

**EFFECT OF FIELD PEA AND MULTICARBOHYDRASE ENZYMES
INCLUSION ON GROWTH, DIET DIGESTIBILITY, AND GREENHOUSE
GAS EMISSIONS OF FINISHING PIGS AND THEIR MANURE**

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By

JISMOL JOSE

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OR

Dean

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University of Saskatchewan

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Saskatoon, Saskatchewan, S7N 5C9 Canada.

ABSTRACT

Introduction of field pea into a crop rotation cycle can reduce the use of nitrogenous fertilizers. Nitrogen fertilizer production and application is one of the largest greenhouse gas (GHG) emission sources from crop production. Multicarbohydase enzymes improve diet digestibility by breaking down resistant starch and non-starch polysaccharides (NSPs), thereby reducing manure organic matter (OM). We hypothesized that the inclusion of field pea and multicarbohydase enzymes in a finisher pig diet would mitigate GHG emissions from pork production. Three studies were conducted to determine the effect of field pea and multicarbohydase enzymes inclusion in finisher pig diet on digestibility, growth performance and GHG emissions of pork production. Inclusion of 40% inclusion of field pea resulted in no difference in average daily gain (ADG), average daily feed disappearance (ADFD), gain to feed ratio (G:F), days to market or carcass traits compared to feeding a conventional wheat-barley based diet. Multicarbohydase enzymes inclusion had no effect on growth performance or carcass parameters. Inclusion of 20 and 40% field pea decreased apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), and N, but increased acid detergent fibre (ADF), neutral detergent fibre (NDF) digestibility compared with feeding the control diet. Inclusion of multicarbohydase enzymes resulted in an increase in ADF digestibility. There were no field pea-enzyme interactions on animal growth or nutrient digestibility. The addition of field pea to the diet resulted in a shift from urinary to fecal N excretion. The 40% dietary inclusion of field pea did not affect carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) production from pig housing and stored manure. Multicarbohydase enzymes inclusion reduced CO₂ emissions from pig housing, however manure associated emissions were unaffected. An LCI (life cycle inventory) evaluating emissions from combined crop production and pig housing found a 13% reduction in global warming potential (GWP). The GWP from pig housing increased with 40% inclusion of field pea, but multicarbohydase enzymes inclusion showed a decrease. The inclusion of up to 40% field pea and multicarbohydase enzymes to the diet of finishing pigs has the potential to reduce overall GHG output of pork production.

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DEDICATION

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TABLE OF CONTENTS

PERMISSION TO USE STATEMENT	ii
DISCLAIMER.....	iii
ABSTRACT.....	iv
ACKNOWLEDGEMENTS	v
DEDICATION.....	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER 1. INTRODUCTION.....	1
CHAPTER 2. LITERATURE REVIEW	3
2.1 <i>General background</i>	3
2.1.1 <i>Role of agriculture and livestock sector</i>	3
2.1.2 <i>Overview of GHG emission from the pig sector</i>	4
2.1.3 <i>Role of feed production</i>	4
2.1.4 <i>Sources of GHG emissions in swine facilities</i>	4
2.2 <i>Field pea in animal nutrition</i>	5
2.2.1 <i>Nutritional value of field pea for swine</i>	5
2.2.2 <i>Anti-nutritional factors</i>	5
2.2.3 <i>Field pea and animal performance</i>	6
2.2.4 <i>Field pea and nutrient digestibility</i>	7
2.2.5 <i>Potential for field pea to mitigate GHG emissions</i>	8
2.3 <i>Enzymes in animal feed</i>	8
2.3.1 <i>Multienzyme combinations</i>	9
2.3.2 <i>Enzyme activity and age of animal</i>	10
2.3.3 <i>Multicarbohydase and animal performance</i>	10
2.3.4 <i>Multicarbohydase and nutrient digestibility</i>	11
2.4 <i>Multicarbohydase and field pea</i>	12
2.5 <i>Estimation of GHG emission and Life Cycle Analysis</i>	12
CHAPTER 3. HYPOTHESES AND OBJECTIVES.....	14
3.1 <i>Research Hypotheses</i>	15
3.2 <i>Objectives</i>	15

CHAPTER 4. GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF FINISHER PIGS FED FIELD PEA BASED DIET SUPPLEMENTED WITH MULTICARBOHYDRASE ENZYMES	16
4.1 Abstract	17
4.2 Introduction	18
4.3 Methodology.....	19
4.3.1 Diets.....	19
4.3.2 Experiment 1	20
4.3.2.1 Animals and Housing.....	20
4.3.2.2 Treatments and Feeding	20
4.3.2.3 Sampling and Data Collection.....	21
4.3.2.4 Statistics.....	21
4.3.3 Experiment 2.....	22
4.3.3.1 Animals and Housing.....	22
4.3.3.2 Treatments and Feeding	22
4.3.3.3 Sampling and Data Collection	22
4.3.3.4 Chemical Analyses and calculations	23
4.3.3.5 Statistics.....	23
4.4 Results	23
4.5 Discussion	24
4.6 Conclusion	27
CHAPTER 5. THE EFFECT OF DIETARY INCLUSION OF FIELD PEA AND MULTICARBOHYDRASE ENZYMES ON GHG EMISSIONS OF FINISHING PIGS AND THEIR MANURE SLURRY.....	35
5.1 Abstract	36
5.2 Introduction	37
5.3 Methodology.....	38
5.3.1 Animals and housing.....	38
5.3.2 Experimental design	39
5.3.3 Sampling and data collection	42
5.3.3.1 Gas sampling from animals housed in the chamber.....	42
5.3.3.2 Stored Manure Sampling	44
5.3.4 Calculations.....	44

5.3.5	<i>Statistics</i>	45
5.4	<i>Results and Discussion</i>	45
5.5	<i>Conclusion</i>	48
CHAPTER 6. EFFECT OF FIELD PEA AND CARBOHYDRASE ENZYMES IN FINISHING PIG DIETS ON GHG EMISSIONS FROM CROP AND ANIMAL PRODUCTION USING A USING A LIFE CYCLE INVENTORY		53
6.1	<i>Abstract</i>	54
6.2	<i>Introduction</i>	55
6.3	<i>Methodology</i>	56
6.3.1	<i>Inventory of environmental inputs and outputs</i>	56
6.3.2	<i>LCI for crop production</i>	58
6.3.2.1	<i>Farm inputs and assumptions for Holo</i>	60
6.3.2.2	<i>Crop inputs</i>	60
6.3.3	<i>LCI for Animal production</i>	62
6.3.3.1	<i>GHG output from pigs and manure</i>	62
6.4	<i>Results and Discussion</i>	63
6.5	<i>Conclusion</i>	65
CHAPTER 7. GENERAL DISCUSSION AND CONCLUSION		69
7.1	<i>General Discussion</i>	70
7.2	<i>Conclusion</i>	73
REFERENCES		74

LIST OF TABLES

Table 4.1 Ingredient and nutrient composition (as fed basis) of the 0%, 20%, and 40% field pea diets for the phase1 (60-90 kg) ^{a,b} growth performance and digestibility study	28
Table 4.2 Ingredient and nutrient composition (as fed basis) of the 0%, and 40% field pea diets for the phase2 (90-127 kg) ^a growth performance study	30
Table 4.3 Effect of high inclusion field pea and carbohydrase supplementation on growth performance of finisher pigs (Experiment 1) ^a	31
Table 4.4 Effect of inclusion of field pea and carbohydrase supplementation on carcass parameters of finisher pigs (Experiment 1)	32
Table 4.5 Effect of high inclusion field pea and carbohydrase supplementation on apparent total tract digestibility of nutrients (Experiment 2) ^a	33
Table 4.6 Effect of high inclusion of field pea and carbohydrase supplementation on N intake, excretion, and retention (Experiment 2) ^a	34
Table 5.1 Ingredient and nutrient composition (as fed basis) of the 0%, and 40% field pea diets fed in the chamber experiment ^a	41
Table 5.2 Effect of 40%inclusion of field pea and multicarbohydrase enzyme on N ₂ O, CH ₄ , CO ₂ emissions from pigs housed in environmental chamber ^{a,b}	51
Table 5.3. Effect of inclusion of 40% field pea and multicarbohydrase enzymes supplementation on N ₂ O, CH ₄ , CO ₂ emissions from stored manure ^{a,b,c}	52
Table 6.1 Ingredient composition of Phase 1 and Phase 2 diets fed in the growth performance study and chamber experiment at PSCI ^{a b}	59
Table 6.2 Yield and fertilizer application rate of feed ingredients as inputs for the Holos model for developing the LCI for crop production ^a	66
Table 6.3 The GWP calculated from emissions associated with crop production of 100 finisher pigs from 60 ± 2.2kg to market ^{a,b,c}	67
Table 6.4 The GWP calculated from finishing pigs fed 0% and 40% field pea diet with or without multicarbohydrase enzymes housed in environmental chambers ^{a b}	68

LIST OF FIGURES

Figure 5.1 Schematic representation of the environmental chamber (acquired from Alvarado, 2011)	39
Figure 5.2 Vacuum sampling apparatus.....	43
Figure 5.3 Schematic representation of the vacuum sampling apparatus (taken from Alvarado (2011)).....	43
Figure 5.4 Water consumption by pigs housed in environmental chambers for 14 d. Values are means of 4 replicates with 6 pigs per treatment. Means with same letters are not significantly different ($P>0.1$).....	49
Figure 5.5 Slurry (feces and urine) output by pigs housed in environmental chambers for 14 d. Values are means of 4 replicates with 6 pigs per treatment. Means with same letters are not significantly different ($P>0.1$).....	50
Figure 6.1 Schematic representation of inputs and outputs of a pork production unit	57
Figure 6.2 Generic farm information input form	60
Figure 6.3 Case ration (control diet) parameters for obtaining the inputs for Holos crop form. .	61
Figure 6.4 Holos annual crops form.....	62

LIST OF ABBREVIATIONS

AIA	Acid insoluble ash
AA	Amino acid
NH ₃	Ammonia
ANF	Anti nutritional factors
AID	Apparent ileal digestibility
ATTD	Apparent total tract digestibility
Arg	Arginine
AOAC	Association of Analytical Chemists
ADFD	Average daily feed disappearance
ADG	Average daily gain
BN	Bacterial nitrogen
BW	Body weight
CCAC	Canadian Council on Animal Care
CO ₂	Carbon dioxide
CTTAD	Coefficient of total tract apparent digestibility
CP	Crude protein
Cys	Cysteine
d	Day/days
DE	Digestible energy
DDGS	Distiller dried grains and solubles
DM	Dry matter
EAA	Essential amino acid
FAO	Food and Agriculture Organisation
G:F	Gain to feed ratio
GC	Gas chromatography
GHG	Greenhouse gas
GE	Gross energy
IDF	Insoluble dietary fibre
IPCC	Intergovernmental Panel on Climate Change

kg	Kilogram
Leu	Leucine
LCA	Life cycle assessment/analyses
LCI	Life cycle inventory
L	Litre
Lys	Lysine
CH ₄	Methane
Met	Methionine
m	Metre
µg	Microgram
NRC	National Research Council
NE	Net energy
NDF	Neutral detergent fibre
N	Nitrogen
N ₂ O	Nitrous oxide gas
NSP	Non-starch polysaccharides
OM	Organic matter
PPC	Pea protein concentrate
PSCI	Prairie Swine Centre Inc.
RCBD	Randomized complete block design
SCFA	Short chain fatty acid
SDF	Soluble dietary fibre
SBM	Soybean meal
SID	Standard ileal digestibility
SAS	Statistical analysis system
Thr	Threonine
TDF	Total dietary fibre
Tis	Trypsin inhibitors
Trp	Tryptophan
VFA	Volatile fatty acids

CHAPTER 1. INTRODUCTION

It is evident that anthropogenic effects have increased global mean surface temperature and are responsible for extensive precipitation changes, extreme hot and cold weather, arctic ice loss, and oceanic salinity changes (Eyring et al. 2021). The human population is predicted to reach 11 billion by 2100 (Rauw et al. 2020). The increasing human population and its demand for food has led to intensification of livestock and crop production, resulting in deforestation and land use changes, contributing to gas emissions to the atmosphere. A collective action from all sectors is required to protect the sustenance of our planet while feeding the growing human population.

Tubiello et al. (2015) found that agriculture, forestry, and land use and land use change activities contributed to 21% of human induced greenhouse gas (GHG) emissions in 2010. Notably, the net emissions from agriculture could be reduced if carbon sequestration from the crops was included in the models (Baum and Bieńkowski 2020). However, agriculture consumes about 70% of the fresh water and takes up to 40% of the land area globally (Panchasara et al. 2021). Relative to the pre-industrial period, atmospheric levels of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) have increased 147%, 259% and 123%, respectively (World Meteorological Organization (WMO) 2019). Livestock production, a major subsection of agriculture, is responsible for 18% of anthropogenic GHG emissions (Steinfeld et al. 2006). Products from ruminants are more carbon intensive relative to those from monogastrics (Dyer et al. 2010; Gerber et al. 2013) however, as pork is the most consumed meat worldwide (Steinfeld et al. 2006), emissions resulting from production of pork should be addressed and mitigation strategies adopted.

The overall objective of this research was to quantify the potential of dietary changes in feedstuffs to mitigate GHG emission from finisher pigs, specifically high inclusion of field pea (*Pisum sativum*) and exogenous feed multicarbohydase enzymes in the diet. Field pea, or legumes in general, convert atmospheric nitrogen (N) to ammonia (NH₃), utilizing the symbiotic relationship with rhizobia in their root nodules. This reduces the use of nitrogenous fertilizers in current and subsequent crop production (Lemke et al. 2007; Macleod et al. 2013). Thus it can be hypothesized that including field pea into swine diets to replace carbon-intensive cereal grains may result in a reduction in the carbon footprint of pork production. Multicarbohydase enzymes break

down resistant starch, complex carbohydrates, and fibre diets improving nutrient digestibility and feed efficiency which could also reduce GHG output. Although animal performance and nutrient digestibility studies have been conducted including field pea (Stein et al. 2006; Landero et al. 2014) or multicarbohydase enzymes in the diet of pigs (Cowieson et al. 2003; Brooks et al. 2009), the effect of this combination on growth, digestibility and GHG emissions has not been evaluated.

The first study in this thesis focused on growth performance, carcass parameters, and nutrient digestibility when feeding field pea, with and without multicarbohydase enzymes included in the diet of finisher pigs. The second experiment determined the dietary effect of field pea and multienzyme inclusion on emissions from finisher pigs housed in environmental chambers. Finally, the results from the first two studies were used to develop a life cycle inventory (LCI) for emissions from crop production and finisher pigs, which is detailed in the last chapter.

CHAPTER 2. LITERATURE REVIEW

2.1 General background

The rise in population and its effect on the environment has been the subject of intense debate and study over the past several decades (Panayotou 2000). One concern is providing food for this growing population in an environmentally sustainable manner. Scientists are trying to find tools to sustain nutrient output while reducing GHG emissions. Greenhouse gases are those gaseous constituents in the atmosphere that absorb and emit infrared radiation and thereby may influence earth's climate (Montzka et al. 2011). Water vapor, CO₂, CH₄, N₂O, and ozone are the important GHGs present in earth's atmosphere (Easterbrook 2016). Greenhouse gas emissions from natural as well as anthropogenic sources are responsible for an overall rise (0.6° C over the 20th century) in surface temperature of the earth resulting in rising sea levels, Arctic ice loss, shrinking glaciers, ocean acidification etc. (Vijayavenkataraman et al. 2012; Yusuf et al. 2012). The high carbon footprint associated with the production of crops to produce animal protein is widely criticized due to its inefficiencies (Reijnders and Soret 2003). More than two thirds of the energy consumed by livestock is used for maintenance or is lost in manure, skin and bones (Röös et al. 2013). Mitigation strategies at every stage of meat production are therefore imperative. Livestock diet modification is one such strategy being employed. Introduction of ingredients with a reduced carbon footprint, such as legumes and the use of technologies such as resistant starch and fibre degrading enzymes may reduce the GHG emission from meat or specifically pork production.

2.1.1 Role of agriculture and livestock sector

Agriculture, specifically the livestock sector, contributes significantly to overall GHG output. According to Garnett (2011), the direct emissions from agriculture contribute 10 to 12% of global emissions and when indirect sources of emissions such as fuel use, fertilizer production and agriculture-induced land use are included the figure goes up to 30%. The livestock sector is alone responsible for 18% of anthropogenic GHG emissions (Steinfeld et al. 2006). The discrepancies in the distribution of emissions in the above examples illustrates the challenges associated with data-intensive studies. From 2005 to 2050 the demand for livestock products, especially meat and milk is expected to increase by 70%, hence the industry must address the growing population demands as well as its related environmental concerns (Macleod et al. 2013).

2.1.2 Overview of GHG emission from the pig sector

Beef and dairy cattle are the major contributors to GHG emissions from the livestock sector and responsible for 65% of emissions (Gerber et al. 2013). The protein-based emission intensity of GHGs from pork production is low (~ 12%) relative to other livestock commodities such as beef and milk (Dyer et al. 2010). However, pork is the most widely consumed meat (Food and Agriculture Organisation (FAO) 2011) accounting for 37% of total meat produced in 2010. A 32% increase in pork production is anticipated by the end of 2030 (Macleod et al. 2013). Therefore, it can be assumed that by adopting mitigation strategies, pork could supplement human protein needs with a reduced carbon footprint.

2.1.3 Role of feed production

It is estimated that feed production constitutes the majority of total GHG emissions from pork production. However, many studies have not considered carbon sequestration in the calculations of the carbon footprint of livestock. Inclusion of carbon sequestration into the calculation of the GWP of resulted in a net decrease in the GWP associated with crop production for animal feed (Baum and Bieńkowski 2020). However, Macleod et al. (2013) conducted a global life cycle assessment and found that feed production accounted for 60 % of total GHG emissions, followed by manure storage or production (27 %), post farm processing activities (27 %), transportation (6 %), enteric methane production (3 %) and various direct and indirect uses of energy (3 %). Similarly, in a study assessing GHG emission intensity and its fluctuation in Norwegian pig production, Bonesmo et al. (2012) found that 80 % of the GHG emission was from the production of feed. The indirect effect of feed production for intensive livestock farming arises mainly from land use change and transportation of crops (Bellarby et al. 2008). Moreover, with a change in feeding practices from grazing to the consumption of feed crops, livestock is the largest user of land (Bellarby et al. 2008). These increasing demands for land for feed production and grazing can result in deforestation and the threat of desertification (Steinfeld et al. 2006).

2.1.4 Sources of GHG emissions in swine facilities

The major GHGs produced from swine production facilities are CO₂, CH₄ and N₂O. Expiration by pigs and release from manure are the two sources of CO₂ whereas bacterial anaerobic degradation of organic matter in the large intestine and manure are responsible for CH₄ emissions.

Incomplete nitrification/denitrification processes by micro-organisms in the manure results in N₂O production (Philippe and Nicks 2015).

2.2 Field pea in animal nutrition

Swine producers are looking for strategies to incorporate alternative feedstuffs into swine rations to reduce feed cost (Woyengo et al. 2014). Field pea is an alternative local ingredient that is competitive in some markets. Canada is the largest pea producer and exporter in the world (Food and Agriculture Statistics database 2019). The replacement of mostly imported soybean meal (**SBM**) with locally-grown field pea in swine rations can be economically feasible, especially when low food grade field pea (splits) or surplus production is available (Landro et al. 2014). Furthermore, inclusion of locally grown pulses into Western Canadian swine diets would reduce dependence on importation of feedstuffs such as soybean and allow the environmental benefits of integrated crop-livestock systems to be realized (Oryschak and Beltranena 2020).

2.2.1 Nutritional value of field pea for swine

The major nutrient in field pea is starch (45.5 – 47.4%) and pea starch is rich in amylose (Shen et al. 2016). Pea starch, relative to cereal starch, is less digestible because of the high amylose content (Jha et al. 2011) Field pea is a good source of essential amino acids (**AA**) and have an average crude protein (**CP**) content of 245 g/kg (Canibe and Eggum 1997; Cowieson et al. 2003). The nutrient profile of peas and cereals compliment each other, as peas are rich in lysine (**Lys**), but deficient in the sulphur containing AAs, whereas cereals are low in Lys (Mariscal-Landín et al. 2002). The two main proteins in pulses are globulins and albumins. Globulins constitute more than 60 to 90% of the protein fraction; albumins constitute 10 to 20% (Mariscal-Landín et al. 2002). Arginine (**Arg**), Leucine (**Leu**), and Lys are the predominant essential amino acids (**EAA**) present in globulins and Lys is the predominant EAA in albumin (Leterme et al. 1990). Albumins contain more threonine (**Thr**), tryptophan (**Trp**) and sulphur containing AA's methionine (**Met**) and cysteine (**Cys**) than globulins. However, the low concentration and relatively low digestibility of albumin when compared with other protein fractions explains the deficiency of Thr, Trp, Met, and Cys in legume grains (Le Guen et al. 1995).

2.2.2 Anti-nutritional factors

High inclusion of field pea in swine rations increases the level of anti-nutritional factors (**ANF**), primarily, trypsin inhibitors (**TIs**), lectins, tannins, α -galactosides and alkaloids in the diet

(Mariscal-Landín et al. 2002). These ANF interfere with the digestion and utilization of protein and other essential nutrients (Huisman et al. 1992). However, when field pea were added to a swine diet supplying 50% of CP and AA, Jezierny et al. (2011) found that the standard ileal digestibility (**SID**) of CP and AA were not affected by ANF content in the pea diet. With advancements in selection and plant breeding programmes, the ANF level in grain legumes has been reduced, resulting in the development of low (zero-) tannin faba bean cultivars and very low alkaloid lupin varieties (Jezierny et al. 2011). According to Gunawardena et al. (2010) grower pigs with a mature gastrointestinal tract are able to tolerate the ANF to a certain extent. Moreover, anti-nutritional factors like TI and lectins are heat labile, and may be partially inactivated by thermal treatments such as pelleting and extrusion (Camire 1991). For example, digestibility of Try and Cys were improved when grower pigs were fed a diet high in extruded field pea compared with pigs fed non-extruded field pea (Mariscal-Landín et al., 2002). Similarly, Stein and Bohlke (2007) reported improved CP, AA, starch and energy digestibility extruded field pea were fed to pigs. Hence, it can be concluded that the level of ANF content should not be a strict limiting factor to the inclusion of field pea in swine diets.

2.2.3 *Field pea and animal performance*

Feed ingredients chosen as part of animal diets should not only maintain but also improve the growth performance of animals. Presence of ANF and deficiency of sulphur-containing AA may challenge growth in young animals with high inclusion of field pea into swine rations. However, inclusion of up to 40 % field pea in weanling pig diets did not affect average daily feed disappearance (**ADFD**), average daily gain (**ADG**) or gain-to-feed (**G:F**), when diets were formulated to comparable net energy (**NE**) and SID Lys levels including crystal amino acids (Landerio et al. 2014). Conversely, replacing corn and SBM with more than 36% field pea in diets formulated to similar metabolizable energy (**ME**) value and SID AA content reduced ADG of weaned pigs (9 to 24 kg BW) by 3% (Stein et al. 2010). Similarly, inclusion of 30% raw field pea in diets formulated to similar digestible energy (**DE**) value and total AA content as that of a positive control, decreased ADG of weaned pigs by 6% (Friesen et al. 2006). Stein et al. (2006) conducted a study to determine the effect of inclusion level of field pea in grower and finisher pig diets and found that an inclusion of 66 % field pea, completely replacing SBM, had no effect on growth performance, carcass composition, carcass quality or pork palatability. Similar results were observed on animal performance by Newman et al. (2011) including 45% field pea. Furthermore,

when SBM was partially replaced with 20% field pea in diets formulated to similar DE value and CP content, inclusion did not affect growth performance and Parma ham quality of finisher pigs (55 to 160 kg BW) (Gatta et al. 2013).

Thus, high inclusion of field pea in finisher diets is feasible provided cost per MJ NE and (or) g SID AA is lower than imported SBM, canola meal or cake or cereal grains. Inclusion of field pea in nursery pig diets, however, should be approached with caution as their immature digestive systems may not be capable of handling ANF content without progressive dietary adaptation.

2.2.4 Field pea and nutrient digestibility

Inclusion of field pea partially or completely replacing dehulled SBM in the diet of pigs increases the fibre content of the diet (Landerio et al. 2014) potentially decreasing nutrient digestibility. However, both resistant starch and largely soluble fibre can be utilized by microbes in large intestine that ferment them to products like short chain fatty acids (**SCFA**), which may contribute to dietary energy (Bakker et al. 1998). When piglets were fed diets formulated with either pea protein concentrate, SBM, full-fat soya bean or soya protein concentrate it was observed that the coefficient of total tract apparent digestibility (**CTTAD**) of organic matter (**OM**), gross energy (**GE**), ether extract (**EE**), CP was not affected by dietary protein source despite varying fibre content of the pea sources (Valencia et al. 2008). Nevertheless, the coefficient of ileal apparent digestibility of OM and GE was greater for diets containing dehulled SBM and soya protein concentrate (Valencia et al. 2008). Canibe and Eggum (1997) examined the effect of toasting on nutrient digestibility of field pea and reported that the apparent ileal digestibility (**AID**) of nutrients (dry matter (**DM**) and energy) in the toasted field pea diet was greater, relative to the dried pea diet, though this effect was not observed for total tract digestibility. In another study, replacing SBM with field pea in the diet of nursery pigs resulted in a linear decrease in CTTAD of DM, CP and NE and reduced growth (Landerio et al. 2014). The reduction in nutrient digestibility in the second phase of study was less pronounced than in the first phase suggesting improved digestibility of the field pea as pigs got older.

Hence, it can be deduced that adverse effects of field pea inclusion diminish with pig age as the GI tract matures and microbiota change with dietary adaptation. Moreover, reduced energy digestibility observed at the terminal ileum can be partially compensated at the total tract level through hind gut fermentation and production of VFA. Although field pea have a greater fibre

content relative to dehulled SBM, they are a good feedstuff for pigs because of reduced rapid fermentation which promotes more bacterial nitrogen incorporation, and helps in sustaining bacterial diversity, yielding beneficial fermentation products (Jha et al. 2011).

2.2.5 Potential for field pea to mitigate GHG emissions

One of the potential benefits of the inclusion of field pea in swine ration comes from symbiotic association of rhizobia with pea roots resulting in the ability to fix atmospheric N (Chen et al. 2021). This symbiotic association results in a reduction in the use of N fertilizers over consecutive years and associated GHG emissions. The biological fixation of N by pulse root nodules not only reduces the fertilizer N requirement for the pulse crop but also reduces the fertilizer requirement for the next cereal or oilseed crop in yearly crop rotations and hence substituting a cereal or oilseed with a pulse crop in yearly rotation might further lower GHG emission (Lemke et al. 2007). Conceivably, this could have an impact on GHG from pork production as 17% of total emissions from the pig sector is accounted for by the application of inorganic and organic nitrogenous fertilizers for crop production (Macleod et al. 2013).

Fecal N is less volatile relative to urinary N (Zervas and Zijlstra 2002). Added fibre in the diet can shift N excretion from urine to feces (Morgan and Whittemore 1988). Fibre fermentation by the hind gut microbiota produces volatile fatty acids (**VFA**) (Jha and Leterme 2012) promoting the growth of “beneficial” microbes (Verstegen and Williams 2002). These microbes would utilize ammonia for bacterial protein synthesis which is subsequently excreted in feces (Zervas and Zijlstra 2002). Due to the high fermentation rate of pea resistant starch and fibre, significant amounts of VFAs are produced which in turn results in high bacterial nitrogen (**BN**) retention. Thus it is hypothesized that the dietary inclusion of field pea as an alternative protein in swine rations could also reduce overall N excretion into the environment (Jha et al. 2011).

2.3 Enzymes in animal feed

The use of exogenous enzymes in animal feed dates back to early 1950s, when amylases and proteases were first added to livestock diets (Adeola and Cowieson 2011). Enzymes used in animal diets are naturally produced by fungi and bacteria as secondary metabolites required to survive in their ecological niches (Schmoll and Schuster 2010). Different fermentation and purification techniques are used to commercially produce the specific enzyme from selected

microbes (Kirk et al. 2002). Genetic engineering techniques have allowed the production of industrial “workhorse” bacteria or fungi, which are chemically engineered to produce a particular molecule. The commonly used microbes in the enzyme industry are *Trichoderma reesei*, *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus subtilis*, and *Sacharomyces cerevisiae* (Adeola and Cowieson 2011). The most common group of enzymes used in animal industry are phytases and carbohydrases, specifically xylanases and glucanases (Adeola and Cowieson 2011). Phytase breaks down the complex phytic acid molecule, liberating primarily inorganic phosphorus (**P**) for absorption by monogastric (Olukosi et al. 2007b). Carbohydrases catalyse the hydrolysis of carbohydrate polymers to shorter chain oligosaccharides available for fermentation.

2.3.1 Multienzyme combinations

Carbohydrases, phytase and other enzymes can be included into animal feed either as single or multienzyme combinations. The wide array of non-conventional feed ingredients and by-products used in feed production results in variable intake of ANF and NSP. Therefore the effectiveness of multienzyme combinations is more beneficial than using a single enzyme (Olukosi et al. 2007b). A common example is the inclusion of phytase and xylanase combinations in wheat-based diets. Theoretically, xylanases can hydrolyze the complex arabinoxylans in ground wheat endosperm and liberate encapsulated phytic acid molecules which would then be exposed to phytase(s) for break down (Woyengo et al. 2008). In a study by Woyengo et al. (2008) designed to determine the effect of phytase and xylanase alone or in combination when added to wheat-based diet for grower pigs, comparable ADFD, ADG, and G:F was observed regardless of treatment. A possible explanation for this given by the authors was insufficient reduction of dietary P in the experimental diets and greater endogenous phytase activity of the enzyme supplemented diets. However, in another study by Olukosi et al. (2007b) supplementation of an enzyme combination of xylanase, amylase and protease improved the growth performance whereas the supplementation of xylanase alone made no difference in animal performance. The different responses observed in the two studies could be due to several factors such as age of animals, diet composition, endogenous enzyme activity of the diet, feed processing, and the type and concentration of enzymes. Another example of enzyme combination is the use of multicarbohydrases. Emiola et al. (2009) found that the negative effects on growth performance and nutrient digestibility when grower pigs were fed a diet based on wheat distiller dried grains and solubles (**DDGS**) low in both energy and protein, was alleviated by the inclusion of

carbohydrase enzymes such as xylanase, glucanase and cellulase. In contrast when a wheat-based diet supplemented with or without either a xylanase mono-enzyme preparation or a multi-carbohydrase preparation was fed to 21-day old piglets, there were no difference in animal performance between enzyme groups (Vahjen et al. 2007). This study measured the intestinal viscosity and found that feeding the control diet and multi-enzyme supplemented diet resulted in similar viscosities, perhaps indicating inactivation of enzyme activity during intestinal passage.

2.3.2 *Enzyme activity and age of animal*

Age of the animal can be an important factor in determining enzyme response. Overall growth and growth of the digestive system in the piglet are allometric as the pancreatic and mucosal tissue grow at an accelerated rate relative to overall body weight, as do their products, the digestive enzymes (Lindemann et al. 1986). Iji et al. (2001) reported that increase in the height of villi and length of intestine as an animal grows is proportional to increased enzyme activity. It can therefore be assumed that dietary exogenous enzymes may be more important for nursery-grower pigs compared with finisher pigs. In support of this argument, inclusion of multicarbohydrase enzymes into post-weaning pig diets including cassava residue and sweet potato vines improved ADG and CTTAD of CP, CF, neutral detergent fibre (**NDF**), OM, and GE, whereas the enzymes failed to show any effect on animal performance or nutrient digestibility, except CP, when included in the diets of growing pigs (Ngoc et al. 2011).

2.3.3 *Multicarbohydrase and animal performance*

Exogenous enzymes are extensively fed in poultry and animal nutrition to enhance growth performance; however, the results are inconsistent. In a study conducted by Mavromichalis et al. (2000), when xylanase was included in a wheat-based diet, there was no effect on nursery pig performance. Similar results were found by Olukosi et al. (2007a), when newly weaned pigs were fed diets containing graded levels of xylanase. In the former study the lack of enzyme response was attributed to the low to medium concentration of pentosans in the wheat fed. In the latter experiment, wheat was added only at the rate of 200g/kg, which might not have contributed sufficient ANFs to cause a reduction in animal performance. Conversely, when 25 day old pigs were fed a corn-SBM and multicarbohydrase supplemented diet for 28 days, ADG and G:F were improved (Omogbenigun et al. 2004).

The studies examining the use of enzymes in livestock feed have used different concentrations and combination of enzymes, different feed ingredients with different fibre profiles,

and animals of different age groups, making it difficult to define an appropriate concentration of enzymes for a particular species or age group. In a study conducted by Cervantes et al. (2004) the AID values of CP and AA were greatest with xylanase inclusion at 11,000 U/kg than when xylanase was included at 16,500 U/kg; AID values were reduced. In a study conducted by Emiola et al. (2009) a negative control diet was supplemented with either a low (2600 U xylanase, 1200 U β -glucanase and 1,300 U cellulase) or high (twice the activities) level of multicarbohydrase enzyme. Although, a significant difference in animal performance was observed between the negative control and enzyme supplemented diets, there was no difference between the low and high levels of enzyme inclusion (Emiola et al. 2009). Apparently, optimum enzyme concentration or an equilibrium between enzyme and substrate concentration is important to achieve the desired effect in animal performance as well as nutrient digestibility (Ravindran 2013).

2.3.4 Multicarbohydrase and nutrient digestibility

The effect of multicarbohydrases on digestibility primarily depends on dietary fibre type and content. Dietary fibres are complex carbohydrates for which endogenous enzymes are incapable of hydrolyzing, thus leave the small intestine undigested and are subjected to bacterial fermentation in the hindgut. In a study conducted by Cervantes et al. (2004) xylanase improved the AID of CP and most of the AA when the growing pigs were fed a diet formulated solely with wheat as protein and energy source. The ability of xylanase to increase the digestibility of arabinoxylans and thereby reducing the viscosity of digesta probably elicited the above response. Multi-enzyme supplementation of amylase, glucanase, cellulase and protease to a diet containing high fibre ingredients (cassava res/sweet potato vines) improved the total tract apparent digestibility in post-weaning pigs (30 to 60 days of age) (Ngoc et al. 2011). Trends for greater digestibilities of DM and N when xylanase was added to wheat-based diet was observed by Mavromichalis et al. (2000). In another study conducted by Olukosi et al. (2007a) when 10 kg pigs were fed a diet containing corn (20%), wheat and rye (45%) and SBM (30%) graded levels of xylanase (0, 400, 800, 1600, 3200, 32,000 U/kg) resulted in a linear improvement on digestibility of DM, CP, and energy. However, there was no effect of enzyme inclusions on growth performance. Hence the improvement in digestibility of nutrients not always results in an improvement in growth performance.

2.4 Multicarbohydase and field pea

While there is extensive literature regarding the use of multicarbohydases in animal feed as well as feeding field pea in swine rations, few are the published reports on the efficacy of multicarbohydases in high field pea diets. Inclusion of 20% field pea and carbohydrase enzyme into a corn-soybean diet replacing 35% corn and 7% soybean improved ADG, ADFD and G:F of nursery pigs, although there was no effect of enzymes (Brooks et al. 2009). Enzymes are highly substrate-specific (Ravindran 2013), therefore it is important to choose enzymes on the basis of the ingredient selection. For instance, grain legumes like field pea contain more starch and oligosaccharides than cereal grains and inclusion of enzymes like amylase and α -galactosidase would be appropriate (Ravindran 2013). Brooks et al. (2009), compared the soluble and insoluble monosaccharides in field pea and corn and found the arabinose, xylose, galactose, glucose and arabinoxylane content to be greater in field pea. Coweison et al. (2003) found that when broiler chicks were fed a diet containing 30% field pea, they had poor growth performance and this negative effect on growth was partially ameliorated by the inclusion of multicarbohydase enzymes and the hydrolysis of the arabinoxylan by xylanase.

2.5 Estimation of GHG emission and Life Cycle Analysis

The biggest challenge associated with GHG emissions studies is the variability that exists in pork production system. This includes the variability in farming system, diet and feeding practices, manure management, variability associated with animals and their genetics, geographical variability and finally the uncertainties in GHG estimation. Life cycle analysis is a comprehensive approach to estimate the carbon footprint of a product by considering the resources used at each stage of its production (Reckmann et al. 2013). The quantitative representation of the environmental burden of the product in an LCA is the functional unit (de Vries and de Boer 2010). Based on the assessment method LCA can be either attributional or consequential (Ekvall 2019). Attributional LCA determines the environmental burden associated with the production of a specified amount of functional unit and a consequential LCA determines how a change in output of the functional unit would affect the impact categories (Thomassen and Dalgaard 2008). The commonly performed assessment in livestock studies are attributional LCA (Thomassen and Dalgaard 2008). Any LCA study is comprised of the following: goal and scope, life cycle inventory (**LCI**) analysis, impact assessment and lastly interpretation. The input and output of a production system that is the raw materials used and substances emitted at each stage is carefully studied in

an LCI and this determines the quality of an LCA (Werf et al. 2007). Although LCA is a robust tool in determining the environmental impact, uncertainty arising either from the data, scenarios or model often downgrades the confidence of an LCA (Bamber et al. 2020).

In summary, techniques like addition of feed enzymes and inclusion of alternate ingredients to replace conventional feed ingredients in diets of finisher pigs may improve animal performance and its economic benefit, however, the environmental impact of this should be quantified for sustainable pork production.

CHAPTER 3. HYPOTHESES AND OBJECTIVES

3.1 Research Hypotheses

1. Inclusion of 40% field pea and multicarbohydase enzymes in the diet of finisher pigs would not affect growth performance (Chapter 4).
2. Growth response would not change with the combined inclusion of field pea and multienzyme (Chapter 4).
3. Inclusion of 40% field pea and multicarbohydase enzymes into swine diets would not affect GHG emission resulting from the production of growing pigs and manure. (Chapter 5).
4. Green house gas emission from crop production and finisher pigs would not change with the combined inclusion of field pea and multicarbohydase enzymes in swine diets. (Chapter 6).

3.2 Objectives

1. To determine the effect of 40% inclusion of field pea with or without carbohydase enzymes on growth performance, days to market and carcass traits of finisher pigs (Chapter 4).
2. To determine the effect of 40% inclusion of field pea with or without carbohydase enzymes on nutrient digestibility and nitrogen excretion (Chapter 4).
3. To determine the effect of 40% inclusion of field pea with or without carbohydase enzymes in GHG emission from growing pigs and manure (Chapter 5).
4. To develop a LCI of pork production for future LCA studies (Chapter 6).

**CHAPTER 4. GROWTH PERFORMANCE AND NUTRIENT
DIGESTIBILITY OF FINISHER PIGS FED FIELD PEA BASED DIET
SUPPLEMENTED WITH MULTICARBOHYDRASE ENZYMES**

4.1 Abstract

Inclusion of field pea and enzymes in the diet of finisher pigs may improve profitability. However, high inclusion of field pea in swine diets may have negative effects on animal performance and digestibility due to high content of ANF. The following experiments were designed to determine the response of finishing pigs to a diet formulated with 40% field pea with or without multicarbohydase enzymes on growth performance and nutrient digestibility. In experiment 1, 4 dietary treatments with two levels of peas (0 and 40%) supplemented with or without multicarbohydase enzymes were fed. A total of 180 crossbred pigs (90 gilts, 90 barrows; 60.0 ± 2.2 kg BW) were assigned to 36 pens, mixed gender, and fed 1 of 4 experimental diets. Pigs were weighed every two weeks to estimate ADFD, ADG, and G:F and were marketed when they attained a target BW of 127 kg. In experiment 2, 6 dietary treatments were used, three levels of peas (0, 20 and 40%), with or without enzymes. Treatment diets were fed to 48 individually housed crossbred barrows (60.9 ± 2.6 kg BW). A 7 d adaptation period was followed by 4 d collection of feces and urine. In experiment 1; ADFD, G:F, days to market and carcass parameters were not affected by dietary inclusion of 40% field pea or multicarbohydases neither there was any interaction between pea and enzymes. In experiment 2, digestibility of DM, GE, and N decreased with the increasing inclusion of field pea ($P < 0.01$) whereas ADF digestibility improved with the increasing inclusion of field pea ($P < 0.001$). Enzyme inclusion improved ADF digestibility ($P < 0.05$). A trend for greater N retention and increased fecal N excretion ($P < 0.001$) were observed with the inclusion of field pea in the diet. Performance of finishing pigs was not affected with the inclusion of 40% field pea in the diet. Inclusion of multicarbohydases improved ADF digestibility.

4.2 Introduction

Field pea is an alternate feed ingredient produced locally in Western Canada. In fact, Canada is the largest pea producer in the world with 4.2 million tonnes, 3.7 million tonnes of which were exported in 2019-2020 (Statistics Canada and Agriculture and Agri-Food Canada (AAFC) 2019). Competition between humans and livestock for food and arable land available for food production can be reduced with the incorporation of by-products or low-quality grains into livestock diets. Feed pea may be cheaper than commonly fed cereal grains, oilseeds, and other protein supplement (Agriculture and Agri- Food Canada, 2019). Fields pea are balanced in protein and energy and thus can be used for both human and animal consumption (Cowieson et al. 2003).

Previous studies have shown that growth and pork quality of pigs were not affected when field pea partially (Gatta et al. 2013) or completely (Stein et al. 2006) replaced SBM in the diet. A recent study to determine the effect of processing of field pea using heat and pressure found that inclusion of up to 40% raw field pea in the diet of weaned pigs maintained animal growth (Hugman et al. 2020). In an earlier study, Stein et al. (2004) showed that, except for Met, Trp, and Cys, SBM and field pea had similar CP and AA digestibility and the energy value of field pea was comparable to that of corn grain.

The main constraint to the inclusion of legumes like field pea into swine rations is the presence of ANF such as trypsin inhibitors, tannins, lectins, saponins and α -galactosides which may adversely affect nutrient digestibility (Mariscal-Landín et al. 2002). However, studies have proven that the deleterious effects of these anti-nutritional factors can be mitigated by feed processing, plant breeding and selection techniques, progressive dietary introduction in nursery diets, and (or) limiting its use to diets fed to older pigs. (Mariscal-Landín et al. 2002; Valencia et al. 2008; Gunawardena et al. 2010; Jeziorny et al. 2011).

Compared to dehulled SBM, the NDF, acid detergent fibre (**ADF**), and total dietary fibre (**TDF**) content of whole field pea are greater (Landro et al. 2014) which can decrease the energy available and may negatively impact the digestibility of other nutrients. Carbohydrases can be added to the diet to improve nutrient digestibility and animal performance (Cervantes et al. 2004; Olukosi et al. 2007a). An increase in dietary fibre favours bacterial proliferation in the hind gut resulting in an overall reduction in volatility of excreted N with the shift from urinal to faecal excretion (Zervas and Zijlstra 2002) potentially reducing the carbon footprint of pork production..

In their study (Zervas and Zijlstra 2002) showed 20% inclusion of sugar beet pulp increased fecal N excretion by 9% and reduced urinary N excretion by 10%.

The overall objectives of the study were to determine whether growth performance of finisher pigs can be maintained feeding a high inclusion level of field pea, how the inclusion of multicarbohydase enzymes would affect growth performance, and whether the effect of enzyme inclusion would be greater in diets containing field pea. Secondly, to determine how these factors affect the partitioning of N excretion between urine and feces.

4.3 Methodology

To achieve the objectives of the study two experiments were conducted:

1. Experiment 1. Effect of a high inclusion of field pea in diets supplemented with multicarbohydase enzymes on growth performance of finisher pigs.
2. Experiment 2. Effect of a high inclusion of field pea in diets supplemented with multicarbohydase enzymes on nutrient digestibility and nitrogen excretion of finisher pigs.

Animal experiments were approved by the University of Saskatchewan Committee on Animal Care and Supply and performed in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 2009) Experiments were conducted at the Prairie Swine Centre Inc. (PSCI) Saskatoon, SK, Canada.

4.3.1 Diets

A multicarbohydase enzymes mix (Superzyme®-W, Canadian Bio-Systems Inc. (Calgary, Alberta, Canada), containing glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) activities was used. The multicarbohydase enzymes was added according to recommendations by the manufacturer at 1000 g per 1000 kg feed. The feed ingredients including field pea were sourced locally through Canadian Feed Research Centre, North Battleford, SK. The peas were ground using a hammer mill equipped with a 3.57 mm screen prior to being incorporated into the diets. Batch diets were prepared with all ingredients except the multicarbohydase enzyme. The batch diet was then divided and the multicarbohydase added to a portion. All diets were then pelleted

through a 4 mm screen following conditioning at 65 °C in a Buhler Conditioner/retentioner. The pellets were immediately cooled in a counter current cooler using ambient air.

Diets were formulated to meet or exceed the National Research Council (NRC) requirements for finisher pigs in phase1 (60-90 kg) and phase 2 (90-120 kg) stages (NRC 2012). The field pea and test diets were sampled and analyzed at Central Testing Laboratory Ltd, Winnipeg, MB. The field pea contained 89.48% DM, 19.43% CP, 54.15% starch, 7.42% NDF, and 6.8% ADF. There were 6 dietary treatments including 3 levels of peas: 0%, 20% and 40% with or without enzymes. Diets were formulated to be comparable Lys/NE ratio but the untested lysine content in the field pea resulted in slightly higher Lys/NE ratio in diets fed. Experiment 1 utilized only control and high inclusion field pea (40%) diet with or without multicarbohydase enzymes, whereas experiment 2 used all the 6 diets. Celite 545, Celite Corporation, Lompoc CA, USA. was added to the diets at 0.4% as source of acid insoluble ash (AIA), an indigestible marker to allow calculation of nutrient digestibility.

4.3.2 Experiment 1

4.3.2.1 Animals and Housing

The experiment utilized 180 crossbred grow-finish pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) equally divided between gilts and barrows with an initial body weight (BW) of 60 ± 2.2 kg. Sixty pigs (males and females) were selected each week and assigned to the pens in room 1 with 5 pigs (mixed gender) per pen ensuring similar initial BW and coefficient of variation among pens. This allowed 3 pens per treatment in each room and 9 pens per treatment overall. The second and third room were started in subsequent weeks. Pens (2.4 x 1.7 m) had fully slatted concrete floors over a shallow manure pit. The pens were separated by concrete walls with polyvinyl chloride front gates. Each pen was provided with a single space dry feeder and a nipple drinker to provide ad libitum access to food and water. The rooms were maintained within the thermo-neutral zone (temperature ~ 15°C and relative humidity ~ 40%) of the pigs with a 10-h light and 14-h dark cycle. The treatments were randomly assigned to the pens.

4.3.2.2 Treatments and Feeding

Treatments were arranged as a 2 × 2 factorial with the main effects of peas (0% and 40% peas) and enzymes, yes or no. The diets were pelleted and formulated in two phases. Phase1 was

formulated to meet or exceed the requirements of early finisher pigs (60 to 90 kg) and phase 2 for late finisher pigs (90 to 120 kg) according to NRC (2012). Ingredient composition of phase 1 and phase 2 diets are outlined in Table 1.

4.3.2.3 Sampling and Data Collection

Animals were weighed on d0 and every two weeks. The weight of pigs on each weigh day when subtracted from the former weigh day, then divided by the days on feed, allowed for the calculation of ADG. The amount of feed offered was recorded daily and the feed left in the barrels were weighed on weigh days to allow the calculation of feed disappearance. The ratio of ADG to ADFD was used to calculate G:F. Pigs were marketed when they attained a minimum target body weight of 127 kg. Marketing was done every two weeks as per the PSCI marketing schedule. Pigs received a shoulder tattoo unique to each pen the day prior to market. Pigs were marketed to Maple Leaf Foods Inc. (Brandon, MB). The estimated carcass parameters obtained from the abattoir were slaughter weight (kg), yield (%), loin depth (mm), and back fat thickness (mm).

4.3.2.4 Statistics

The experiment design was a randomized complete block design (**RCBD**). The residual error data of performance parameters were analysed using Shapiro-Wilk test and found to be non-normal. Data were therefore analyzed using a generalised linear mixed model procedure, GLIMMIX (Keselman et al. 2016) of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). To allow a comparison between 0% field pea, 40% field pea, and yes or no enzymes, data were analyzed by analysis of variance for a 2×2 factorial treatment structure. To accommodate the time related variation in BW, ADG, ADFD, and G:F, a repeated measure analysis was performed. The covariance structure with the lowest Akaike and Bayesian value were chosen. The SLICE option of SAS was used for partitioned analysis of the least square means for treatment by period interactions and reported the overall treatment by period effect. The model included inclusion level of field pea, multicarbohydase enzyme, and interactions as fixed effects and block as random effect. Pens with 5 pigs was the experimental unit. Means were separated using the adjusted Tukey option. A P value of < 0.05 was considered significant and < 0.1 was considered a trend.

4.3.3 Experiment 2

4.3.3.1 Animals and Housing

A total of 48 barrows (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) weighing 60.9 ± 2.6 kg BW (mean \pm SD) were selected. The experiment was conducted in 4 blocks utilizing 12 pens per block. The barrows were individually housed in metabolism pens (1.42 m x 1.49 m), with polyvinylchloride walls and plastic slatted floors. One plexi-glass window between pens allowed visual, but not tactile contact. Plastic balls were provided for enrichment. Pens had an individual feeder and a single nipple drinker. The metabolism room was environmentally controlled with an average temperature around 20°C (19.96 ± 0.88), ventilated using negative pressure and a 12-h light and 12-h dark cycle.

4.3.3.2 Treatments and Feeding

The dietary treatments were arranged as a 3×2 factorial with 3 levels of peas (0%, 20%, and 40%) and with or without enzymes in a RCBD. Celite was added to the diets (0.4%) as an indigestible marker. Animals were fed three times their maintenance requirement (110 kcal DE/kg BW^{0.75}; NRC 2012) which approximates 95% of ad libitum intake and were given as two equal meals at approximately 0800 h and 1500 h. Water was available from a nipple drinker in each pen.

4.3.3.3 Sampling and Data Collection

Each experimental period lasted 11 d, 7 d for adaptation and 4 d for sample collection. Feces were collected by grab sampling twice daily at approximately 0800 h and 1500 h in plastic bags and frozen immediately at -20°C. A metal screen was placed under each pen to separate the feces from urine. Urine was collected in collection vessels placed under the metabolic pens for a period of 24 h for 4 d and the container was emptied every 24 h. A 10% aliquot was subsampled from the collected urine every day and stored. These subsamples were pooled on the final collection day and an aliquot was preserved at -20°C until lab analyses. Glass wool was used in the funnels to filter contaminants from the urine. To prevent the volatilization of urinary nitrogen and bacterial contamination an adequate amount of 12 N HCl were added to the collection container prior to the collection.

4.3.3.4 Chemical Analyses and calculations

Fecal samples were oven-dried at 55 °C for 48 h (Jacobs et al. 2011). Fecal and diet samples were ground through a 1 mm mesh screen (Retsch Mill ZM1, Newton, PA, USA) and subsequently analysed for DM (Association of Official Analytical Chemists (AOAC) 930.15), N (AOAC 990.03), ADF (ANKOM 3/98), NDF (ANKOM 3/98), and AIA (AOAC 920.08), GE was analyzed using an isoperibol bomb calorimeter, (model 6400, PARR Instrument Co., Moline, IL, USA) with benzoic acid as standard at the General Nutrition laboratory of the Department of Animal and Poultry Science at the University of Saskatchewan. The diet and fecal samples were also analyzed for P by spectrophotometry (method 965.17; AOAC, 2007) at Central Testing Laboratories in Winnipeg, Manitoba. Urinary nitrogen was analyzed (LECO) at University of Manitoba. Apparent total tract digestibility (**ATTD**) was calculated using the following equation (Adeola 2000):

$$ATTD, \% = [1 - (NF \times MD) / (ND \times MF)] \times 100 \quad [4.1]$$

where MD and MF are the percent of AIA in the diet and feces, respectively while NF and ND are the percent of nutrient in the diet and feces, respectively.

4.3.3.5 Statistics

The normality of the residuals were analyzed using Proc UNIVARIATE in SAS (SAS Institute Inc., Cary, NC, USA) and those with a P value > 0.05 for Shapiro-Wilk test was considered normally distributed. Any observation which was not within a ± 2 standard deviations was considered an outlier. The data were analyzed using Proc MIXED in SAS and data which was not normally distributed were analyzed in Glimmix. To allow a comparison between 0%, 20%, and 40% field pea, and yes or no enzymes, data were analyzed by analysis of variance for a 3 \times 2 factorial arrangement of treatments. The model contained the main effects of field pea and enzymes and their interactions as fixed effects while the block was considered random. The pig in the metabolism crate was the experimental unit. Mean separations were carried out using the adjusted Tukey option of SAS. A P value of <0.05 was considered significant and < 0.10 was considered as a trend.

4.4 Results

Experiment 1. Pigs remained healthy during the trial. Table 4.3 shows the least square means of data from growth performance experiment. Treatment had no effect on BW at any period

of growth ($P > 0.1$). Inclusion of field pea had no effect on ADG on d 0-14, 15-28 or 43-56 ($P > 0.1$) but improved growth was observed from d 29-48 ($P < 0.05$) and there was a trend for the overall experimental period ($P = 0.059$). Similarly, ADFD tended to be increased with 40% pea inclusion in the diet on d 15-28 ($P = 0.052$) and overall ($P = 0.094$). Except for an improvement in G:F observed on d 15-28 ($P < 0.05$), the inclusion of enzymes in the diet had no effect on growth performance.

Carcass parameters (slaughter weight, yield %, loin depth, and back fat, mm) were not different among treatments, but days to market tended to be reduced with the inclusion of field pea in the diet (67.9 vs 66 ± 2.14 ; $P = 0.094$; Table 4.4).

Experiment 2. The effect of treatment on nutrient digestibility is described in Table 4.5. Dry matter, GE, and N digestibility decreased with the increasing inclusion of field pea ($P < 0.01$) and there was no effect of multicarbohydase enzyme inclusion ($P > 0.1$). In contrast, ADF digestibility was improved when the diet was supplemented with field pea ($P < 0.0001$) or the multicarbohydase enzymes ($P = 0.02$). The 40% field pea diet and control diet had improved NDF digestibility compared to the 20% field pea diet ($P < 0.0001$), but enzyme had no effect on NDF digestibility.

The N intake ($P < 0.0001$) and N retention ($P = 0.03$) increased with the increasing inclusion of field pea in the diet. The fecal excretion of N was also increased with increasing level of field pea in the diet ($P < 0.0001$). However, the N excretion via urine tended to be reduce with increasing level of pea inclusion ($P = 0.054$). There was no effect of field pea inclusion on total N excretion ($P > 0.1$). Addition of multicarbohydase enzymes to the diet had no effect on N intake, excretion, or retention. Results are presented in Table 4.6.

4.5 Discussion

Comparable to the results of others (Stein et al. 2006; Landero et al. 2014), data from these experiments indicates that 40% field pea can be included in the diets of finishing pigs (60 to 120 kg BW), partially replacing SBM with no negative effect on growth performance or carcass parameters. In fact, the overall ADG tended to improve, although there was no effect on G:F. These results agree with a study conducted by Stein et al., (2004), feeding up to 36% inclusion of South Dakota grown field pea in grower and finisher pig diets. However, the growth response of

nursery pigs towards field pea inclusion was not always positive. For instance, inclusion of 40% field pea in the diet of weaned pigs completely replacing SBM had no overall effect on growth, although a reduction in ADG and G:F was observed during the first week of the trial (Landro et al. 2014). And in another study, Stein et al. (2010) fed greater inclusion of field pea up to 48% or 60% in the diet of weaned pigs and found a reduction in growth performance, whereas growth was maintained at 360g field pea/kg of diet. Hence, the level of inclusion of field pea in the diet of nursery pigs is important and techniques like genetic selection and feed processing may further reduce the negative effects of ANFs and make it a more suitable ingredient for monogastric animal nutrition.

Inclusion of up to 40% field pea in the present study feeding 60 kg BW pigs had a negative effect on digestibility of DM, GE, N, and P, relative to a wheat-barley based diet. This is similar to results of Landro et al. (2014), but in their work it was shown that growth performance and digestibility of nutrients improved in grower pigs fed 40% field pea diet compared to post-weaned pigs, suggesting animals with a more mature digestive system tolerated the field pea better. Conversely, Valencia et al. (2008) reported that, when pea protein concentrate (PPC) was added to the diet of weanling pigs as the sole source of protein, it did not alter the nutrient digestibility at total tract level when compared to SBM or soya protein concentrate diet. Pea protein concentrate is a refined form of pea protein produced after dehulling, grinding and air classification and has a higher CP and lower fibre content than SBM. The negative effect on CP digestibility by field pea incorporation is mainly due to the NSP content. The presence of NDF-bound protein in peas can further reduce protein digestibility (Gdala 1998). However in the current study the digestibility of NDF and ADF improved with the inclusion of peas, suggesting that field pea fibre and resistant starch fermented better as reported by other studies (Goodlad and Mathers 1991; Jha et al. 2011).

Inclusion of whole field pea can increase the dietary fibre content of the diet. Goodlad and Mathers (1991) found that inclusion of 30% field pea to a wheat-based diet doubled the NSP content, with a five-fold increase in resistant starch. According to Canibe et al. (1997), pea hull contains more insoluble fibre than pea cotyledon, constituted mainly by cellulose, uronic acid, arabinose and xylose. The inclusion of multicarbohydase enzymes to a pea diet was expected to improve nutrient digestibility. Enzyme efficacy is influenced by different factors, and an important one is substrate specificity (Ravindran 2013). Hence, the inclusion of enzymes specific to the feed

ingredients used in feed formulation is important. Moreover, with the incorporation of multiple feed ingredients and by-products in an effort to produce a least cost ration, a multienzyme combination would be more beneficial than a mono-enzyme preparation (Olukosi et al. 2007b). The carbohydrases fed in our study contained glucanase, xylanase, cellulase, amylase and invertase activities. Pea starch is more resistant to endogenous enzyme digestion when compared to starch from cereals (Wiseman 2006). Hence, the addition of exogenous enzymes like amylases, cellulases and xylanases would be expected to improve both starch and fibre digestibility. However, in our experiment, , supplementation of enzymes did not improve digestibility of DM, GE, N, or P. Similar results were observed by Dadalt et al. (2016) when PPI (pea protein isolate) was supplemented with carbohydrase enzymes in the diet of growing pigs, whereas in a study conducted by Ngoc et al. (2011) using a multi-enzyme mixture composed of α -amylase, β -glucanase, cellulase and protease supplemented to a high fibre diet fed to post-weaning pigs improved CP, CF, NDF, OM, and GE digestibility. However, the enzymes improved the CTTAD of only CP and NDF during the growing period. This result suggest that enzymes might have a better influence on nutrient digestibility in the diet of young pigs than in grower pigs with a fully developed digestive system. In contrast to other nutrient digestibilities, enzymes had a significant effect on ADF although not on NDF digestibility. The improvement in ADF digestibility by enzymes in our study is probably because of the action of cellulase on fibres and NDF was not influenced because hemicellulose degrading enzymes was not incorporated. In a study conducted by Emiola et al. (2009) when wheat DDGS and carbohydrases was added to the diet of grower pigs, although enzymes improved the ATTD of DM, N, GE and ether extract, there was no effect on NDF digestibility. Similarly, post-weaned piglets fed with a diet containing wheat and wheat bran supplemented with either a mono-enzyme (xylanase) or multi-enzyme preparation, neither the mono-enzyme nor the multi-enzyme produced an improvement in digestibility of total fibre, ADF and NDF. Moreover, multi-enzyme preparation reduced fibre digestibility numerically when compared with the control group (Vahjen et al. 1998).

A shift in N excretion from urine to feces feeding a high field pea diet with no change in total N excretion was observed. A tendency was observed for increased N retention and reduced urinary N output as field pea level in the diet increased. Work conducted by Zervas and Zijlstra (2002) demonstrated a shift in N nitrogen excretion from feces to urine without altering total N excretion with increased fermentable fibre in the diet. Another study with a similar objective was

conducted by Bindelle et al. (2009) and found that inclusion of up to 30% sugar beet pulp (source of soluble fibre) reduced urinary N to fecal N ratio linearly with no effect on N retention. They also compared the effect of insoluble and soluble dietary fibre in nitrogen excretion patterns and found insoluble dietary fibre did increase urinary N to fecal N ratio compared to soluble fibre source. Nitrogen excreted through feces is less volatile and will reduce the concentration of ammonia excreted from manure (Stevens et al. 1989; Mroz et al. 2000). Similarly Canh et al. (1998) found that with an increase in dietary NSP, the pH and ammonia emission from manure decreased. When expressed as % N intake, urinary N and total N excretion decreased with the inclusion of field pea, despite increased N intake by pigs fed the high field pea diet. High inclusion of field pea can therefore possibly reduce the N₂O emissions from manure and can be considered a better ingredient for monogastric animals with the potential to reduce carbon footprint.

4.6 Conclusion

Field pea can be considered as an alternate plant starch and protein source and can partially or completely replace dehulled soybean meal when diets are balance for NE and SID AA. Growth performance and carcass traits of finisher pigs were not affected even at an inclusion level of 40% field pea in the diet. Enzymes showed no effect in growth as well as in carcass traits. However, nutrient digestibility (DM, GE, N, and P) was reduced with the inclusion of field pea in the diet while digestibility of fibre was improved with the high inclusion of field pea in the diet. Supplementation of enzymes improved ADF digestibility. The results of this study failed to demonstrate an interaction between pea and enzymes on growth or nutrient digestibility.

Table 4.1 Ingredient and nutrient composition (as fed basis) of the 0%, 20%, and 40% field pea diets for the phase1 (60-90 kg)^{a,b} growth performance and digestibility study

Treatments	0%	20%	40%
<i>Ingredients</i>			
Field pea	0.00	20.00	40.00
Wheat	61.85	41.86	21.87
Barley	24.80	27.135	29.47
Canola oil	1.50	1.25	1.00
Soybean meal	9.00	7.00	5.00
L-Lysine (78%)	0.34	0.17	0.00
DL-Methionine (99%)	0.03	0.05	0.08
L-Threonine (98.5%)	0.08	0.08	0.08
Limestone	1.20	1.20	1.20
Dicalcium phosphate	0.30	0.35	0.40
Salt	0.30	0.30	0.30
Vitamin and mineral premix ^c	0.20	0.20	0.20
Celite	0.40	0.40	0.40
<i>Analyzed nutrient composition</i>			
Dry matter, %	92.28	91.92	92.11
Gross energy, Mcal kg ⁻¹	3.84.	3.83	3.82.
Crude protein, %	17.01	17.50	17.98
Ether extract, %	2.60	3.03	2.85
Calcium,%	0.63	0.69	0.71
Phosphorus, %	0.46	0.46	0.45
Acid detergent fibre, %	3.89	4.64	5.33
Neutral detergent fibre, %	9.50	9.87	9.83
Soluble dietary fibre, %	3.18	2.16	3.01
Insoluble dietary fibre, %	12.94	15.74	14.88
Total dietary fibre, %	16.12	17.90	17.89
<i>Calculated nutrient composition</i>			
NE Kcal kg ⁻¹	2.42	2.40	2.40
SID Lysine, %	0.78	0.81	0.84

^a Celite was not added to the diet used for performance study.

^b The diets were partitioned to two equal parts and enzymes were added to one half, giving 3 additional diets. The enzyme combination contained the activities of glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) (Superzyme®-W from Canadian Bio-Systems Inc. Calgary, Alberta, Canada). Enzyme was added to one set of diet at 1000g/tonne of completed feed.

^c The swine premix provided the following vitamins and minerals per kilogram of 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, 2 mg. Iron, 100 mg; Zinc, 100 mg; Manganese, 40 mg; Copper, 15 mg; Selenium, 0.30 mg; and Iodine, 1 mg.

Table 4.2 Ingredient and nutrient composition (as fed basis) of the 0%, and 40% field pea diets for the phase2 (90-127 kg)^a growth performance study

Treatments	Control	Peas
<i>Ingredients</i>		
Field pea	0.00	40.00
Wheat	45.72	15.00
Barley	43.00	42.57
Canola oil	1.20	0.50
Soybean meal	8.00	0.00
L-Lysine (78%)	0.20	0.00
DL-Methionine (99%)	0.03	0.03
L-Threonine (98.5%)	0.05	0.08
Limestone	1.00	1.00
Dicalcium phosphate	0.30	0.40
Salt	0.30	0.30
Vitamin and mineral premix ^b	0.20	0.20
<i>Calculated nutrient composition</i>		
DM %	88.45	89.27
CP %	15.54	15.69
NE Kcal kg ⁻¹	2.40	2.37
SID Lysine, %	0.64	0.73

^a The diets were partitioned to two equal parts and enzymes were added to one half, giving 2 additional diets. The enzyme combination contained the activities of glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) (Superzyme®-W from Canadian Bio-Systems Inc. Calgary, Alberta, Canada). Enzyme was added to one set of diets at 1000g/tonne of completed feed.

^b The swine premix provided the following vitamins per kilogram of diet: Vitamin A, 8,000 IU; Vitamin D, 1,500 IU; Vitamin E, 30 IU; Vitamin B12, 0.02 mg; Menadione, 2 mg; Thiamine, 2 mg; Biotin, 0.1 mg; Niacin, 20 mg; Riboflavin, 12 mg; Pantothenate, 12 mg; Folic acid, 0.50 mg; and Pyridoxine, 2 mg. The swine premix also provided the following minerals per kilogram of diet: Iron, 100 mg; Zinc, 100 mg; Manganese, 40 mg; Copper, 15 mg; Selenium, 0.30 mg; and Iodine, 1 mg

Table 4.3 Effect of high inclusion field pea and carbohydrase supplementation on growth performance of finisher pigs (Experiment 1) ^a

	Peas, %		Enzymes			P-Value		
	0	40	N	Y	SEM	Peas	Enzymes	Peas × Enzymes
Bodyweight, kg								
Initial	59.85	59.70	59.55	60.00	0.43	0.797	0.459	0.847
d 14	75.89	75.72	76.22	75.39	0.73	0.872	0.426	0.878
d 28	92.10	92.19	92.16	92.13	0.71	0.926	0.974	0.914
d 42	107.29	108.75	108.10	107.94	0.83	0.222	0.887	0.612
d 56	119.87	121.85	120.96	120.76	1.02	0.180	0.889	0.594
ADG, kg d⁻¹								
d0 – 14	1.13	1.14	1.19	1.08	0.05	0.848	0.133	0.500
d15 – 28	1.14	1.17	1.12	1.20	0.03	0.589	0.083	0.224
d29 – 42	1.09	1.17	1.13	1.13	0.03	0.030	0.977	0.181
d43 – 56	0.98	1.03	1.00	1.01	0.02	0.142	0.765	0.322
d0 – 56	1.09	1.13	1.11	1.11	0.03	0.059	0.881	0.960
ADFD, kg d⁻¹								
d0 – d14	2.24	2.28	2.29	2.23	0.09	0.718	0.638	0.941
d15 – d28	2.84	3.00	2.96	2.88	0.05	0.052	0.330	0.169
d29 – d42	2.87	2.98	2.93	2.92	0.05	0.132	0.794	0.488
d43 – d56	3.25	3.25	3.23	3.27	0.09	0.968	0.718	0.977
d0 – d56	2.80	2.88	2.85	2.82	0.04	0.094	0.513	0.546
G:F								
d0 – d14	0.52	0.51	0.53	0.50	0.03	0.795	0.406	0.852
d15 – d28	0.40	0.39	0.38	0.42	0.01	0.474	0.023	0.061
d29 – d42	0.38	0.39	0.39	0.39	0.01	0.243	0.903	0.682
d43 – d56	0.30	0.32	0.31	0.31	0.01	0.248	0.904	0.579
d0 – d56	0.40	0.40	0.40	0.40	0.02	0.574	0.735	0.735

^a Data are presented as least square means of 9 replicate pens with 5 pigs per pen. SEM; standard error of means

Table 4.4 Effect of inclusion of field pea and carbohydrase supplementation on carcass parameters of finisher pigs (Experiment 1)

	Peas		Enzymes		SEM	P-Value		
	0	40	N	Y		Peas	Enzymes	Peas × Enzymes
Days to market ^b	67.9	66.0	66.9	67.0	2.14	0.094	0.901	0.409
Slaughter weight, kg	105.8	105.6	105.8	105.7	0.52	0.731	0.878	0.829
Back fat depth, mm	16.7	16.4	16.7	16.4	0.79	0.469	0.542	0.057
Loin depth, mm	66.9	67.2	67.2	66.8	0.93	0.825	0.765	0.905
Carcass yield, %	61.8	62.0	61.8	62.0	0.41	0.509	0.496	0.103
Dressing, %	79.9	79.7	79.9	79.7	0.31	0.654	0.587	0.664

^b Days to market represent the period of time when the pigs remained in trial or until they reached a minimum target weight of 127 kg.

SEM; standard error of means

Table 4.5 Effect of high inclusion field pea and carbohydrase supplementation on apparent total tract digestibility of nutrients (Experiment 2) ^a

Item	Peas, %			SEM	Enzymes			SEM	P-Value		
	0	20	40		N	Y	Peas		Enzymes	Peas× Enzymes	
Total tract digestibility											
DM, %	87.87	86.15	86.73	0.28	87.02	86.81	0.23	<0.001	0.509	0.123	
GE, %	87.41	85.89	86.10	0.31	86.58	86.36	0.25	0.001	0.108	0.389	
N, %	86.28	84.34	83.27	0.59	84.24	85.02	0.51	0.001	0.212	0.439	
ADF, %	34.61	37.95	47.57	1.41	38.05	42.04	1.15	<0.0001	0.019	0.076	
NDF, %	49.98	45.33	53.03	1.11	49.61	49.29	0.91	<0.0001	0.804	0.069	

^a Data are presented as least square means of 8 replicate pigs individually house in metabolism crates

SEM; standard error of means

Table 4.6 Effect of high inclusion of field pea and carbohydrase supplementation on N intake, excretion, and retention (Experiment 2) ^a

Item	Peas, %			SEM	Enzymes			SEM	P-Value		
	0	20	40		N	Y	Peas		Enzymes	Peas × Enzymes	
N intake (g/d)	59.35	60.70	62.23	0.36	60.47	61.05	0.30	<0.0001	0.166	0.001	
Fecal N output	8.14	9.54	10.39	0.36	9.46	9.25	0.31	<0.0001	0.578	0.228	
Urinary N output	13.21	10.31	10.25	1.91	10.81	11.70	1.81	0.089	0.465	0.599	
Total N excretion	21.35	19.85	20.63	2.06	20.27	20.96	1.96	0.639	0.594	0.429	
N retention	38.00	40.85	41.60	2.03	40.20	40.10	1.92	0.072	0.938	0.054	

^aData are presented as least square means of 8 replicate pigs individually housed in metabolism crates
SEM; standard error of means

**CHAPTER 5. THE EFFECT OF DIETARY INCLUSION OF FIELD PEA
AND MULTICARBOHYDRASE ENZYMES ON GHG EMISSIONS OF
FINISHING PIGS AND THEIR MANURE SLURRY**

5.1 Abstract

Changes in dietary feedstuffs is one potential strategy to reduce GHG emission from the swine sector. The objective of this study was to determine GHG emissions from finishing pigs as well as from stored manure when the diets supplemented with field pea and a multicarbohydase enzymes were fed to finisher pigs. The treatments, arranged as a 2 by 2 factorial, were 0 and 40% peas with or without a multicarbohydase enzyme. Two identical environmental chambers were used to house the pigs. At the start of each of the 16 replicates, 6 pigs, either all male or female (Camborough Plus females \times C337 sires; PIC Canada Ltd., Winnipeg, MB), weighing 60 ± 2.2 kg were selected and adapted to the experimental diets for 7 d prior to being moved into the chambers (d 0). Baseline samples were collected on d 0 before introducing the pigs to the chamber. Air samples were collected and analysed for CO₂, CH₄, and N₂O by gas chromatography on d 0 and on d 14. Immediately after gas collection, manure was subsampled into 65 L barrels and samples were collected in month 1, 3, and 6. Inclusion of 40% field pea had no effect on emissions from the chamber. However, inclusion of multicarbohydase enzymes decreased CO₂ emissions ($P = 0.036$) and a trend for reduction of N₂O emission was observed ($P = 0.99$). Emissions from the stored manure were not affected by the 40% inclusion of peas or carbohydrase enzymes, except for a reduction in CH₄ emission with inclusion of peas in month 3 ($P = 0.012$). To conclude, inclusion of field pea did not affect emissions from chambers or stored manure and enzyme addition only reduced chamber CO₂ emissions.

5.2 Introduction

The livestock sector is a major contributor to GHG emissions accounting for 18% of human-produced emissions on a global basis. Emissions from swine currently account for only 13% of global emissions from livestock species (Steinfeld et al. 2006), however, pork is the most widely consumed meat. By the end of 2050, a 38% increase in pork consumption is predicted (FAO 2011). The anticipated increase in pork production requires that mitigation strategies be adopted to reduce the GHG emissions to tackle adverse effects on environment.

Animals and their manure slurry are the major sources of GHG emission from swine facilities. Carbon dioxide is released by animal exhalation, produced as a by-product of cellular respiration. Manure slurry CO₂ emissions are caused by urea hydrolysis, anaerobic fermentation of OM to produce VFA, and aerobic degradation of OM (Philippe and Nicks 2015). Anaerobic degradation of OM in the hind gut and manure slurry are responsible for CH₄ emissions (Dennehy et al. 2017). Unlike CO₂ and CH₄, N₂O is exclusively produced from manure slurry from degradation of N containing compounds by micro-organisms. According to Olsen et al. 2003, CH₄ and N₂O liberated from manure slurry storage represented approximately 20% of Canada's total agricultural emissions in 2001. These emissions are influenced by the physiological state of the animal, diet, housing, ventilation, manure storage, as well as techniques used for gas collection and quantification (Philippe and Nicks 2015).

Different mitigation strategies have been proposed to reduce the GHG emission from pigs and manure slurry. Dietary changes of feedstuffs is among the many proposed techniques and it can be achieved by several means including reducing protein content with the inclusion of crystalline AA, changing the fibre content of the diet, including feed enzymes, and use of acidifiers (Montalvo et al. 2013). Field pea can be added to swine diets as an alternative protein and starch source replacing cereal starch and SBM protein (Stein et al. 2006). High inclusion of field pea in swine diets can increase fibre content and possibly increase GHG emission. Also, field pea resistant starch and fibre are readily fermentable (Jha et al. 2011). Feed enzyme inclusion may reduce the GHG emission from pigs and manure (Chen et al. 2020). The objective of this study was to determine the effect of inclusion of field pea and multicarbohydase enzyme in mitigating GHG emission from finisher pigs and stored manure slurry.

5.3 Methodology

The animal experiments were performed in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 2009), and approved by the University of Saskatchewan Committee on Animal Care and Supply. The experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada).

5.3.1 Animals and housing

A total of 96 animals (males and females) were used for the experiment. At the start of each block, 7 pigs of the same sex, weighing 60 ± 2.2 kg, were selected, and adapted to the control or test diet for 7 d. The pigs were then weighed and 6 out of these 7 pigs within a similar weight range were housed in an environmentally controlled chamber for 14 d. This process was repeated 16 times providing a n of 4 per treatment.

Two identical environmental chambers at PSCI were used for this trial. Each chamber had interior dimensions of $4.2\text{m} \times 3.6\text{m} \times 2.7\text{m}$ (L×W×H) and contained a pen with half solid concrete and half slatted flooring (Fig 5.1). The ceilings and internal walls were sealed with stainless steel sheets to eliminate emissions from these surfaces. Each chamber had a separate manure pit under the slatted concrete flooring with inside dimensions of $2\text{m} \times 1.25\text{m} \times 0.3\text{m}$ (L×W×H) and an approximate capacity of 900 L. The outside of the pen was plastic matrix flooring which could be dismantled easily to access the manure pit. Each chamber had an individual air outlet and inlet. The pens were equipped with commercial feeders and bowl-type water drinkers. Animals had ad libitum access to food and water

Each chamber was operated on a negative pressure ventilation system. The outside air entered the chambers through ceiling inlets and passed either through a 5-ton 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). After conditioning, the room air was then received by 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) before entering each chamber through an actuated inlet located on the ceiling of each chamber. A sidewall fan (H18, Del-Air Systems Inc., Humboldt, SK, Canada) expelled the air out of each chamber. The chamber was washed and disinfected before the start of each batch of pigs.

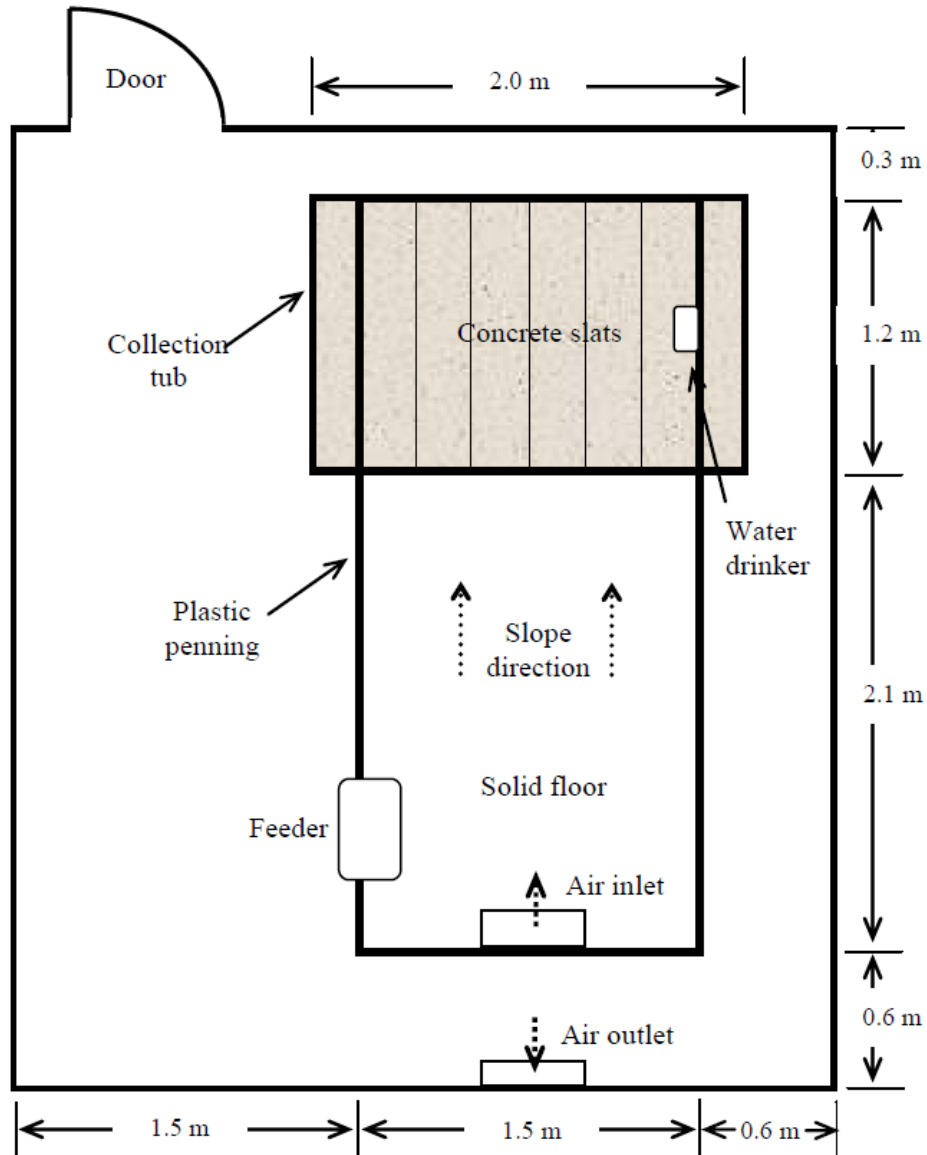


Figure 5.1 Schematic representation of the environmental chamber (acquired from Alvarado, 2011)

5.3.2 *Experimental design*

The experiment was conducted as a RCBD with two levels of field pea (0% and 40%) with or without multicarbohydase enzymes. The environmental chamber with pigs was the experimental unit. Each dietary treatment was replicated 4 times. As only two chambers were available, the experiment was carried out with 2 treatments at a time. Diets used were similar to growth performance and digestibility trial (Table 5.1) and formulated to meet or exceed the NRC (2012) requirements of 60 kg pigs. The ingredients were locally sourced through Canadian Feed

Research Centre, North Battleford, SK and were ground, mixed and pelleted before mixing half batch with enzymes. The enzyme used was a multi-carbohydrase enzyme, “Superzyme®-W” from Canadian Bio-Systems Inc. (Calgary, Alberta, Canada), and contained glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) activities. The carbohydrase mix was added according to the recommendations of the manufacturer at the rate of 1000 g t⁻¹ of feed.

Table 5.1 Ingredient and nutrient composition (as fed basis) of the 0%, and 40% field pea diets fed in the chamber experiment ^a

Treatments	0%	40%
<i>Ingredients</i>		
Field pea	0.00	40.00
Wheat	61.85	21.87
Barley	24.80	29.47
Canola oil	1.50	1.00
Soybean meal	9.00	5.00
L-Lysine (78%)	0.34	0.00
DL-Methionine (99%)	0.03	0.08
L-Threonine (98.5%)	0.08	0.08
Limestone	1.20	1.20
Dical 21/16	0.30	0.40
Salt	0.30	0.30
Vitamin and mineral premix ^b	0.20	0.20
Celite	0.40	0.40
<i>Analyzed nutrient composition</i>		
Dry matter, %	92.28	92.11
Crude protein, %	17.01	17.98
Gross energy, Mcal kg ⁻¹	3.84	3.82
Ether extract, %	2.60	2.85
Calcium, %	0.63	0.71
Phosphorus, %	0.46	0.45
Acid detergent fibre, %	3.89	5.33
Neutral detergent fibre, %	9.50	9.83
Soluble dietary fibre, %	3.18	3.00
Insoluble dietary fibre, %	12.94	14.88
Total dietary fibre, %	16.12	17.89
<i>Calculated nutrient composition</i>		
NE Mcal kg ⁻¹	2.42	2.40
SID Lysine, %	0.78	0.84

^a The diets were partitioned to two equal parts and enzymes were added to each half, giving 3 additional diets. The enzyme combination contained the activities of glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) (Superzyme®-W from Canadian Bio-Systems Inc. Calgary, Alberta, Canada). Enzyme was added to the diet at 1000g/tonne of completed feed.

^b The swine premix provided the following vitamins and minerals per kilogram of 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, 2 mg. Iron, 100 mg; Zinc, 100 mg; Manganese, 40 mg; Copper, 15 mg; Selenium, 0.30 mg; and Iodine, 1 mg.

5.3.3 Sampling and data collection

5.3.3.1 Gas sampling from animals housed in the chamber.

Prior to introducing the pigs to the chamber, gas samples were collected to determine baseline CO₂, CH₄, and N₂O levels. Animals were introduced to the chambers on d 0 for a period of 14 d. Airflow was measured from ceiling inlets on d 0 and 14 using an anemometer (Model 8330 VelociCheck, TSI Incorporated, St. Paul, MN; accuracy of ± 5% of reading) to determine volumetric airflow rate. On d 14, gas samples were collected from the room, the pigs were then moved out of the chamber and another sampling was done after 6 hours to measure the CO₂, CH₄, and N₂O emissions solely from chamber manure slurry.

A sampling line was placed close to the exhaust in order to collect a representative sample of the air leaving the chamber. A tedlar bag (Saint-Gobain ChemwareTM FEP Gas Sampling Bags, 4.7L) connected to a vacuum apparatus was used for collection of gas samples. The tedlar bags were repeatedly purged with zero air (Praxair) after each sampling and reused. Purging with zero air (< 0.1 ppm of hydrocarbons) prevented cross contamination between samples. The vacuum apparatus was made of an airtight plastic bucket and sealed using a glass lid with four sampling ports and a center port which was connected to a peristaltic pump (Master flex L/S tubing pump, Model 7017-52 pump head, Cole-Parmer, Vernon Hills, USA), which created negative pressure inside the sampling apparatus. One of the other 4 ports was connected to the sampling line and as the negative pressure was built inside the apparatus the air from the chamber was drawn in to the tedlar bag via the sampling line. Immediately after collection the gas samples were subsampled in to 12 ml exetainer vials (LabCo Inc., High Wycombe, UK) and transported to the soil science laboratory at the University of Saskatchewan for analysis via gas chromatography (GC). The samples from the vials were then transferred to the GC analyzer (Scion 456-GC, Scion Instruments, Livingston, UK). CO₂, CH₄ and N₂O were quantified by thermal conductivity detector (TCD at 120 °C), flame ionization detector (FID at 120 °C) and electron capture detector (ECD at 330 °C), respectively.



Figure 5.2 Vacuum sampling apparatus

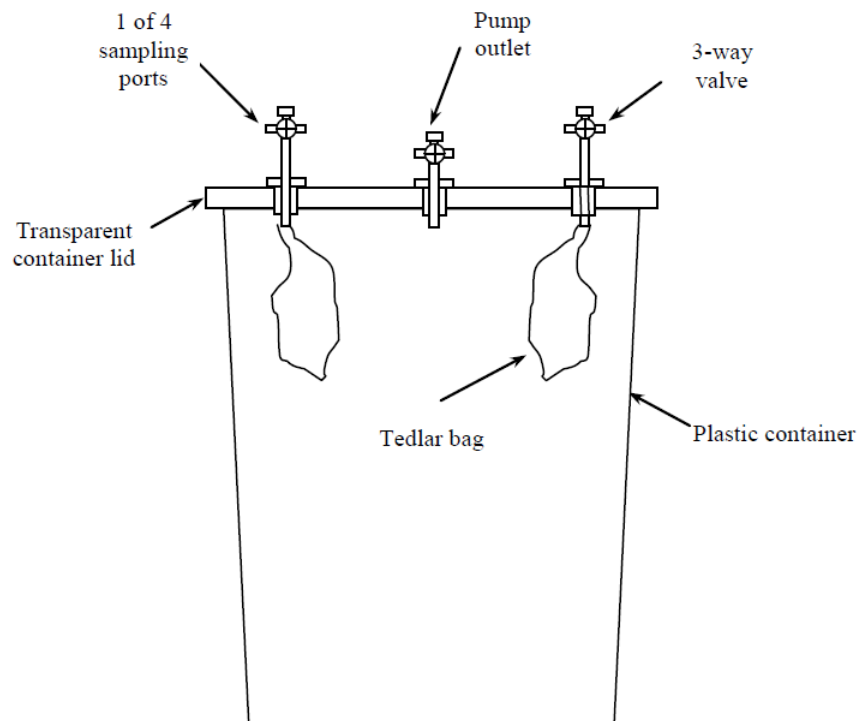


Figure 5.3 Schematic representation of the vacuum sampling apparatus (taken from Alvarado (2011))

5.3.3.2 *Stored Manure Sampling*

Following the chamber gas sample collection the manure from the chamber pit was thoroughly stirred using a rake and transferred to a 65L container using a submersible utility pump. The open container was left undisturbed for a week in an isolated room which was not accessed by barn workers due to potential toxic gas emission. The first sampling was done after 7 d and subsequent sampling were conducted on month 3 and 6.

The containers were stored uncovered to simulate typical storage conditions in a barn. The headspace gas was measured at 0, 5, and 10 min. A plastic lid covered the barrels before collection to allow the headspace concentration to accumulate. The gas samples were collected using a 20 mL syringe, by sliding off the plastic lid slightly at each time point without agitation. After collection, the samples were immediately transferred to a 12ml exetainer vial (LabCo Inc., High Wycombe, UK). Two samples were taken for each of the time points. A meter stick was used to measure the height of the headspace and also the radius of the container to measure the headspace volume and area.

5.3.4 *Calculations*

Estimation of GHG emission from the chambers

Gas concentrations from the chamber were estimated on d 0 (baseline concentration) before introducing the pigs and on d 14 at 0900 h with pigs. The air velocity was also measured before and after sampling to determine the volumetric air flow rate. The gas emissions from the chamber was then estimated utilizing the above parameters using the following equations (Kpogo et al. 2021),

$$\text{Gas emission (mg/s)} = \text{estimated gas concentration from the chamber (mg/m}^3\text{)} \times \text{volumetric airflow rate (m}^3\text{/s)} \quad [5.1]$$

$$\text{Volumetric airflow rate (m}^3\text{/s)} = \text{Air velocity (m/s)} \times \text{Fan duct area (m}^2\text{)} \quad [5.2]$$

Estimation of GHG emission from the manure

Emissions from manure was calculated using regression analysis as follows (Agnew 2010),

$$F = \rho (V / A) \times (\Delta C / \Delta t) \quad [5.3]$$

Where F = gas flux ($\mu\text{g m}^{-2} \text{s}^{-1}$),

ρ = gas density (kg m^{-3})

V = headspace volume of the barrel (m^3)

A = area of the barrel (m^2)

$\Delta C/\Delta t$ = rate of change in gas concentration at $t = 0$ (by regression, ppm min^{-1})

$\Delta C/\Delta t$ was calculated using linear regression by plotting concentration vs time.

5.3.5 Statistics

The effect of inclusion of field pea and enzymes on GHG emission from animals were analyzed using Proc MIXED of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). For the evaluation of GHG emissions from the chamber and stored manure, the experimental unit was the chamber with 6 pigs. The experiment was conducted as an RCBD with a 2×2 factorial arrangement of treatments. The model contained the fixed effect of field pea, enzyme inclusion and interactions while the chamber was included as a random effect. To accommodate the time related variation in CO_2 , CH_4 , and N_2O emissions from stored manure, a repeated measure analysis was performed. The covariance structure with the lowest Akaike and Bayesian value were chosen. The normality of the data was checked using Shapiro-Wilk test. The manure data was non-normal, thus logarithmic transformation was done, and the transformed values were subjected to one-way analysis of variance. The SLICE option of SAS was used for partitioned analysis of the least square means for treatment by period interactions and reported the overall treatment by period effect. Reported least square values are from the untransformed data. Mean separations were carried out using the adjusted Tukey option of SAS. A P value of > 0.05 was considered significant and < 0.1 was considered a trend.

5.4 Results and Discussion

Pigs were healthy throughout the trial. The ADG, ADFD, G:F were not determined for this trial. Figure 5.1 and 5.2 shows the effect of inclusion of field pea and multi carbohydrase enzymes in a finisher diets on water consumption and manure output, respectively. Inclusion of 40% field pea resulted in increased water consumption ($P = 0.053$) by 15% and manure output ($P = 0.005$) by 29%. Table 5.2 describes emissions of CO_2 , CH_4 , and N_2O . Inclusion of field pea did not

influence CO₂, CH₄, and N₂O emissions from animals. However, the inclusion of multicarbohyrase enzymes reduced CO₂ emissions (P = 0.036) and a tendency for decreased emission was observed with N₂O (P = 0.099). Table 5.3 describes the mean gas flux of CO₂, CH₄, and N₂O in µg m⁻² s⁻¹ from stored manure in the barrels when field pea and multicarbohyrase enzymes were included in the diet. Inclusion of field pea and multicarbohyrase enzymes did not affect the CO₂, CH₄, and N₂O missions from the manure .

Increased consumption of water would be expected to increase urine slurry production. Moreover, pigs fed 40% field pea diet tended to eat more (Table 4.3). As discussed below the increase in fibre content of field pea can also be a potential factor in the increased consumption of water by pigs fed 40% pea diet. In an environmental system analysis study, Eriksson et al. (2005) found that pigs fed field pea produced more manure when compared with pigs fed a SBM diet or a diet formulated with synthetic amino acids and similarly in the current study the feed disappearance of pigs fed pea diet was greater.

Others have shown that abatement of GHG emission from pigs and manure can be achieved through dietary changes of feedstuffs (Clark et al. 2005; Montalvo et al. 2013). As 40% field pea were added to the diet of finisher pigs the TDF, soluble dietary fibre (**SDF**), and insoluble dietary fibre (**IDF**) were 17.89%, 3.0%, and 14.88% compared with 16.12%, 3.18%, and 12.94% respectively of the control diet. A diet higher in fibre content changes nitrogen excretion patterns, an increase of fecal nitrogen excretion is expected over urinary nitrogen. Because the undigested fibre and ammonia in the large intestine would result in bacterial nitrogen intake and eventually excreted via feces (Zervas and Zijlstra 2002). Fecal nitrogen is less volatile and less susceptible to bacterial decomposition to ammonium ions (Canh et al. 1998; Mroz et al. 2000). The only source of N₂O emissions from pig houses is the manure slurry. Pea resistant starch and fibre are readily fermentable. The VFAs produced as a result of resistant starch and fibre fermentation can lower the manure pH and in turn reduce ammonia emission (Zervas and Zijlstra 2002; Montalvo et al. 2013). A reduction in the emission of ammonia decreases N₂O emission which are the product of incomplete nitrification and denitrification (Dennehy et al. 2017). However, in the current study the inclusion of 40% peas to the diet had no effect on N₂O emissions from either the chambers or from stored manure slurry barrels. This might be explained by greater N intake by pigs fed 40%

field pea diet (Table 4.6) compared with controls. Moreover, comparable to other studies (Park et al. 2006) N₂O emissions in the current study were negligible.

Methanogenic bacteria in the hindgut of pigs concentrated in the colon and cecum produce CH₄ (Butine and Leedle 1989). The level of NSP in the diet has a positive correlation with CH₄ produced in the hindgut of pigs, suggesting that high fibre diets may increase methane production from pigs (Jensen and Jorgensen 1994; Jensen 1996). In the current study, although the field pea diet had greater TDF and ADF content than the control diet no significant increase in CH₄ emissions were observed in air samples taken from the chambers housing the pigs. This may be due to the physico-chemical properties of the fibre present in the diets and their degradation products. When pigs were fed a high fibre diet from sugar beet pulp there was a 23% increase in CH₄ production (Montalvo et al. 2013). Similarly, in our study high inclusion of field pea in the diet did not influence CH₄ production from stored manure on d 0 or 180. However, a decrease in CH₄ production from manure slurry with feeding 40% field pea was observed on d 90. The primary precursor of manure-generated CH₄ is acetate and this process is hindered by a pH less than 7 (Park et al. 2006). As field pea fibre is readily fermentable and favours VFA production, this might decrease the manure pH and potentially reduces CH₄ production. Nevertheless, the effect of feeding peas on manure CH₄ was only manifested on d 90. This could be due to the high variability caused by differences in manure composition, pH, and temperature between and within treatments.

Inclusion of 40% field pea had no effect on CO₂ emissions from either the chamber or manure slurry. In some LCA studies the direct release of CO₂ from pigs and manure is disregarded as it is presumed to be compensated by the uptake of CO₂ for photosynthesis by the plants that supplied the feed (Philippe et al. 2007). The major contributor of CO₂ emissions in pig houses is by animal expiration. The current study did not observe any steady increase or decrease in CO₂ emissions over time between treatments. Temperature is a key factor in determining emissions from manure (Dennehy et al. 2017). The samples were collected and stored throughout the year and the manure storage room was not environmentally controlled. The varying temperature, therefore, is the potential cause for variation in emissions over time.

Multicarbohydrase enzymes break down the complex carbohydrate polymers increasing digestibility of nutrients (Adeola and Cowieson 2011). As a result, it is expected that less organic matter will be excreted and microbial fermentation in both the hindgut as well as in manure will

be reduced. The results obtained from the current study substantiate this. With the inclusion of multicarbohydase enzymes to the diet a significant reduction in emissions from the chamber was obtained with CO₂, a tendency for reduced emission for N₂O was also found. Similarly, in a study conducted by Chen et al., (2020), when NSP degrading enzymes were used in the diet of finisher pigs fed a corn-soybean based diet, CO₂ emission from the pigs were reduced and there was no effect on emission intensities of CH₄, N₂O ,or NH₃. Clark et al., (2005) found that the addition of xylanase to a high protein diet reduced the CO₂ and CH₄ emissions from manure. On the contrary, in the current study manure emissions were not affected by the inclusion of multicarbohydase enzymes.

5.5 Conclusion

Inclusion of 40% field pea did not affect CO₂, CH₄ and N₂O emissions from pigs housed in chambers or their stored manure slurry. The inclusion of multicarbohydase enzymes reduced CO₂ emissions from the chamber and showed no effect on the emissions from stored manure. The increase in manure production by pigs fed peas and its effect on large scale land application needs to be studied further. Overall LCA considering the emissions generated during crop production, growth, and manure management would give an explicit picture of the effect of dietary inclusion of field pea and enzymes to swine diets.

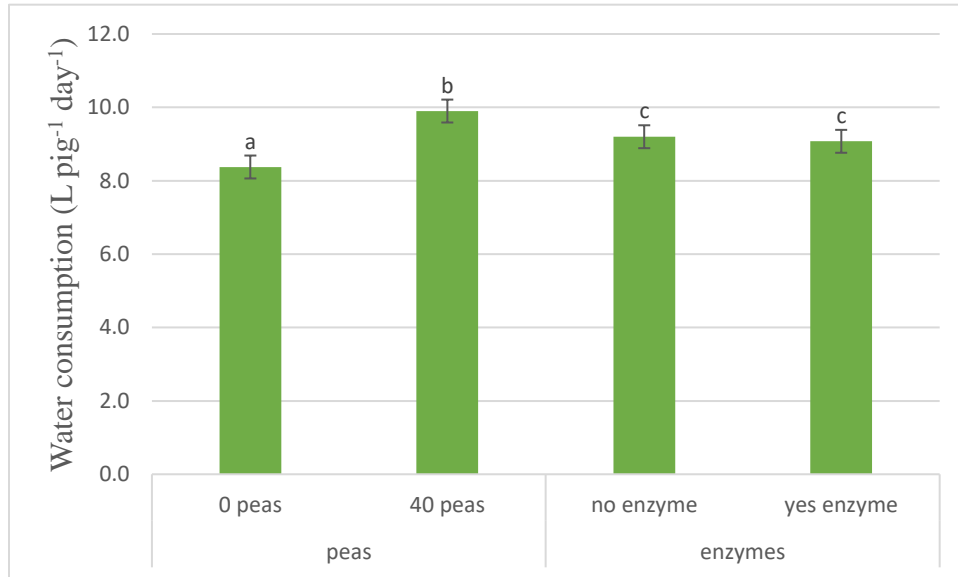


Figure 5.4 Water consumption by pigs housed in environmental chambers for 14 d. Values are means of 4 replicates with 6 pigs per treatment. Means with same letters are not significantly different ($P > 0.1$).

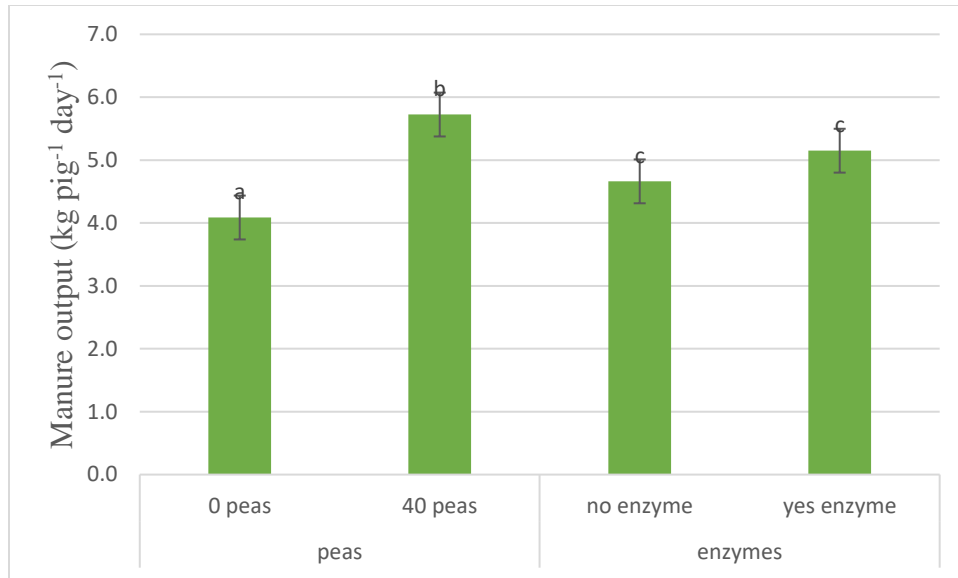


Figure 5.5 Slurry (feces and urine) output by pigs housed in environmental chambers for 14 d. Values are means of 4 replicates with 6 pigs per treatment. Means with same letters are not significantly different ($P>0.1$).

Table 5.2 Effect of 40% inclusion of field pea and multicarbohydase enzyme on N₂O, CH₄, CO₂ emissions from pigs housed in environmental chamber ^{a,b}

Item ^c	Peas,%		Enzymes		SEM	P-value		
	0	40	N	Y		Peas	Enzymes	Peas × Enzymes
Gas flux, mg s ⁻¹								
N ₂ O	0.4	0.4	0.5	0.4	0.05	0.849	0.099	0.633
CH ₄	4.4	4.4	5.2	3.6	0.65	0.982	0.118	0.124
CO ₂	1506.1	1357.1	1642.7	1220.5	139.66	0.391	0.036	0.811

^a Pigs were housed in the chambers for 14 d and sampling was done on d 14 of each trial with pigs and manure. The gas flux in mg s⁻¹ was then quantified using gas chromatography

^b Data are presented as least square means of 4 replicate chambers with 6 pigs

^c N₂O: Nitrous oxide, CH₄: Methane, CO₂: Carbon dioxide, mg: milligram, s: second
SEM; standard error of mean

Table 5.3. Effect of inclusion of 40% field pea and multicarbohydrase enzymes supplementation on N₂O, CH₄, CO₂ emissions from stored manure ^{a,b,c}

Item ^d	Peas, %		Enzymes		SEM	P-value		
	0	40	N	Y		Peas	Enzymes	Pea × Enzymes
Gas flux, µg m ⁻² s ⁻¹								
<i>CH₄</i>								
d 0	11.8	65.6	46.6	30.8	9.29	0.514	0.681	0.804
d 90	23.0	4.7	9.4	18.3	11.20	0.016	0.956	0.080
d 180	17.7	9.2	12.4	14.5	9.55	0.785	0.518	0.899
<i>CO₂</i>								
d 0	518.4	299.9	577.9	240.4	9.54	0.961	0.112	0.409
d 90	435.1	314.9	238.7	511.3	11.90	0.622	0.273	0.632
d 180	305.6	390.2	325.6	370.2	9.31	0.795	0.587	0.119

^a Manure were stored in 65L barrels and headspace gas was measured on d 0, d 90, d 180 using regression analysis

^b Data are presented as least square means of 4 replicate manure barrels

^c The P values were obtained after logarithmic transformation of data and least square means reported are from non-transformed data

^d CH₄: Methane, CO₂: Carbon dioxide, µg: microgram, m: metre, s: second
SEM; standard error of mean

N₂O emissions from the stored manure slurry were negligible.

**CHAPTER 6. EFFECT OF FIELD PEA AND CARBOHYDRASE
ENZYMES IN FINISHING PIG DIETS ON GHG EMISSIONS FROM
CROP AND ANIMAL PRODUCTION USING A USING A LIFE CYCLE
INVENTORY**

6.1 Abstract

Field pea is produced abundantly in western Canada and can be a good source of starch and protein when included in swine diets. A major contribution to the carbon footprint from pork production is feed. We hypothesized that primarily due to reduction in N fertilizer required for pea production, including field pea in a swine diet would reduce overall GHG emission from pork production. Inclusion of multicarbohydrase enzymes would improve nutrient digestibility resulting in further decrease in GHG emissions. The objective of this study was to develop a life cycle inventory (LCI) for combined crop production and animal emissions to estimate and compare the GWP when field pea and multicarbohydrase enzymes were fed to finishing pigs. The HOLOS software (Agriculture and Agri-Food Canada, Lethbridge, AB) was used for compiling the LCI from crop production which emissions based on information from individual farms. The LCI for animal emissions were from a study conducted in environmental chambers experiment at PSCI. The treatments, arranged as 2 by 2 factorial, were 0 and 40% field pea with or without multicarbohydrase enzyme (glucanase, xylanase, cellulase, amylase, and invertase) inclusion. Each dietary treatment was repeated four times and randomized between the two chambers. Six pigs weighing 60 ± 2.2 kg BW were maintained in the chamber for 14 d. Gas samples were collected and analyzed for CO₂, CH₄ and N₂O by gas chromatography. The results obtained from HOLOS for crop production alone were respectively 4429 (0% peas with enzyme), 4308 (0% peas without enzyme), 3742 (40% peas with enzyme), 3873 (40% peas without enzyme) kgCO_{2e} per 100 pigs. Including field pea in the finishing pig diets reduced emission from crop production by 13%. Inclusion of multicarbohydrase enzymes reduced emissions from animal production by 26%, but 40% field pea in the diet increased animal emissions by 17%. The inclusion of field pea reduced crop associated emissions, but the emissions from animal production increased. A complete LCA would require determining the carbon footprint of both field pea as crop and pork production.

6.2 Introduction

Pork is the most consumed meat worldwide (FAO 2011) and third most consumed meat in Canada after chicken and beef (Statistics Canada and Agriculture and Agri-Food Canada (AAFC) 2020). According to Statistics Canada, the Canadian per capita consumption of pork for the year 2019 was 22.1 kg and this was anticipated to increase in the following years (Macleod et al. 2013). The requirements of the livestock sector for land, water, feed, and energy can be an environmental burden. The most important output from the livestock sector is meat, which is one of the food products with the greatest carbon footprints, primarily due to inefficiencies of converting feed grains to meat (Röös et al. 2013). Therefore, to facilitate further development of the meat industry, potential means for sustainable meat production should be investigated.

The choice of feed ingredients can influence the environmental footprint of pork production. For example, the protein requirement of the pig is often achieved by adding soybean meal to the diet to supplement protein provided by cereal grains. The extensive cultivation of soybean in Brazil has been implicated in the loss of habitat, biodiversity and soil erosion (Mattsson et al. 2000). An LCA study conducted by Eriksson et al. (2005) determined the effect of three feed scenarios: SBM scenario, field pea scenario, and synthetic amino acid scenario on energy use, GWP, acidification and eutrophication. It found that the SBM scenario had the greatest impact on energy use, GWP and eutrophication, followed by pea scenario, but synthetic amino acid scenario had the least impact. Hence, alternate feed ingredients or feed additives with reduced carbon footprint are required. Field pea is an alternate protein as well as energy source capable of replacing SBM in pig diets. Carbohydrase enzymes are used in swine rations as a feed additive to improve overall nutrient digestibility, and therefore have the potential to reduce manure output.

To establish a novel or an alternate feedstuff as sustainable when compared to another, a complete study of the entire processes should be undertaken. A gain in sustainability in one stage might be nullified by increased emission in the next stage. For instance, use of legumes when compared to cereal grains may reduce carbon footprint because of their ability to fix atmospheric N in symbiosis with rhizobia. However, if these ingredients, when fed to pigs and are not efficient in feed conversion or result in more emissions, then sustainability gains from feed production may be nullified. Hence a complete life cycle assessment is key to determine overall carbon footprint. The overall objective of this study was to determine the effect of field pea or multicarbohydrase

enzymes inclusion in finishing pig diet on carbon footprint of pork production and to develop a life cycle inventory which can be used for future LCA studies

6.3 Methodology

6.3.1 Inventory of environmental inputs and outputs

Life cycle inventory of a complete LCA accounts for all the inputs going to the system and outputs produced from the system and the associated emissions will be reported using an impact factor. The current study developed an LCI for emissions from crop production and animal production (finisher pigs). And the impact factor used in this study to represent carbon footprint is GWP. According to IPCC, GWP is the relative effect of a GHG on climate change over a period of time (100 years), compared with the same mass of CO₂. The manure produced from the finisher pigs were stored for 6 months, and emissions were estimated. However, in order to develop an LCI for manure emissions the field level application of manure and the related emissions should be estimated, and which was outside the scope of this study.

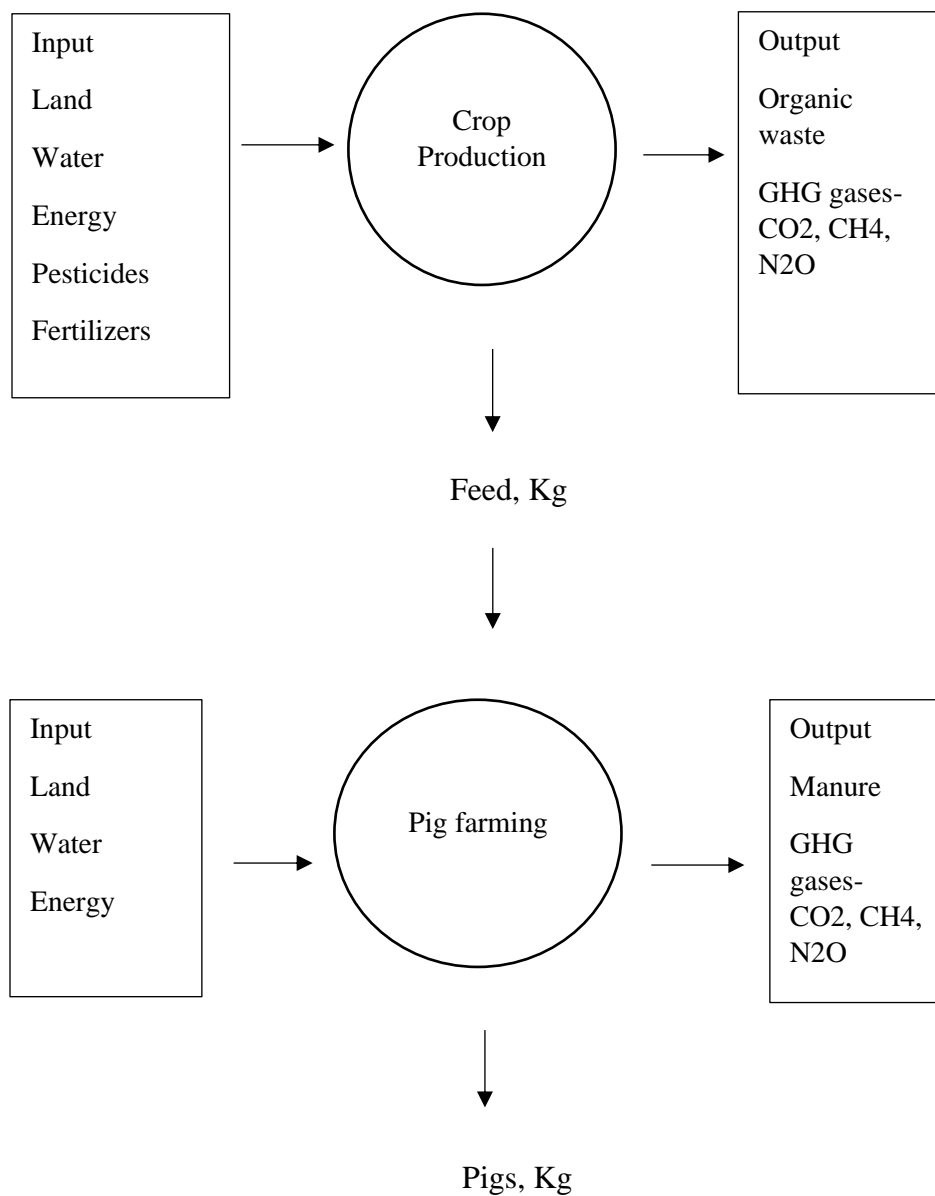


Figure 6.1 Schematic representation of inputs and outputs of a pork production unit

6.3.2 LCI for crop production

To estimate the GHG emission from crop production Holos software (Agriculture and Agri-Food Canada, Lethbridge, AB) was used. This whole-farm model determines the GHG emissions on an individual farm basis using the farm specific information provided. The first step of crop production LCI was in collaboration with scientists at PAMI (Humboldt, SK) to develop an Excel spreadsheet allowing the calculation of land area in hectares required to feed 100 finisher pigs. Feed was given in 2 phases and the emissions associated with phase 1 and phase 2 feed were determined separately. Table 6.1 shows the ingredient composition of diets fed. The data obtained from a previously conducted experiment (Chapter 4) at PSCI was used for calculation of ADFD as well as number of days the pigs were on a particular diet. This information together with crop yield information obtained from average values reported by the Saskatchewan Crop Planning Guide (Government of Saskatchewan 2018) allowed the estimation of the total land area used by each ingredient. The yield and land area were then used as inputs for modelling along with the details of pesticide and fertilizer application rates (Government of Saskatchewan 2018). Holos then generated the GHG emission from crop production for the ingredients included in the diets.

Table 6.1 Ingredient composition of Phase 1 and Phase 2 diets fed in the growth performance study and chamber experiment at PSCI ^{a b}

Treatments	Phase 1		Phase 2	
	Control	Peas	Control	Peas
<i>Ingredients</i>				
Field pea	0.00	40.00	0.00	40.00
Wheat	61.85	21.87	45.72	15.00
Barley	24.80	29.47	43.00	42.57
Canola oil	1.50	1.00	1.20	0.50
Soybean meal	9.00	5.00	8.00	0.00
L-Lysine (78%)	0.34	0.00	0.20	0.00
DL-Methionine (99%)	0.03	0.08	0.03	0.03
L-Threonine (98.5%)	0.08	0.08	0.05	0.08
Limestone	1.20	1.20	1.00	1.00
Dical 21/16	0.30	0.40	0.30	0.40
Salt	0.30	0.30	0.30	0.30
Vitamin and mineral premix ^c	0.20	0.20	0.20	0.20
Celite	0.40	0.40	0.00	0.00

^a The diets were partitioned to two equal parts and enzymes were added to each half, giving 3 additional diets. The enzyme combination contained the activities of glucanase (300 LGU units/g), xylanase (1000 XYL units/g), cellulase (1900 CMC units/g), amylase (4,200 FAA units/g) and invertase (150 INV units/g) (Superzyme®-W from Canadian Bio-Systems Inc. Calgary, Alberta, Canada). Enzyme was added to the diet at 1000g/tonne of completed feed.

^b Chamber experiment utilized phase 1 diet only

^c The swine premix provided the following vitamins and minerals per kilogram of 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, 2 mg. Iron, 100 mg; Zinc, 100 mg; Manganese, 40 mg; Copper, 15 mg; Selenium, 0.30 mg; and Iodine, 1 mg.

6.3.2.1 Farm inputs and assumptions for Holos

For the purpose of this model, it was assumed that the crops were produced in Saskatchewan Ecodistrict number 774, a medium soil texture, and Black/Gray Chernozem soil type. Reduced tillage intensity was selected so that the GHG emissions associated with soil disturbances were neglected. Figure 6.2 shows how the information was entered into the Holos farm information form.

Farm Information Form	
Details <input type="radio"/> On <input checked="" type="radio"/> Off	
Farm Information	
Farm Name	Input New Farm Name
Farm Year	2019
Ecodistrict	774 <input type="button" value="Ecodistrict Map"/>
Province	Saskatchewan
Description	
Soil Texture	Medium
Soil Type	Black/Gray Chernozem
Tillage Management Practice	
Present Intensity	Reduced
Past Intensity	Reduced

Figure 6.2 Generic farm information input form

6.3.2.2 Crop inputs

Ingredients which contributed less than 0.5% and non-organic ingredients were not considered. In total there were four diets. A control diet with and without multicarbohydase enzymes and 40% field pea diet with and without multicarbohydase enzymes. Emissions attributed to crop production were calculated based on the area of land used for the production of a particular feed ingredient and its proportion in the particular diet. The Excel worksheet (Figure 6.3) determined the land requirement based on the following inputs: feed disappearance, number

of animals, days on feed, and ingredients as percent weight basis for each diet. For by-product and co-product ingredients such as soybean meal and canola oil the associated fraction of the raw commodity was used so that emissions associated with their production were appropriately distributed. Soybean meal and canola oil represent 79% and 44% of whole kernel soybean and canola respectively on weight basis (U.S. Soybean Export Council. 2015; Canola Council of Canada. 2017). The densities of various agricultural commodities and yield values were based on averages reported by the Saskatchewan Crop Planning Guide (Government of Saskatchewan 2018).

Total Ration	2.6	kg																				
# of Pigs	100			% of Whole	Raw Commodity		Yield			Land Ratio			Land Area									
# of Days on Feed	30.3			Kernel	Mass	Density																
Feed Barley	24.80	%			1,954	kg	21.8	kg/bu	72.9	bu/ac	180.1	bu/ha	1589.2	kg/ac	3927.0	kg/ha	1.2	ac	0.50	ha		
Spring Wheat	61.85	%			4,873	kg	27.2	kg/bu	51.1	bu/ac	126.3	bu/ha	1389.9	kg/ac	3434.6	kg/ha	3.5	ac	1.42	ha		
Field Peas	0.00	%			-	kg	27.2	kg/bu	45.7	bu/ac	112.9	bu/ha	1243.0	kg/ac	3071.6	kg/ha	0.0	ac	0.00	ha		
Other		%			-	kg		kg/bu		bu/ac	0.0	bu/ha	0.0	kg/ac	0.0	kg/ha	0.0	ac	0.00	ha		
Other		%			-	kg		kg/bu		bu/ac	0.0	bu/ha	0.0	kg/ac	0.0	kg/ha	0.0	ac	0.00	ha		
Soybean Meal	9.00	%	709	kg	79	%	560	kg	27.2	kg/bu	23.5	bu/ac	58.1	bu/ha	639.2	kg/ac	1579.5	kg/ha	0.9	ac	0.35	ha
Canola Oil	1.50	%	118	kg	44	%	52	kg	22.7	kg/bu	45.2	bu/ac	111.7	bu/ha	1026.0	kg/ac	2535.4	kg/ha	0.1	ac	0.02	ha
Wheat Millrun		%	-	kg	15	%	-	kg	27.2	kg/bu		bu/ac	0.0	bu/ha	0.0	kg/ac	0.0	kg/ha	0.0	ac	0.00	ha
Other		%	-	kg		%	-	kg		kg/bu		bu/ac	0.0	bu/ha	0.0	kg/ac	0.0	kg/ha	0.0	ac	0.00	ha

Figure 6.3 Case ration (control diet) parameters for obtaining the inputs for Holos crop form.

The calculated land area per commodity required to produce each ingredient was then input into the Holos annual crops form (Figure 6.4). Yield, nitrogen fertilizer application rates, and phosphorus fertilizer application rates for input in to the Holos were obtained from the 2018 Saskatchewan Crop Planning Guide (Government of Saskatchewan 2018) (Table 6.2). The analysis assumed that the crop acres were not irrigated, and an appropriate herbicide management plan was followed based on conventional agricultural practices in Saskatchewan. Once the crop form was saved, the emissions report for the case ration were viewed under the ‘Results’ tab and the emissions in kg CO₂ were selected for this study. The GHG emissions associated with crop production are shown in Table 6.3.

File Window Tools Results Help

Farm Crops Beef Dairy Swine Sheep Poultry Other Animals

Details On Off

Annual Forages, Legumes, Cereals, Oilseeds, Specialty Crops

Type	Area (ha)	Yield (kg ha ⁻¹)	Irrigated	Herbicide	N Fert Rate (kg N ha ⁻¹)	P Fert Rate (kg P2O5 ha ⁻¹)
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0
None	0.0	0.0	No	Yes	0	0

Figure 6.4 Holos annual crops form

6.3.3 LCI for Animal production

For developing the LCI for emissions associated with animals and manure, the data obtained from a previous GHG emission study (Chapter 5) utilizing the specialised environmental chambers in the PSCI was used. A wheat-barley based control diet and a high inclusion field pea diet was formulated with and without enzymes and were fed to finisher pigs. The field pea was included at the rate of 400mg/kg of diet (Table 6.1).

6.3.3.1 GHG output from pigs and manure

A total of 96 animals (males and females) were used for the experiment. At the start of each experiment, 6 pigs weighing around 60 kg, same sex was housed in an environmentally controlled chamber. Two identical environmental chambers at PSCI were used for this trial. Detailed information on experimental design, housing, and sample collection was provided in Chapter 5.

Prior to introducing the pigs to the chamber, gas samples were collected to determine the baseline CO₂, CH₄, and N₂O levels. Animals were introduced to the chambers on d 0 and maintained there for 14 d. On d 14, gas samples were collected from the room. Immediately after collection the gas samples were subsampled in to exetainer vials (LabCo Inc., High Wycombe, UK) and transported immediately to the laboratory for gas chromatography.

The following equation was used for determining the total emissions from the finisher pigs housed in the chamber (Montalvo et al. 2013),

$$E_{gas} = (C_{gas\ out} - C_{gas\ in}) / 10^6 \times (\beta_{gas} \times Q \times 86400) / M_{pig} \times 10^6 \quad [6.1]$$

Where E_{gas} = gas emissions (mg_{gas} kg_{pig}⁻¹ day⁻¹)

$C_{gas\ out}$ = gas concentration in the room measured on d 14 (mg m⁻³)

$C_{gas\ in}$ = baseline gas concentration measured on d 0 (mg m⁻³)

β_{gas} = mass of gas by air volume (kg_{gas} m⁻³)

Q = room ventilation rate (m³ s⁻¹)

M_{pig} = total mass of the pigs in the room (kg)

86400 s d⁻¹ = 24h d⁻¹ × 3600 s h⁻¹.

6.4 Results and Discussion

Life cycle analysis determines the impact of a product on the environment by considering the resources used and emissions produced at each stage of its production (Werf et al. 2007). The impact categories generally used for agricultural products includes global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), non-renewable energy, and land use change (LUC) (Nguyen et al. 2011). This study followed an attributional LCA modelling, like the most of the LCAs on livestock products (Thomassen and Dalgaard 2008) and aimed to develop an LCI for the emissions from crop production and pig housing when field pea and enzymes were incorporated into the finisher pig diet. Pigs from 60 kg to a market weight of 127 kg were used. Studies reported that the finishing stage results in the majority of emissions because of the increased demand of nutrients for growth (Reckmann et al. 2013; Lamnatou et al. 2016). As discussed above, an LCI for manure associated emissions were considered outside of the scope of this study. To determine the environmental impact of crop production and emissions from animal

production the impact category used in this study was GWP, expressed in kg CO₂ equivalents (kgCO₂e). To calculate the GWP, the quantified CO₂, CH₄, and N₂O emissions were converted to their respective CO₂ equivalence factor (CH₄ emissions × 28, N₂O emissions × 265 CO₂ emissions × 1) of the 100 year time horizon (IPCC 2014) and then summed.

Table 6.3 shows the emissions from crop production. It also shows the days to market and ADFD of pigs (extracted from growth performance experiment; Chapter 4). Using these inputs HOLOS software calculated the crop associated emissions for feeding 100 pigs weighing around 60 kg body weight until they reached their target market weight, when fed the respective diets. When 40% peas were included in the diet of finisher pigs, the GWP from crop production was 37.4 and 38.7 kg CO₂e/pig (with and without enzyme) and 44.3 and 43.1 kg CO₂e/pig (with and without enzyme) for the 0% peas diet. Including 40% field pea reduced the crop emissions used to provide feed for the finishing stage of 100 pigs by 13%. In contrast an LCA study conducted by Lamnatou et al. (2016), which solely looked at the impact of animal nutrition on pork production, it was found that the GWP of feed alone contributed to 336 kgCO₂e per market pig with an initial body weight of 25 kg and final body weight of 105 kg. The current study used pigs with an initial body weight around 60 kg and market weight of 127 kg, which might explain the difference in emissions between the two studies. In our study CO₂ and N₂O were the main contributors to feed associated emissions, representing 52% and 48% respectively. The CH₄ emissions from crop production were almost nil. These findings were similar to a study conducted by Reckmann et al. (2013), in evaluating the carbon footprint of German pork production where the emissions from feed mainly came from CO₂ (49%) and N₂O (49%). Eriksson et al. (2005) conducted a study to find the impact of feed choice in pig production and found, within feed associated emissions a major share was constituted by N₂O from soil. The reduced usage of N fertilizers and the biological N fixation to the soil with the inclusion of 40% field pea to the diet probably reduced the direct and indirect N₂O emissions from soil and thus the carbon footprint of crop associated emissions.

Table 6.4 shows the effect of diets formulated with and without field pea and multicarbohydrase enzymes from finishing pigs. This includes emissions from animal expiration, enteric fermentation, and manure. When finisher pigs consumed a diet with 40% peas, the GWP from pig housing was 8.17 and 12.19 kgCO₂e kg_{pig}⁻¹ day⁻¹ (with and without enzyme) and 10.82 and 14.58 kgCO₂e kg_{pig}⁻¹ day⁻¹ (with and without enzyme) for the control diet with 40% field pea.

Montalvo et al. (2013) looked at the effect of different dietary treatments on GHG emission from post weaned pigs and the estimated emissions ranged from 0.025 – 0.028 kgCO₂e kg_{pig}⁻¹ day⁻¹. Compared to our findings the emission obtained by Montalvo are very low and the difference can be attributed to the age of pigs and the differences in room ventilation rates used in two studies. In our findings, 93% of the animal emission were contributed by CO₂, followed by CH₄ (4%) and N₂O (3%). Likewise, Montalvo et al. (2013), observed that 97% of emissions from swine facilities was accounted by CO₂. On the contrary, Reckmann et al. (2013) found the emissions from pig housing were mainly composed of 88% CH₄, 7% CO₂, followed by 5% N₂O. However, the study by Reckmann et al. (2013) did not take into account the direct CO₂ emissions from animals. Moreover, the direct CO₂ emissions from animals and manure are often disregarded in LCA studies as they can be compensated by the consumption of CO₂ during crop production (Philippe and Nicks 2015). The inclusion of multicarbohydase enzymes to control diet and high field pea diet reduced the emissions at pig production stage. It would be interesting to look at how enzymes would affect the emissions from manure and further research would answer it.

6.5 Conclusion

Including field pea in the diet of finisher pigs resulted in reduced carbon footprint from crop production stage but not from the swine facility. Multicarbohydase enzyme has the potential to reduce emissions from animal houses. A complete LCA considering manure production and associated emissions is required to conclude if field pea reduced the overall carbon footprint of pork production. It would also be important to consider other impact factors like acidification potential, eutrophication potential, energy use, and land use change.

Table 6.2 Yield and fertilizer application rate of feed ingredients as inputs for the Holos model for developing the LCI for crop production ^a

Item	Wheat	Barley	Field pea	Soybean	Canola
Yield (kg ha ⁻¹)	3437	4903	3073	1580	3040
N fertilization rate (kg ha ⁻¹)	88.6	77.3	7.9	5.6	98.6
P fertilization rate (kg ha ⁻¹)	34.8	37.0	34.8	26.9	53.8

^a Data obtained from the Government of Saskatchewan crop planning guide, 2018 (assuming the crops planted in a dark brown soil zone in Saskatoon).

Table 6.3 The GWP calculated from emissions associated with crop production of 100 finisher pigs from $60 \pm 2.2\text{kg}$ to market ^{a,b,c}

	0% Peas		40% Peas	
	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No
Days to market	68	67	66	66
ADFD, kg	2.75	2.72	2.78	2.83
<i>Feed ingredients, kgCO₂</i>				
Barley	1502	1449	1210	1255
Wheat	2654	2589	1069	1115
Canola	43.2	43.2	32.39	32.39
SBM	230	227.1	133.9	136.9
Peas			1297	1335
Total, kgCO₂e/100 pigs	4429	4308	3742	3873
KgCO₂e pig⁻¹	44.3	43.1	37.4	38.7

^a CO₂e were estimated using the following conversion factors; CH₄ emissions × 28, N₂O emissions × 265, and CO₂ emissions × 1 (IPCC 2014)

^b The GWP is determined with the HOLOS software using ADFD and Days to market data for 100 pigs for the particular diet

^c The kgCO₂e for each ingredient is based on the proportion in the diet, feed intake and emissions to grow that crop as determined by the model

Table 6.4 The GWP calculated from finishing pigs fed 0% and 40% field pea diet with or without multicarbohydrase enzymes housed in environmental chambers ^{a b}

	0% Peas		40% Peas	
	Enzyme Yes	Enzyme No	Enzyme Yes	Enzyme No
Gas, kgCO ₂ e kg _{pig} ⁻¹ day ⁻¹				
Carbon dioxide	0.12	0.17	0.15	0.21
Methane	0.008	0.005	0.007	0.008
Nitrous oxide	0.004	0.007	0.007	0.003
GHG total	0.132	0.182	0.164	0.221
GHG x days on feed	8.97	12.19	10.82	14.58

^a CO₂e were estimated using the following conversion factors; CH₄ emissions × 28, N₂O emissions × 265, and CO₂ emissions × 1 (IPCC 2014)

^b Results from the GHG emission study were used for determining the GWP as described by Montalvo et al. (2013) by taking into account the baseline gas concentration

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSION

7.1 General Discussion

In 2015, food systems contributed 34% of the global anthropogenic GHG emissions with a little over two-third (71%) of these emissions arising from agricultural and land use change activities (Crippa et al. 2021). The only plausible scenario to mitigate emissions to feed the expanding global human population is to find sustainability in food production. The exponential growth in population is accompanied by increased production and consumption of meat, for example 46% increase in per capita meat consumption from 1961 to 2013 (Rauw et al. 2020). Despite the wide range of benefits from meat consumption, it is also one of the least sustainable sectors of food production (Djekic 2015).

Improving sustainability of pork production through dietary changes in feedstuffs was the objective of this study. Field pea, locally grown in Western Canada are a good source of dietary protein and starch. Nitrous oxide is a potent GHG comprising 5 to 6% of total atmospheric GHG (Crutzen et al. 2008) and most of this is emitted from agricultural soils from fertilizer and manure application (Reay et al. 2012). The production of N fertilizer requires high fossil energy inputs, releasing approximately 300 Tg of CO₂ to the atmosphere each year for every 100 Tg fertilizer N manufactured (Jensen et al. 2012). Incorporation of legumes into the cropping cycle can reduce the overall use of nitrogenous fertilizers from biological nitrogen fixation (Ma et al. 2018). A meta-analysis conducted by MacWilliam et al. (2018) found that the introduction of pulses (dry peas and lentils) into the rotation of cereals and oilseeds resulted in an overall GHG emissions savings of up to 475-719 kgCO₂e/ha over a two year period when N-fertilizer inputs were unchanged. A further reduction of 489-1185 kg CO₂e/ha could be achieved by reducing N-fertilizer to the minimum. Hence, we hypothesized that high incorporation of field pea in swine diets could be beneficial in mitigating GHG emissions from pork production.

Exogenous enzymes can be added to the diet of livestock to improve nutrient digestibility, resulting in better utilization of feed (Kiarie et al. 2013). This is especially important when alternate ingredients are used which are often more economical but high in NSPs. Addition of enzymes can also reduce the endogenous losses induced by polymeric carbohydrates (Acamovic 2001). Further addition of enzymes to the diet formulated with alternate ingredients can combat the anti-nutritional factors by breaking down complex polysaccharides and improve the nutrient digestibility. The incorporation of multi-carbohydrase enzymes to high field pea diet might

improve nutrient digestibility and feed efficiency of the diet. Jha et al. (2011) found field pea to be readily fermentable with an NSP, NDF, and ADF content of 254, 214, and 81 g/kg DM respectively.

This research was comprised of three studies which looked at the impact of high inclusion of field pea and multi-carbohydrase enzyme on mitigating GHG emission from finishing pigs. We hypothesised that the inclusion of field pea and multicarbohydrase enzymes would lower the carbon footprint (expressed as GWP) due to possible N savings during crop production. The data required to develop an LCA framework was obtained from growth performance-nutrient digestibility and GHG emission studies. The methodology and results are detailed in Chapter 4 and Chapter 5 of this thesis. The process of development of the LCA inventory is explained in Chapter 6.

High inclusion of field pea in the diet of finishing pigs has previously shown negative effects on performance (Friesen et al. 2006; Stein et al. 2010) in some studies. However, 66% inclusion of field pea in the diet of grower pigs maintained animal performance and carcass parameters (Stein et al. 2006). In the growth performance study, pigs weighing around 60 kg BW were selected and fed either a control diet (wheat-barley) or a diet with 40% field pea until the target market weight of 128 kg was attained. Performance and carcass quality were unaffected by with the addition of 40% field pea to finisher pig diet. Peas are relatively higher in fibre compared to dehulled SBM (Jha et al. 2011) which could adversely affect nutrient digestibility. Pigs fed the field pea diet had reduced GE, DM, N and P digestibilities but higher ADF and NDF digestibility compared with control diet and the incorporation of the multicarbohydrase enzyme had no effect on either animal growth or nutrient digestibility. Despite, reduced digestibility of DM, GE, and N, the animals fed high field pea diet showed a tendency to eat more, had improved their growth performance, and reduced days to attain target body weight. Hence, if high field pea diets are formulated to similar NE and SID AA as of a standard finisher pig diet, growth performance and carcass parameters can be maintained. These production data was used in the model to estimate GHG production from pigs fed control or 40% field pea diets.

The potential of 40% field pea diet with or without enzymes to affect emissions from animals and manure was estimated utilizing small groups of pigs housed in environmental chambers. Following an adaptation period of 14 d, air samples were collected and CO₂, CH₄, and

N₂O emissions from pigs and the manure were quantified and analyzed using gas chromatography. Manure samples were also collected from the chamber and emissions of CO₂, CH₄ and N₂O measured on d 0, d 90, and d 180 of storage were determined.

Inclusion of 40% field pea in the diet had no effect on CO₂, CH₄ and N₂O emission from the pigs as well as CO₂ and CH₄ emissions from the stored manure. These results agree with a study conducted by Mihaela et al. (2020), where 16% inclusion of field pea, partially replacing corn and SBM, did not affect emissions of CO₂ and CH₄ from pigs. Reduced N₂O emissions from manure by pigs fed high field pea diets were not observed. Similar to results observed by others (Park et al. 2006), the N₂O emissions from manure was negligible. An important and expected finding from the digestibility trial was the shift in N excretion patterns and N retention feeding the high field pea diet. This might reduce the N₂O emissions from manure, as faecal N is less volatile (Zervas and Zijlstra 2002). The expected reduction in N₂O emission was not found, however, N₂O emissions were negligible from stored manure. The inclusion of the multicarbohydase enzymes reduced CO₂ emissions and tended to reduce N₂O emissions from pigs, but not from the stored manure. There is a positive correlation between fibre in the diet and methane emissions (Jensen 1996), however, the current study observed no such rise in CH₄ emissions when field pea were included in the diet. Further research would answer how dietary inclusion of field pea and multicarbohydase enzymes would affect large scale manure storage and land application.

Field pea in symbiosis with rhizobia are biological nitrogen fixers and can reduce the use of nitrogenous fertilizers in crop rotation cycles (Lemke et al. 2007). We hypothesized that the inclusion of field pea in swine rations would reduce GWP when the overall life cycle of pork production is considered, provided diet maintains animal performance. Utilizing Holos software developed by Agriculture and Agri-Food Canada and the results from growth and chamber experiment, a partial LCA framework was developed and detailed in the third study. LCA is a holistic approach to estimate the environmental burden of a product by evaluation of inputs and outputs at each stage of production (Reckmann et al. 2013). The three main emissions from pork production are from crop production, pig housing, and manure. Emissions from pig housing showed an increase feeding the 40% field pea diet as per the LCI, although the emissions per unit time were not statistically different as shown in Chapter 5. Overall, the major finding from the LCA was that the inclusion of 40% peas in the diet reduced crop associated emissions by 13%

providing an indication that dietary ingredient changes may have an effect on sustainability of pork production. Manure management and associated emissions were not a part of this study. Further research of feed production, pig housing and manure management, considering more impact factors like acidification potential and eutrophication potential would give more comprehensive idea of the carbon footprint of pork production.

7.2 Conclusion

As the demand for feed for livestock to produce food for humans has increased it is imperative to find sustainable solutions in agriculture and farming. This calls for the inclusion of alternate feed ingredients as well as technologies like enzyme inclusion for enhancing the digestibility of available ingredients in animal nutrition. Field pea is locally produced in Western Canada and Canada is the largest pea producer in the world. Incorporation of field pea and pea splits (by-product from split pea production for human consumption) in swine is likely sustainable. Inclusion of pea as a primary ingredient in finisher pig diet can maintain animal performance, days to market, and carcass qualities and also has the potential to reduce GHG emissions. And the data from LCI study can contribute to Global Feed LCA Institute (GFLI) database to support future environmental assessment studies.

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