), which seek to reduce GHG emissions from N fertilizer application.

Table 5. 1 Average yield and fertilizer application rate of feed crops used in the LCA framework as inputs into the Holos model [a,b]

Category	**Wheat**	**Barley**	**Soybean**	**Canola**
Yield (kg ha^{-1})	3437	4903	1580	3040
N fertilizer rate (kg ha^{-1})	88.6	77.3	5.6	98.6
P fertilizer rate (kg ha^{-1})	34.8	37.0	26.9	53.8

[a] Data extracted from the Government of Saskatchewan crop planning guide, 2018.

[b] Results based on crops planted in a dark brown soil zone in Saskatoon.

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Data input for GHG emissions in GWP from the performance study are presented in Table 5.2. The GWP calculated per daily consumption of feed from the performance study over the growing period from 100 pigs show that wheat millrun inclusion resulted in the lowest GWP (Figure 5.1). At the inclusion level of 30 % wheat millrun, the GWP was 30.52 and 31.47 kg CO_2eq (with and without enzyme) and 44.28 and 43.08 kg CO_2eq (with and without enzyme respectively) for the no wheat millrun diet. The results are comparable to results from a study by Mackenzie et al. (2016), who reported a reduction in GWP when growing-finishing pigs were fed with co-products such as wheat shorts and meat meal. The production of co-products leads to offsets in the amount of fossil fuels, energy inputs, and emissions associated with the production of the crops (Bremer et al. 2010).

Table 5. 2 Effects of dietary treatments from performance data from pigs (60 to 128 kg)[a,b]

	0 % wheat millrun		**30 % wheat millrun**	
Items	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No
Days on feed	68	68	68	71
ADFI (kg pig^{-1})	2.75	2.72	2.87	2.76
ADG (kg pig^{-1})	1.08	1.04	1.01	0.94

[a] Data used as inputs into the Holos model.

[b] Data from the performance study from 180 pigs.

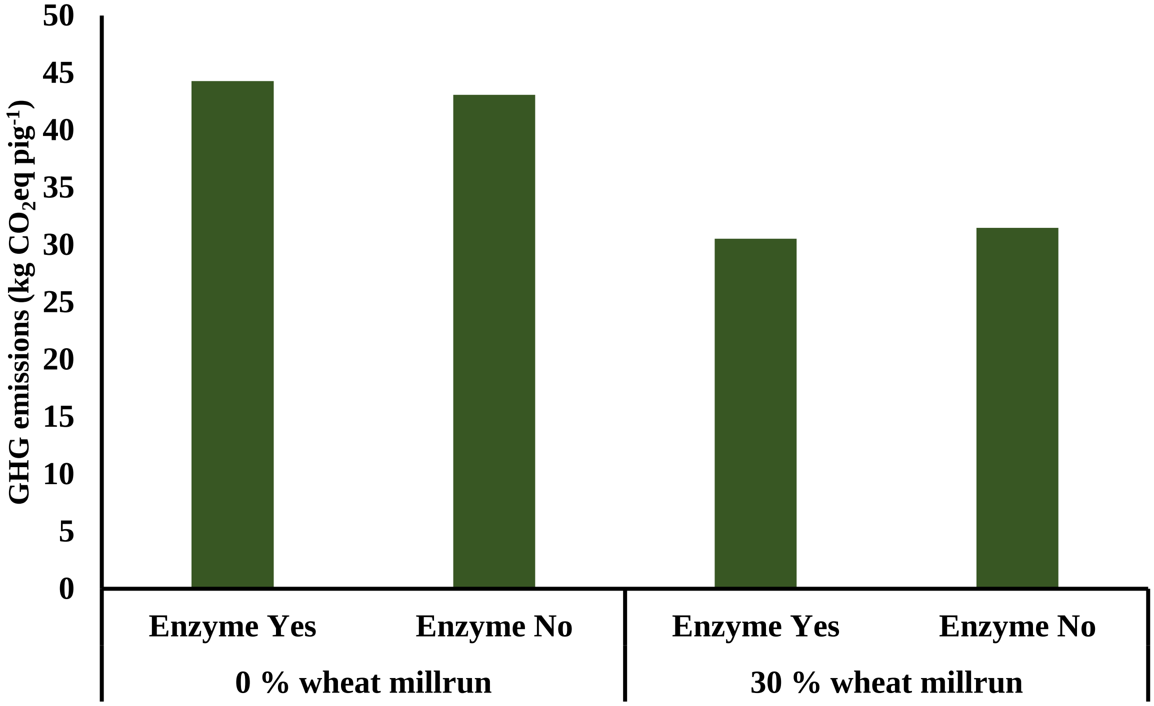

Figure 5. 1 Global warming potential calculated from emissions from feed production and consumption. The GWP is calculated from the Holos model using ADFI per 100 pigs by the number of days on a particular feed.

The environmental impact for 1 kg growing pig for the different diets is presented in Table 5.3. It is noted that CO_2 contributed the most emissions among the gases (Figure 5.2). The CO_2 is mainly influenced by respiration and less by dietary composition (Atakora et al. 2011; Montalvo et al. 2013). However, the inclusion of CO_2 in LCA is controversial (Herrero et al. 2011). In a case study by Beauchemin et al. (2010) on the LCA of GHG in beef production CO_2 emissions were excluded from the animals in the GHG intensity estimation because the CO_2 emissions from livestock may be considered to be sequestered in the animal and plant interactions in biological systems (Herrero et al. 2011). The emissions of CH_4 and N_2O from pigs in the GHG emission study were 18 % and 3 %, respectively, of total output.

Table 5. 3 Effect of dietary treatments on GWP (Kg CO_2 eq $kg_{pig}^{-1}day^{-1}$) from growing pigs (60 kg) housed in environmental chambers

	0 % wheat millrun		**30 % wheat millrun**		
Gas[a]	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No	SD
Carbon dioxide	0.13	0.19	0.17	0.15	0.020
Methane	0.03	0.02	0.03	0.02	0.005
Nitrous oxide	0.005	0.008	0.008	0.002	0.002
GHG total [b]	0.165	0.218	0.208	0.172	0.070
GHG × days on feed	**11.22**	**14.82**	**14.14**	**12.21**	

[a] CO_2 eq = (methane emissions × 28) + (nitrous oxide × 265) + carbon dioxide (IPCC 2014).

[b] calculated from results from the GHG production study.

SD; standard deviation.

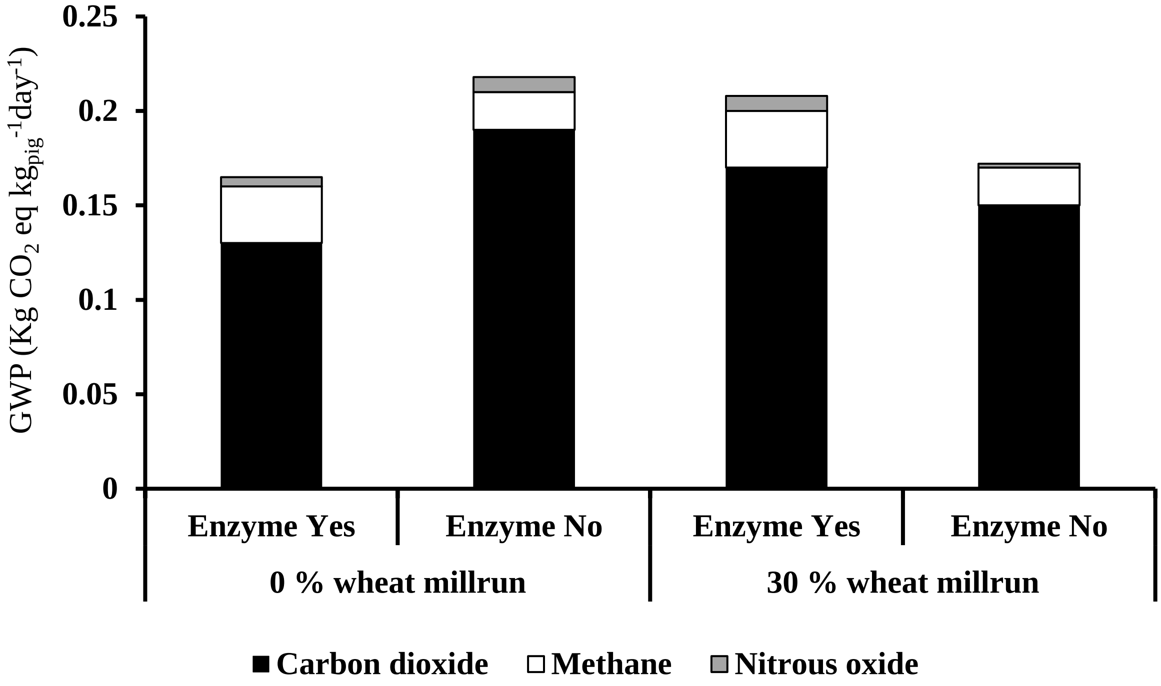

Figure 5. 2 Global warming potential calculated from the GHG production study where pigs were housed in environmental chambers for 14 days with methane emissions (× 28), nitrous oxide (× 265), carbon dioxide (× 1) (IPCC 2014) as conversion factors.

5.5 General discussion

The use of co-products from the biofuel industry and human food production sector has increased in recent years, aiming towards a reduction in the diet cost and competition between humans and livestock feed (Woyengo et al. 2014). The high fibre content of some co-products means they have the potential of increased enteric fermentation, resulting in increased production of gases like CH_4 (Philippe and Nicks 2015). However, the overall implications of feeding pigs with co-products using tools such as LCA is imperative, as these take a more holistic approach towards quantifying GHG emissions from production systems. Accordingly, this study used an LCA framework, within the Holos model developed by Agriculture Canada, to investigate the effect of including wheat millrun in the diets of growing-finishing pigs. Global warming potential (GWP), eutrophication potential (EP), acidification potential (AP), non-renewable energy use (NRE), and non-renewable resource use (URRU) may be included in quantifying total environmental impacts (Mackenzie et al. 2016; McAuliffe et al. 2016). For the scope of this study, only GWP was considered.

The results from the GHG emission study examining GHG emissions from pigs and manure in environmental chambers (Table 4.2) highlights our overall finding that the inclusion of wheat millrun in the diets of the growing pigs did not significantly increase GHG emissions. If emissions from only the pigs are considered, it could be concluded that the inclusion of wheat millrun did not affect GWP. However, the inclusion of wheat millrun in the pig diets in the LCA framework which also considered feed production showed major reductions in the overall GWP.

Although earlier research indicated that feed production was the highest contributor to GHG emissions in pigs (Macleod et al. 2013), manure management in an LCA framework is also considered critical. However, emissions from manure are sometimes excluded in GWP calculations because it is assumed to be added as fertilizer to the farmland during the feed crop production (Zhou et al. 2018) and it has also been reported that pig manure generates more GWP than synthetic fertilizers (Stephen 2012). The inclusion of manure management in LCA is complex. Several factors and scenarios are required when considering the addition of manure to the LCA, such as storage, treatment, and differences in land application techniques (McAuliffe et al. 2016). In the study by Mackenzie et al. (2016), 30 % corn was replaced with wheat shorts, and the model assumed that the pig manure was kept in the barn for about 7 days before it was transferred to an outside storage and assumed to be applied twice to the land in autumn and spring.

There was a 12 % reduction in the GWP from the wheat shorts diet per kg of feed when compared to the corn control diet. This suggests that the application of manure from wheat by-products on the field as fertilizer could result in further savings and reduction in GWP.

5.6 Conclusion

This current study was designed to determine whether the inclusion of wheat millrun will reduce emissions when not only the pigs are considered but also other production processes and systems. The results suggest overall benefit in terms of environmental impact, in the use of wheat millrun in the diets of growing-finishing pigs when crop production, manure management, and emissions from the pigs are considered. It should be identified that quantifying all production systems that can have environmental impacts on pig production can be very challenging and very broad; however, the findings of this study adds to the knowledge base and helps towards identifying nutritional strategies to reduce environmental impacts in pig production.

5.7 Acknowledgments

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CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

6.1 General discussion

Livestock production contributes about 14.5 % of global anthropogenic GHG emissions and increases environmental burdens through the pollution of air, soil and water (Steinfeld et al. 2006; Gerber et al. 2013). Efforts to reduce GHG emissions from livestock production by increasing the efficiency of resource use has gained prominence (van Zanten 2016). Relative to ruminants, on a per kg meat basis, pig production poses less of a threat in terms of GHG emissions, however, pork is globally the most consumed meat (OECD and FAO 2019) thus overall emissions due to pork production are significant, and efforts to reduce these emissions merit consideration.

Production and utilization of feed are major factors influencing environmental impacts from livestock production (de Vries and de Boer 2010). Adjusting diet formulation, such as reducing CP content and the inclusion of feed additives, can reduce GHG emissions (Meul et al. 2012; Philippe and Nicks 2015). The use of "human grade" grains as the primary source of energy in pig diets increases competition for these grains and the cost of the feed (Woyengo et al. 2014; Jha and Berrocoso 2015). Pigs can convert co-products from the food and biofuel industries into pork (Zijlstra and Beltranena 2013b). Reducing the cost of production, coupled with the efficient use of co-products, has led to an increase in the inclusion of co-products in pig diets (Woyengo et al. 2014). Wheat millrun, which is made up of various wheat flour processing by-products, is commonly used in Western Canada as an alternative feedstuff for swine (Nortey et al. 2007a). However, co-products such as wheat millrun, are typically high in fibre. The fibre in co-products is not well hydrolyzed by the digestive enzymes of the pigs, limiting the availability of nutrients for the pigs (Woyengo and Nyachoti 2011). This can lead to a reduction in growth (Nortey et al. 2007b), increasing the duration of time required by the pigs to attain market weight. The inclusion of carbohydrase enzymes may improve fibre digestibility (Nortey et al. 2008; Zijlstra et al. 2010) by improving the availability of smaller oligosaccharides for digestion or fermentation. The low digestibility of the co-products also leads to fibre fermentation in the hindgut, which can increase CH_4 output (Philippe and Nicks 2015). Therefore, while swine producers are encouraged to increase co-products usage in their diets to reduce costs, it is critical to determine if this will negatively impact GHG mitigation efforts.

The studies in this thesis focused on determining if increasing the wheat millrun content in the diets fed to growing pigs will change overall emissions due to pork production. We used an LCA framework, which included feed production. Our theory was that GWP due to feed production would be lessened if increased usage was made of the co-products. The impact of a multi-carbohydrase enzyme on nutrient digestibility and the potential for mitigation of GHG emissions from the pigs was also considered.

Several studies were required to provide data required for the LCA framework. In the first study (Chapter 3), two experiments were conducted to determine the effect of increasing inclusion of wheat millrun supplemented with a multi-carbohydrase enzyme on nutrient digestibility, growth performance, feed intake and feed efficiency of growing pigs. In the first experiment, a growth performance trial, pigs from 60 to 128 kg BW, were fed a 0 % or 30 % wheat millrun diet without or with a multi-carbohydrase supplementation. The energy content of the high millrun diets was allowed to decrease, allowing for potential cost savings with the addition of the wheat millrun. The second experiment was a nutrient digestibility trial where pigs were fed diets with either 0 %, 15 %, or 30 % wheat millrun, again without or with multi-carbohydrase enzyme supplementation. They were housed individually in metabolism crates, which allow for fecal and urine collection.

Although the growth performance and nutrient digestibility were reduced, and there was a tendency for BW to be reduced in pigs fed the 30 % wheat millrun diets, the cumulative effect did not result in increased days to attain market weight relative to the pigs on the 0 % wheat millrun diets. This information is crucial in accounting for GHG production. In the second study, actual GHG production data was collected in an experiment where pigs were housed in specialized environmental chambers that allowed for the collection of air samples. The air samples were analyzed for CH_4, CO_2 and N_2O. Manure from the chambers was also collected, stored, and air samples collected and analyzed for CH_4, CO_2 and N_2O.

There was no significant difference in GHG production between the pigs fed the 0 % wheat millrun diets, and the pigs fed the 30 % wheat millrun diets. Enzyme supplementation also had no effect on GHG production.

The results from the study were contrary to the meta-analysis by Philippe and Nicks (2015), where they established a linear relationship between enteric CH_4 production and high fibre diets. Similarly, an increase in CO_2 production with these diets was observed in an earlier study (Philippe et al. 2009). Most of the studies which observed increases in CH_4 production with added dietary fibre included highly soluble fibre ingredients such as SBP (Clark et al. 2005; Philippe et al. 2009; Montalvo et al. 2013). Sugar beet pulp is a highly soluble fibre source, with 25 % of the TDF being soluble compared to the wheat millrun, which has just 6 % (Garcia et al. 2015; Wang et al. 2016). In our study, the proportion of SDF (19.8 %) and IDF (80.2 %) to the TDF were the same for the 0 % wheat millrun diets and the 30 % wheat millrun diets. Although the proportions were the same, the amount of IDF was higher in the wheat millrun diets. This could be the reason why we did not observe any significant difference in our study as compared to other reports. High variability in GHG emissions from pig slurry/manure was observed in our study as well as others (Wolter et al. 2004; Viguria et al. 2015).

The third study, which was an LCA framework study, involved the use of the Holos model, a whole farm LCA model and software program developed by Agriculture and Agri-Food Canada, to determine emissions from the production of treatment diets, and data from the performance study as well as the GHG production (chamber) study, to determine GHG emissions when wheat millrun is used as a feed ingredient in diets of growing pigs.

The key finding from the LCA framework study was a 25 % reduction in GHG emissions when pigs were fed 30 % wheat millrun diets. The reduction was mainly from feed production as emissions from the chamber study showed no significant differences when wheat millrun was fed to the growing pigs. The differences in the allocation of co-products during the estimation of GHG emissions (Meul et al. 2012) and the economic value of the co-products leads to savings in the usage of co-products in an LCA (Mackenzie et al. 2016).

The overall objective of this research was to investigate and determine if increasing the wheat millrun content of swine rations and, if the supplementation of the wheat millrun with a multi-carbohydrase enzyme will change the overall GHG emissions when the LCA considers just output inside the barn, or the entire production cycle, including feed production.

In summary, it was discovered from the studies that, feeding growing pigs up to 30 % wheat millrun diets decreased growth, but not sufficient to affect the number of days it took to reach market weight. There was also no significant difference in GHG (CH_4, CO_2, N_2O) emissions when pigs were fed 30 % wheat millrun diets.

6.2 Conclusion

There was no significant difference in GHG (CH_4, CO_2, N_2O) emissions when pigs were fed 30 % wheat millrun diets compared to the 0 % wheat millrun diets. However, the usage of wheat millrun, a co-product, which is not suitable for human consumption, reduced overall emissions by 25 % when substituted at 30 % at the expense of wheat in diets of growing-finishing pigs. Therefore, it can be concluded that the inclusion of up to 30 % wheat millrun in the diets of growing-finishing pigs will not only reduce the dependence on human-grade feed to feed pigs and reduce the overall cost of feed, but also reduce GHG emissions and environmental impact from the pig production industry when emissions from feed production are considered. This information is crucial as the impetus for sustainable pork production increases. This research will be beneficial to pork producers in western Canada, nutritionists, environmentalists, and policymakers.

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APPENDIX

A.1 Conversion of gas chromatography values and sample calculations

Below are examples of gas concentration values from gas chromatography of two sampling periods from the same treatment. The air samples were collected into a Tedlar bag and sub-sampled into two exetainer tubes

Baseline CO_2 concentration (ppm) = 1075, 1088

Day 14 CO_2 concentration (ppm) = 1288, 1276

The following outlines the conversion of the gas chromatography results (values) from ppm (or $\mu L\ L^{-1}$) to $mg\ m^{-3}$.

The formula used was:

Concentration ($mg\ m^{-3}$) = Concentration ($\mu L\ L^{-1}$) × [Molecular mass ($g\ mol^{-1}$) / Molar volume (L)] (Eq 1)

The molar volume of the gases at 1 atm and 25°C (standard ambient temperature and pressure) was 24.45 L.

Molecular mass for CO_2, N_2O = 44.01; CH_4 = 16.04

A.1.1 Sample calculation for gas concentration conversion

$$\text{Concentration (mg m}^{-3}) = \mu L\ L^{-1} \times (44.01\ g\ mol^{-1} / 24.45\ L)$$

$$\text{Concentration} = 1958.4\ mg\ m^{-3}$$

A.1.2 Sample calculation for emission rate

The following equations were used to calculate gas emissions:

$$E = C \times V \quad [4.1]$$

$$V = Q \times A \quad [4.2]$$

Where E=gas emissions ($mg\ s^{-1}$)

C = gas concentration in room measured close to the exhaust duct ($mg\ m^{-3}$)

V = volumetric airflow rate ($m^3\ s^{-1}$)

Q = airspeed (flow rate) measured with anemometer (3.49 m s^{-1})

A = Exhaust fan duct area (0.25 m^2).

$E_{d\ 14} = [((2318.4+2296.8)\ mg\ m^{-3}/2) \times (3.49\ m\ s^{-1} \times 0.25\ m^2))]$

$E = 2307.6\ mg\ m^{-3} \times 0.873\ m^3\ s^{-1}$

$E_{d\ 14} = 2014.5\ mg\ s^{-1}$

$E_{baseline} = [((1958.4+1935)\ mg\ m^{-3}/2) \times (1.00\ m\ s^{-1} \times 0.25\ m^2))]$

$E = 1946.7\ mg\ m^{-3} \times 0.25\ m^3\ s^{-1}$

$E_{baseline} = 486.7\ mg\ s^{-1}$

$E = E_{d\ 14} - E_{baseline}$

$E = 2014.5 - 486.7\ mg\ s^{-1}$

$E = 1527.8\ mg\ s^{-1}$

A.1.3 Sample calculation for GHG flux using regression analysis

Table A. 1 Sample of gas concentration data from headspace in barrels.

Time (min)	CO_2 concentration (ppm)
0	2844.47
5	3113.24
10	3243.06

$$F = \rho\ (V/A) \times (\Delta C/\Delta t) \qquad [4.3]$$

Where F = surface gas flux (μg m^{-2} s^{-1})

ρ = density of gas (1.96 kg m^{-3})

V = volume of headspace (0.032 m^3)

A = area of headspace (0.159 m^2)

ΔC/Δt = rate of change of gas concentration by regression (ppm min^{-1})

$F = 1.96\ (0.318/0.159) \times 39.859$

$F = 15.722\ (1000/60)$

$F = 262.03\ \mu g\ m^{-2}\ s^{-1}$

Illustration of variability in GHG concentration results from pigs housed in controlled conditions in environmental chambers.

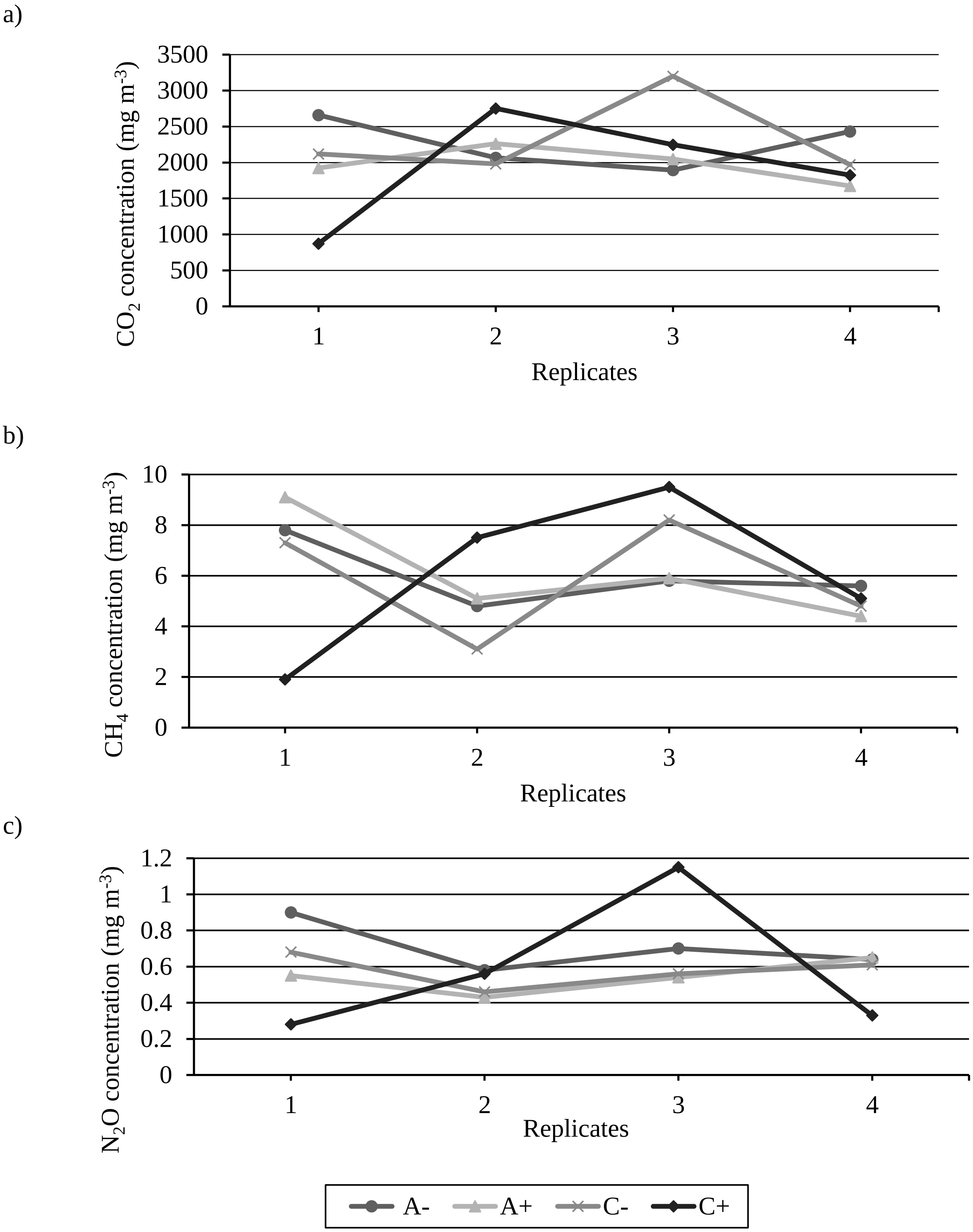

Figure A. 1 Concentration (mg m^{-3}) of (a) carbon dioxide, (b) methane, (c) nitrous oxide from gas chromatography (average of two gas chromatography concentrations for each treatment). A- (0 % wheat millrun; without enzyme), A+ (0 % wheat millrun; with enzyme), C- (30 % wheat millrun; without enzyme), C+ (30 % wheat millrun; without enzyme).