

Nutritional value of heat-processed field pea and lentil grains as alternative feedstuffs for pigs

By

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## ABSTRACT

Field pea and lentil grains are alternative starch and protein sources for swine; however, pulse grains have a lower energy digestibility in pigs than conventional feedstuffs, contain trypsin inhibitors that may reduce protein digestion, and may reduce feed efficiency (G:F). Processing using treatments with heat and friction and whether it reduces trypsin inhibitor activity (TIA) and increases nutritive value of field pea and lentil grains remains unknown. The objectives of this thesis were to gain insight into the effects of heat processing on nutrient digestibility of field pea and lentil grains and further our understanding of the feeding value of raw and heat-processed field pea and lentil grains in weaned and growing pigs. In Chapter 2, weaned pigs ( $n = 236$ ) were fed for 21 d diets including 400 g/kg raw, cold-pelleted, steam-pelleted or extruded field pea replacing 300 g/kg soybean meal (SBM) and 100 g/kg wheat grain. Average daily feed intake of pigs fed field pea diets (853–882 g/day) was greater than pigs fed SBM (813 g/day); however, the predicted net energy value was 0.7–0.76 MJ/kg lower in field pea diets than in the SBM diet. Average daily gain and final body weight did not differ between SBM diet and field pea diets, nor did it differ among field pea treatments. Thus, G:F was lower for pigs fed field pea diets (0.60–0.63) than for pigs fed SBM diet (0.68). In Chapter 3, the same raw and processed field pea grain (cold-pelleted, steam-pelleted or extruded) were included in 4 diets at 956 g/kg and fed to 8 ileal-cannulated weaned pigs in a double  $4 \times 4$  Latin square. A N-free diet was also fed to pigs to measure basal endogenous losses of amino acids (AA). Cold-pelleting reduced TIA by 0.8 mg/g and increased digestibility of energy by 0.92 MJ/kg in field pea grain and extrusion reduced TIA by 1.1 mg/g and increased ileal digestibility of some AA compared with raw field pea grain in weaned pigs. In Chapter 4, energy and protein digestibility of raw, steam-pelleted, or extruded lentil grain was measured using 9 ileal-cannulated growing-finishing pigs. Diets containing 956 g/kg of raw or

processed lentil grain were fed to pigs in a triple  $3 \times 3$  Latin square with an additional N-free diet. Steam-pelleting and extrusion increased measured starch content (91 and 89 g/kg, respectively) and digestibility of dry matter, gross energy, and crude protein of lentil grain by 2.0 to 6.5%. Ileal digestibility of all amino acids except glutamic acid were increased by steam-pelleting and some were increased further by extrusion. The TIA was reduced by extrusion by  $>1.2$  mg/g, but not steam-pelleting. In summary, nursery pigs fed 400 g raw field pea/kg maintained growth, and pigs compensated for the reduced energy value of field pea with a greater average daily feed intake, which is indicative of an absence of negative effects of TIA on feed intake. Cold-pelleting and extrusion of field pea grain reduce TIA, though only cold-pelleting increased energy digestibility and only extrusion increased digestibility of some AA. Both steam-pelleting and extrusion increase digestibility of AA and energy value of lentil in growing-finishing pigs. This thesis provides information that enhances the understanding of opportunities and limitations to feed pulse grains to both weaned and growing-finishing pigs. In conclusion, feeding value and nutritive value of lentil and field pea grain may be affected by heat-processing, as indicated by increased energy digestibility, increase protein digestibility or maintained growth.

## PREFACE

This thesis is an original work by Jill M. Hugman. The thesis includes three studies for which animal use was approved and procedures were reviewed by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care (CCAC, 2009) as described in Chapters 2, 3 and 4.

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## LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ANF	Anti-nutritional factor
BW	Body weight
CAID	Apparent ileal digestibility coefficient
CATTD	Apparent total tract digestibility coefficient
CP	Crude protein
CSID	Standardised ileal digestibility coefficient
DE	Digestible energy
DM	Dry matter
GE	Gross energy
G:F	Feed efficiency (ADG/ADFI)
Lys	Lysine
NDF	Neutral detergent fibre
NE	Net energy
SBM	Soybean meal
SID	Standardised ileal digestible
TIA	Trypsin inhibitory activity

## **Chapter 1. Nutritional value of field pea and lentil grain in pig nutrition: A review**

### **1.1 Introduction**

In the swine industry, feed is the single largest cost to producers and has the greatest impact on profitability (Niemi et al., 2010). On a daily basis, cereal grain prices fluctuate; however, increasing long term demand for grains for biofuels may cause a rising trend in price (Shrestha et al., 2019). Consequently, feeding of alternative feed ingredients in swine diets is explored. The majority of these alternative feedstuffs, in the form of cereal and pulse grains, are a food source for humans. However, excess or non-food grade product and co-products are viable for use in the swine industry and offer a low-cost alternative to cereal grains (Stein and de Lange, 2007; Landero et al., 2014; Woyengo et al., 2014a). Exploring alternative ingredients available for use in swine diets may be effective at reducing feed cost (Woyengo et al., 2014a).

Legume grains and their co-products are an example of these low-cost alternatives. Amongst legumes, there are oilseeds such as soybean and peanut or non-oilseed pulse grains such as lentil, field pea, bean, faba bean and chickpea. Soybean is not a new ingredient in the swine industry. In the form of soybean meal (SBM), it has become the most popular protein source in swine diets around the world due to its balanced amino acid (AA) profile, high digestibility, and good palatability (Genove et al., 2019). On the other hand, pulse grains are still relatively new to the industry. Because the use for pulse grains is under development, research has yet to clearly define limits of dietary inclusion of these ingredients and the conditions under which they can best be utilized. For example, little is known about how factors such as age or physiological state of the pig affect dietary inclusion, or how properties and characteristics of the pulse grain may affect feeding (Woyengo et al., 2014a). Some reports state growing-finishing pigs may be able to tolerate

up to 700 g field pea/kg feed (Durga et al., 2019). Other reports limited inclusion to 200-400 g field pea/kg (Shelton et al., 2001; Degola, 2015). A better understanding of how these pulse grains affect growth performance and nutrient digestibility may aid in increasing the use of these ingredients to further reduce feed cost.

In terms of production in Canada, soybean has the largest tonnage at 7.4 million mt in 2018/2019, much larger than the 3.6 million mt of dry pea and 2.1 million mt of lentil produced in the same year (AAFC, 2019a). However, Canada is the world's largest producer and exporter of lentil and dry pea and is a substantial stakeholder in the production of these crops (AAFC, 2019b). One of the reasons these crops are so well cultivated in Canada is due to the agronomic conditions that make them thrive (Castell, 1990). Pulses grow well in cool and moist climates, such as in Alberta and Saskatchewan, whereas soybean may not grow as well in temperate latitudes (Woyengo et al., 2014a; Statistics Canada, 2015). In addition, pulse cultivars can be grown in rotation with other crops like soybean, other oilseeds and cereal grains to disrupt the spread of disease and fix nitrogen in the soil (Statistics Canada, 2015). By fixating atmospheric nitrogen, the producer can decrease the need for fertilizer to be applied to subsequent crops that may reduce cost of cereal or oilseed production (Castell, 1990). Thus, the feeding of pulse grains in swine feed as an alternative energy or AA source may be highly relevant to Canadian swine producers.

## **1.2 Nutrient composition of pulse grains**

Pulse grains as alternative feedstuff can be a good source of starch, protein and AA, fibre, vitamins and minerals in swine diets (Woyengo et al., 2014b; Singh, 2007). Many pulse grains are nutrient dense, containing high amounts of carbohydrates, protein, fibre, mineral and vitamins



(Nosworthy et al., 2017). Field pea grain contains approximately 222 g CP, 62 g crude fibre, 28 g ash, and 12 g ether extract/kg (NRC, 2012). Data on the nutritional composition of lentil is lacking, as is evident by the single sample that is included in the USA-database (NRC, 2012). Multiple factors limit the dietary inclusion of alternative feedstuffs (Woyengo et al., 2014a). Information on the feeding value of lentil is sparse and field pea has been mostly evaluated in growing-finishing pigs, not sows or weaned pigs. Protein and AA, carbohydrates, fat and anti-nutritional factors (ANF) are discussed in the following sections.

### **1.2.1 Carbohydrates**

Starch accounts for the major portion of the carbohydrates in pulse grains. A benefit of pulse carbohydrates is that slow-digestible pea starch serves as fermentable carbohydrates, contributing to a slow glucose release and thus lower glycaemic index compared with that of cereal grains (Fledderus, 2004; Berrios et al., 2010). Health benefits to humans of diets rich in pulse grains include little or no effect on blood glucose, reduced caloric intake due to high non-starch polysaccharide content, and overall improved heart health (Chibbar et al., 2010). These human health benefits can be attributed to high concentration of amylose in pulse grains that increase resistant starch and slow the rate of digestion, promoting hindgut fermentation (Chibbar et al., 2010). Starch content of lentil and pea grains are within the range of 370-590g and 300-490g/kg, respectively (Hall et al., 2017). Starch amylose content is greater in field pea (230-490g/kg) than in lentil (190-250g/kg), thus field pea has a slower rate of carbohydrate digestion (Hall et al., 2017). Increased energy digestibility in field pea and lentil versus other legume crops can be attributed to slowly digested starch (Landerio et al., 2012; Woyengo et al., 2014b).

The fibre content of field pea grain varies. Field pea total dietary fibre (TDF) content ranged from 140-260 g/kg, of which 20-90 g/kg is soluble fibre (Tosh and Yada, 2010). The TDF of lentil is greater and contains a similar portion of soluble fibre (Tosh and Yada, 2010). Soluble fibre slows gastric emptying and nutrient absorption whereas insoluble fibre decreases intestinal transit time, decreases energy digestibility, and increases bulk density of faeces (Renteria-Flores et al., 2008; Serena et al., 2008). Decreases in energy digestibility associated with increased fibre content contribute to lower energy digestibility in field pea grain, particularly in instances with increased NDF (Zijlstra et al., 1990; Htoo et al., 2008).

Pulse grains contain substantial oligosaccharides. The primary oligosaccharides in pulse grains are comprised of a group of  $\alpha$ -galacto-oligosaccharides known as the raffinose family (Berrios et al., 2010; Tosh and Yada, 2010). The raffinose family remains undigested in the small intestine yet is fermented in the large intestine by microbiota (Chibbar et al., 2010). Raffinose and stachyose (another galactoside) positively affect physiological functions and characteristics, such as normalizing bowel function and increasing the concentrations of beneficial microbiota in the intestine, such as lactobacilli and bifidobacteria (Berrios et al., 2010). The raffinose family of oligosaccharides is not constant among pea and lentil grain and can vary with environmental and genetic factors (Vidal-Valverde et al., 2003; Wang and Duan et al., 2006; Tosh and Yada, 2010). Although these oligosaccharides have prebiotic benefits to the digestive tract, they also pass through to the lower intestines where they are fermented and can cause flatulence and occasional abdominal pain or diarrhoea (Berrios et al., 2010). Soaking of pulse grains in water or bicarbonate for 12 h, or cooking of un-soaked pulse grains in boiling water, decreased sucrose and raffinose sugars (Abdel-Gawad, 1993). However, the combination of moisture and heat using autoclaving

or extrusion decreased raffinose and other oligosaccharides more (Abdel-Gawad, 1993; Berrios et al., 2010).

### **1.2.2 Protein and amino acids**

The amount of protein and the quality of protein make pulse grains a potential alternative, if formulated properly into diets. In field pea, variability in crude protein (CP) is affected by many factors. These factors include environmental condition, genetics and physical characteristics of the seed (Hlodversson, 1987; Gâtel and Grosjean, 1990). Cultivar is reported to have a small effect on AA content (Wang and Duan 2004; Wang and Duan 2006; Hall et al., 2017). Most pulse grains range in protein around 210-250 g/kg but are low in sulphur containing AA (Singh, 2007). Field pea contains less CP and lysine than soybean meal, but more fibre than soybean meal, corn or wheat grain (Woyengo et al., 2014b). Versus cereal grains, field pea and many other pulse grains contain more CP and AA (Stein et al., 2007). Field pea has similar standardized ileal digestibility (SID) lysine and AA to that of soybean meal (Woyengo et al., 2014b).

Alternatively, lentil contain more protein than field pea, though digestibility data for lentil has not been reported and sources are scarce, especially in the pig model (NRC, 2012). In comparison to soybean meal, lentil grain has nearly half the CP and AA content (NRC, 1998). Glutamic acid and aspartic acid are the most abundant in lentil, with high concentrations of arginine, leucine and lysine (Hall et al, 2017). Cysteine and methionine contents in lentil were also affected by soil sulphur content (Kesli and Adak, 2012). In both lentil and pea grain, increases in protein content was observed when cooked (Wang et al, 2008; Wang et al., 2009; Wang et al., 2010). Under the appropriate conditions, lentil and field pea may replace SBM inclusion as a protein source that

also provides an abundance of AA with the exceptions of cysteine and methionine (Woyengo et al., 2014a).

### **1.2.3 Fat**

Fat content in legumes is negligible in terms of nutrition and practical diet formulation. Though lipids can provide an excellent source of energy, much of the energy in pulse grains is due to carbohydrates. Ether extract in field pea and lentil is <20-60 g fat/kg dry matter (NRC, 1998) and linoleic acid makes up 450-500 g/kg of total fatty acids (Hall et al., 2017; NRC 1998). Lentils have high lipoxygenase enzyme activity, about 4 to 5 times higher activity than that of field pea (Chiang and McCurdy, 1985; Bhatta, 1988). The lipoxygenase catalyses degradation of polyunsaturated fatty acids and may cause off-flavours in legumes, especially lentil (Bhatta 1988; Roland et al., 2017), leading to shorter shelf-life. Therefore, proper storage of pulse grains with greater ether extract content is important to maintain quality.

### **1.2.4 Anti-nutritional factors**

Antinutritional factors (ANF) are plant-based factors that affect digestibility, availability, and utilization of nutrients (van der Poel, 1990; Gilani et al., 2005). ANF are mostly naturally occurring, such as tannins, lectins, and phytate; however, ANF can also be formed during heat/alkaline processing, resulting in Maillard compounds, oxidized AA, and unnatural forms of AA (Gilani et al., 2005).

Legume grains contain various ANF that each have a different mechanism of action on nutrient digestion and utilization. Tannins decrease digestibility of proteins whereas phytate and phytic acid limits bioavailability of specific minerals (Rehman and Shah, 2005). Consequently, use of

legumes as feedstuff for monogastric animals is limited and further investigation is required to understand maximum dietary inclusion of these feedstuffs (van der Poel, 1990; Adamidou et al., 2011).

#### **1.2.4.1 Protease inhibitors and lectins**

Many animals, including swine, use proteolysis to degrade and digest proteins they consume (Ryan, 1990). Proteolysis is the breaking of protein bonds in the lumen of digestive tract, performed by various endogenous proteases. Proteins are broken into small peptides and single AA to be absorbed into the blood stream. The mechanism of protease inhibitors is to permanently bind to protease enzymes and prevent these enzymes from effectively breaking down protein molecules, hindering digestion and absorption of protein (Lajolo and Genovese, 2002).

There are two types of protease inhibitors largely found in legume grains: Kunitz and Bowman-Birk (Lajolo and Genovese, 2002). The Kunitz protease inhibitor is classified as having a larger molecular weight than the Bowman-Birk, fewer disulphide bonds, and specificity mainly for trypsin (Gilani et al., 2012). The Bowman-Birk protease inhibitors, in addition to the aforementioned characteristics, have an affinity for both chymotrypsin and trypsin at independent binding sites (Werner and Wemmer, 1992).

Protease inhibitors in excess in swine diets may result in increased pancreatic function of and secretion of proteases such as trypsin (Ryan, 1990). Due to increases in pancreatic secretion, it is implied that trypsin has an active role in the regulation of the pancreas (Ryan, 1990). In the presence of TIA, the body can sense that proteins and peptides are in high concentration in the intestine and a feedback loop signals to the pancreas to release more trypsin, a process known as pancreatic hypertrophy (Ryan, 1990; Gilani et al., 2012). Trypsin may linearly increase the

endogenous and exogenous flow of nitrogen, as well as the total nitrogen flow (Károly Dublec., 2011). Exogenous nitrogen losses would occur due to decreased protein digestion and increased endogenous losses would be a result of pancreatic hypertrophy. Therefore, reducing TIA in swine diets has potential to increase protein digestion decreasing nitrogenous losses (Károly Dublec., 2011).

Lectins, also called haemagglutines, are proteinous ANF generally found in the form of glycoproteins that bind to gut epithelial cells in animal tissues (Vasconcelos and Oliveira, 2004). The method of action of lectins is to bind to terminal N-acetyl-D-galactosamine and/or D-galactose in the gut, causing clinical signs of drastically reduced feed conversion and reduced feed intake (Károly Dublec., 2011). Purified soybean lectins in a diet fed to pigs caused increased endogenous nitrogen losses at the terminal ileum, resulting from damage to the brush border membrane (Schulze et al., 1995). Damage to the gut wall would result in immunological reactions and increased synthesis of mucosal proteins to protect the host (Vasconcelos and Oliveira, 2004; Károly Dublec., 2011). Exogenous nitrogen flow is less affected by lectins, concluding that lectins have minor effects on true protein digestibility (Károly Dublec., 2011); However, there may be an increase in total dry matter (DM) flow as a result of lectins, potentially resulting in decreased digestibility of other nutrients (Oliviera et al., 1994).

In general, field pea has low protease inhibitors and lectins as well as low levels of other ANF, which increase the interest in studying field pea as a major feedstuff in swine diets (Njoka, 2008). Protease inhibitors are the ANF of highest concentrations in field pea grains, ranging around 0.2-0.5 mg TIA per g of CP (Jeziorny et al., 2011). Lentil grains, however, have a range of 1.0-4.0 g tannins/kg dry matter, and lower TIA at 2-3 mg TIA/g CP in raw lentil (Wange and Duan, 2006).

#### **1.2.4.2 Phytate and tannins**

Phytate, also called phytic acid is a complex compound naturally found in plants that binds phosphorus and other minerals such as magnesium, calcium, and potassium (Gilani et al., 2012). Phytate chelates several essential nutrients for the pig, rendering them less bioavailable for the animal and unable to be hydrolysed by enzymes secreted in the gut (Károly Dublicz., 2011, Gilani et al., 2012). In addition to limiting the bioavailability of minerals, phytate can also bind to proteins in the gut (Gilani et al., 2012). Phytate binds either directly or indirectly to proteins, and the binding can vary with pH and concentration (Gilani et al., 2012). This binding is known to interfere with pepsin and possibly trypsin that can significantly interfere with protein digestion (Gilani et al., 2012). Due to the structure of phytate, it is considered heat stable. A common method to reduce the negative effects of phytate is to add exogenous phytase, as enzyme which breaks down phytate and reduces binding to nutrients (Károly Dublicz., 2011).

Tannins are bitter tasting polyphenolic compounds present in various cereal and legume grains (Jansman, 1993). They are classified into hydrolysable and condensed tannins (Gilani et al., 2012). Hydrolysable tannins are easily hydrolysed by acids, alkali, or some enzymes whereas condensed forms are not (Jansman, 1993; Gilani et al., 2012). Zero-tannin faba bean cultivars have been developed that have significantly lower tannin content at about 10 g/kg, compared with regular faba bean that contain 80-90 g tannins/kg (Oomah et al., 2011).

Tannins form complexes with enzymes and feed proteins, reducing protein and AA digestibility in swine (Mullins and Lee, 1991). Indeed, pigs fed increasing dietary purified condensed faba bean tannin decreased apparent total tract digestibility (ATTD) of CP, largely from a decline in ileal digestion (Jansman et al., 1993). When tannins are present in a diet, pigs may not absorb sufficient AA to meet their requirement despite having enough AA in the diet. In these

scenarios, it is likely pigs would display decreased protein deposition in response to insufficient AA for growth (Marquardt et al., 1977; Jansman et al., 1993). In addition to binding of dietary tannins to proteins, tannins may also bind to digestive enzymes such as trypsin and chymotrypsin (Jansman et al., 1993).

Tannins may reduce the nutritional quality of diets. In swine fed high tannin diets, average daily gain (ADG) decreased, feed efficiency decreased, and the apparent ileal digestibility (AID) of nitrogen/protein and AA decreased (Jansman, 1993). In other monogastric animals, results are not as conclusive and variation occurs among species (Jansman, 1993). Lentil contains 1.28 g tannins and 4.11 g phytic acid/100 g of lentil grain on a DM basis (Hefnawy, 2011). These tannin levels are comparable to other legumes; however, the phytic acid content in lentil is much greater, especially compared to the 1.20 g phytic acid/100 g field pea grain (Adamidou et al., 2011). The high content on ANF is a major limiting factor to the inclusion of pulse grains in swine diets.

### **1.3 Feeding value of lentil and field pea**

To determine the usefulness of field pea and lentil grains in swine diets, this section explores the benefits and challenges of feeding these pulse grains to swine. In particular, attention is focused on indicators of quality such as protein and AA digestibility and energy utilization, and subsequent effects on growth performance.

#### **1.3.1 Protein and AA digestibility**

Field pea and lentil grains can be included as a protein source in swine diets, provided diets are formulated to appropriate AA content. The AID of AA is an acceptable measure of AA



availability in most feedstuffs for growing pigs (Tuitoek et al., 1997; NRC, 1998). Although accepted, AID does not correct for the basal endogenous losses of AA, unlike standardized ileal digestibility (SID; Stein et al, 2007). If pulse grains are to partially replace a protein source in swine diets, calculating AID of multiple individual protein ingredients could underestimate digestibility, versus calculating the dietary AID of AA values for combined protein sources together (Stein et al, 2005; Xue et al., 2014; Zhou, 2016). Therefore, formulating diets to equal SID AA may be necessary to minimize the negative effects of feeding these alternative ingredients.

The AID values of essential AA in field pea are similar to that of soybean meal (NRC, 1998). The AID of AA has been measured at 840, 780, 730, and 700 g/kg field pea grain for lysine, methionine, threonine, and tryptophan, respectively compared to SBM measuring at 850, 860, 780, and 800g/kg for lysine, methionine, threonine, and tryptophan, respectively (NRC 1998; Njoka, 2008). In contrast, the AID of essential AA in lentil were generally lower than both field pea and SBM (Woyengo et al, 2014b). Lower digestibility of AA in lentil and field pea could be due to increased levels of fibre compared with SBM (Woyengo et al, 2014b; Zhou, 2016). Fibre in lentil grain contains around 130 g/kg more lignin than dehulled SBM fibre that would reduce the fermentability of fibre, thereby reducing digestibility (Khan et al., 2007; Woyengo et al., 2014b). Alternatively, SID of protein and AA in peas have a negative relationship with TIA but were not affected by fibre content, further supporting the benefits of formulating to equivalent SID of AA when able (Grosjean et al., 2000).

### **1.3.2 Energy utilization**

Starches, non-starch polysaccharides (NSP), and sugars in pulse grains provide an important energy source to swine (Bach Knudsen, 1997). Compared to cereal grains, field pea grain contains

less starch, and less sugar and NSP (Jezierny et al., 2010). The mean digestible energy (DE) content of field pea is 16.2 MJ/kg DM, with a range of 15.2-17.3 MJ/kg DM (Crépon, 2006). Variation in digestibility of energy is related to the presence of fibre components such as NSP including cellulose and lignin (Noblet et al., 2000). Although fibre may be high in pulse crops grains, the relatively high amounts of starch make them a suitable alternative as an energy source for swine (Navarro et al., 2019).

### **1.3.3 Growth performance**

SBM is the most commonly used protein source in livestock feeds around the world. Its importance has increased recently after concerns about the biosafety of animal by-products like plasma, blood meal, or meat and bone meal regarding potential viral disease transmission (e.g. porcine epidemic diarrhoea; Kim et al., 2007; Jezierny et al., 2010). Often, other protein feedstuffs are priced in relation to SBM (Willis, 2003). Field pea and lentil in Canada are generally priced lower (\$303/mt less) than SBM and are used to partially replace SBM to reduce feed cost (AAFC, 2019). When evaluating alternative feedstuffs, the feeding value and effect on animal performance should be considered.

Research on the feeding of field pea to commercial herds is important in understanding the relative use of this ingredient. In a study performed on 1000 crossbred pigs in a commercial facility, one of the test diets fully replaced SBM with field pea grain (Beltranena et al., 2008). Regardless of test diet, weight gain, feed disappearance, feed efficiency, backfat and loin depth, yield, or carcass index did not differ, indicating that field pea can fully replace SBM as a dietary protein source without affecting carcass traits and growth performance (Beltranena et al., 2006). In addition, field pea diet had the lowest cost/kg of weight gain (Beltranena et al., 2006).

Conversely, young pigs react differently to diets containing field pea compared with their older counterparts. Young pigs fed field pea had a greater decrease in growth performance indicators such as average daily feed intake (ADFI), ADG, and feed efficiency than older pigs (Stein et al., 2010; Landero et al., 2014). In growing pigs of 30 to 100 kg body weight, raw field pea inclusion of 400 g/kg reduced growth rate and feed conversion, although extruded field pea did not (O'Doherty and Keady, 2000). When field pea is fed to young pigs, the reduction on performance is more prominent. Dietary inclusion of 400 g field pea grain/kg reduces growth and feed conversion in pigs 2-3 weeks post-weaning (Landero et al., 2014). Similarly, young pigs fed diets containing 600 g raw field pea grain/kg had reduced growth in the first week and during the overall 28-day trial despite maintained feed intake (Stein et al, 2010).

Feeding of lentil may have little effect on growth performance and carcass traits in pigs (Woyengo et al., 2014a). Inclusion of lentil in weaned pig diets can reduce feed costs by \$4.13/mt of diet and 0.64 cents/kg of gain in weaner pigs (Woyengo et al., 2014a). In addition, dietary inclusion of 200 g damaged lentil grain/kg did not affect growth rates or feed efficiency (Bell and Keith, 1986). The lack of decreased performance in pigs fed lentil suggest this ingredient may have greater potential for use in young swine diets, especially compared to field pea.

#### **1.4 Effect of processing on feeding value of lentil and field pea**

The feeding value of pulse grains is limited due to their ANF, NSP content and lower energy digestibility (Jezierny et al, 2010). Most commonly, we see these limitations in young pigs, with a negative effect on growth performance and digestibility; However, in growing-finishing pigs we tend not to see such effects and limitations to pulse inclusion levels (Degola, 2015). Growing-

finishing pigs fed diets that are properly balanced and supplemented with sulphur-containing AA resulted in dietary inclusion ranges from 200-400g pulse grains/kg feed in practical diets (Bell and Keith, 1990; Gâtel and Grosjean, 1990; Landblom and Poland, 1998; Brand et al., 2000; Shelton et al., 2001; Degola, 2015). Particularly in young pigs, to increase possible inclusion levels, processing could reduce the undesirable traits in pulse grains and reduce the negative effect on nutrient digestibility, performance, and health (van der Poel et al., 1998; Singh et al., 2007; Stein and Bohlke, 2007; de Vries et al., 2012). Processing techniques include dry techniques like grinding, sieving, and air classification, as well as heat-induced techniques such as cold-pelleting, steam-pelleting, and extrusion. Further reasons to process feed, aside from improving nutrient availability, are to alter the physical form, facility storage and transportation, improve palatability, or improve feed uniformity in a diet.

#### **1.4.1 Dry processing**

Dry processing refers to a processing method which does not add moisture to the feed when processed. Dry processing alters the physical properties of the feedstuff, most commonly through grinding, mixing, and sieving. These methods are used as ways to alter the quality of the feed by reducing particle size, having even distribution of ingredients in feed, or by separating feed components based on density, shape, or size.

##### **1.4.1.1 Particle size reduction**

Grinding is commonly used to reduce particle size and can be achieved through various milling, such as use of a hammer mill, disk mill or roller mill (Hancock and Behnke, 2001). Particle size reduction increases the surface area of particles, allowing for more contact and action by

digestive enzymes, thereby increasing nutrient digestibility in pigs (Kim et al., 2002; Liu et al, 2013; Rojas and Stein, 2015). Distillers dried grains with solubles (DDGS) and SBM are co-products of prior processing and are usually presented with a reduced particle size, thereby not requiring further particle size reduction (Rojas and Stein, 2017). Cereal and pulse grains, however, are raw when they arrive at the feed mill and typically require particle size reduction (Rojas and Stein, 2017).

Particle size should be an important consideration in swine feeds. Too fine of particle size, such as a reduction of finely ground barley, from 785 to 434  $\mu\text{m}$  fed to pigs weighing 31 kg to slaughter, resulted in increased stomach ulcers in pigs (Morel and Cottam, 2007). The pig's stomach has 4 regions: oesophageal region, cardiac region, fundic region, and pyloric region. The cardiac, fundic, and pyloric regions are all glandular, whereas the oesophageal region is more like an extension of the oesophagus. Feeding pigs diets containing too low a particle size can increase the risk of pigs developing ulcers, causing the functions of the stomach regions to be disrupted or impaired (Rojas and Stein, 2017). More specifically, the oesophageal region, which lacks glands to produce mucus, is most at risk for developing ulcers (Mahan et al., 1966; Pickett et al., 1969; Maxwell et al., 1970) due to the lack of protective functions the mucus and glandular portions provide to the gut wall (Ohara et al, 1993; Varum et al., 2010). However, a reduction in particle size is not the sole cause of an ulcer, and other factors may elicit ulcers.

Recommendation for optimal particle size reduction depends on various factors, including age and physiological status of the pig, type of grain, as well as method of particle size reduction being used (Rojas and Stein, 2017). On average, however, a particle size range between 480 and 600  $\mu\text{m}$  is generally considered to have positive effects on energy and nutrient digestibility, as well as growth performance of the animal (Wondra et al., 1995a; Rojas and Stein, 2017). When particle

size of diets containing barley, SBM, and field pea was reduced from 900  $\mu\text{m}$  to 600  $\mu\text{m}$ , ileal and total-tract energy digestibility in cannulated growing pigs increased 11 and 3%, respectively (Oryschak and Zijlstra, 2002). In addition, reducing particle size increased N digestibility. Consequently, the site of energy and N digestion shifted from the large to small intestine (Oryschak and Zijlstra, 2002). When pigs were fed barley-field pea diet of 400  $\mu\text{m}$ , increased measures in ATTD of GE, DM, and CP was observed compared with pigs fed the same diet ground to a particle size of 700  $\mu\text{m}$  (Oryschak et al., 2002).

Although a reduction of particle size may increase enzyme surface action that increases energy and nutrient digestibility, an increase in digestibility does not always increase pig growth performance (Rojas and Stein, 2017). This finding is in large part because pigs may compensate for low digestibility by eating more feed (Rojas and Stein, 2017). Optimal growth performance is obtained if wheat is ground to a particle size of 600  $\mu\text{m}$  for weaning and 500  $\mu\text{m}$  for finishing pigs (Mavromichalis et al., 2002; Camargo, 2020).

#### **1.4.2 Heat processing**

Heat processing is the application of high temperatures to a diet or ingredient in order to cook the feed to increase palatability and food safety, or nutrient digestibility, availability, or bioavailability (van der Poel, 1988; Watzke, 1998; Dong and Pluske, 2007). Thermal treatments generally increase the nutritional value of feed and feedstuff (Zijlstra et al., 2009). The purpose of focusing on heat treatment is because heat may have a greater effect on ANF than other dry processing techniques. Heat can alter protein and starch molecules, it can change the digestibility and availability of other nutrients and heat treatment can combine other processing techniques (Rehman and Shah, 2005). For example, extrusion uses a combination of heat, pressure,

mechanical shear, and moisture to alter the nutrients and ANF in feed (Singh et al., 2007; Masoero et al., 2005).

On the other hand, because heat processing can be combined with other methods to alter feed, heat may not be the sole contributing factor to the changes in feeding value. For example, a significant reduction in tannins and phytate in Bambara groundnut seed was achieved by boiling and roasting (Ndidi et al., 2004). Boiling involved placing the seed in water at 100°C for over 3 hours, and then mashing them into a paste. Roasting involved cooking the seed over a heat source for about 1 hour over burning firewood as approximately 300°C and pulverizing them into a fine powder (Ndidi et al., 2004). Possibly in both these cases, the reduction in particle size and other factors may have played a role in reducing ANF and altering digestibility.

#### **1.4.2.1 Cold-Pelleting**

Cold-pelleting is a method that does not require steam and thus the use of a boiler. Cold-pelleting can still result in an increased feeding value of diets and ingredients to swine. With pelleting at 70°C, broiler chicken performance was increased (Bayley et al., 1968). This observation was further supported in swine by multiple authors who observed that pelleting increased feed efficiency, in part by reducing ADFI and by increasing ADG (Hanke et al., 1971; Baird et al., 1973; Wondra et al., 1995b). Pelleting reduces ADFI due to a reduction in feed wastage compared with a mash diet, which in turn could reduce feeding cost to producers. To contrast, in regards to digestibility of pelleted feed, AID or SID for CP of field pea diets that were pelleted at 75°C did not differ compared to the untreated control diet (Stein and Bohlke, 2007). The AID of AA did not differ, except for Arg, or SID of AA, except for Pro, and AID for starch and energy did not differ (Stein and Bohlke, 2007). When a corn-soybean meal diet was pelleted at 75°C, digestibility

increased 5 to 8% for DM, nitrogen and GE (Wondra, et al., 1995b). In regard to the effect heat processing has on ANF content and activity, temperatures of 60°C and 70°C had no effect on lectin activity of whole soya bean seed (Armour et al., 1998). To conclude, cold-pelleting may not increase nutrient digestibility such as AID and SID of CP and AA; however, it still has practical uses in reducing dust and fine grains in feed, improving flowability and reducing feed wastage.

#### **1.4.2.2 Steam-Pelleting**

Proper steam conditioning of substrate prior to pelleting is necessary to combat the loss of performance from dust, fines, and feed wastage. Pelleting feed has gained in popularity among the swine industry for the benefits of reducing ingredient separation, reducing dust, and being a more cost-effective alternative to expensive extrusion technology (Hancock and Behnke, 2000). Pellet durability and hardness can be influenced by the nutritional composition of ingredients in the diet (Wilson, 1994; Thomas et al., 1998). For example, gelatinized starches and raw proteins improve pellet quality (Wood, 1987), but fats tend to lessen pellet quality (Van Vliet, 1981; Thomas et al., 1998). Effects of fibre on pellet quality have been both positive and negative, some research stating resilient fibre strands weaken the pellet whereas others state the fibre strands may entangle other particles and strengthen the pellet (Rumpf, 1958; Thomas et al., 1998). There is also a large variate among ingredient feedstuffs due to the environmental and growing conditions, which can affect nutrient composition.

Many studies regarding the effects of pelleting on nutritional value of feeds have been done. Feed intake was reduced by 2% and feed intake and feed utilisation were increased by 7 and 8%, respectively when diets were pelleted (Thomas et al., 1998). Further, steam pelleting may affect feed intake and pelleting often increases ADG as well as a 4-12% increase in feed conversion



(Rojas and Stein, 2017). Alternatively, steam pelleting did increase feed efficiency, but not ADG, in weanling pigs compared to those fed a raw mash diet (Skoch et al., 1983). Furthermore, DM and energy digestibility were increased by steam-pelleting compared with feeding a raw mash diet (Skoch et al., 1983). In regards to calcium and phosphorus absorption, steam pelleting decreased the absorbability of both calcium and phosphorus, largely due to a reduction in the phytase enzyme (Jongbloed and Kemme, 1990).

### **1.4.2.3 Extrusion**

Extrusion is commonly used to produce pet food or aquaculture feed. Nearly all dry pet food is extruded, emphasising the importance this technology has on the industry. Extrusion processing is comprised of steam and pressure forcing product through a barrel with either a single or double-screw extruder, which results in the generation of heat (Fellows, 2000; Hancock and Behnke, 2001). Extrusion is relevant to the animal nutrition industry because can increase the ATTD of GE and AID of starch and indispensable AA (Stein and Bohlke, 2007; Htoo et al., 2008). Extrusion may also turn a portion of insoluble fibre into soluble fibre, which may increase energy digestibility (Urriola et al., 2010; de Vries et al., 2012). Compared with pelleting, extrusion results in an 8% increase of feed conversion, DM digestibility by 3%, and CP digestibility by 6% (Sauer et al., 1990). Similarly, extrusion of field pea diets at 115°C increased AID and SID of AA, starch, and energy, whereby pelleting did not (Stein and Bohlke, 2007). The increased AA digestibility may also result in a diet formulated for lower concentrations of CP and AA, which lowers the cost of diets and the levels of nitrogen excreted by animals (Stein and Bohlke, 2007).

A downside to extrusion is that it can be costly (Hancock and Behnke, 2001). Whether or not extrusion will be economical to swine producers depends on the cost to extrude and the savings

earned from cheaper diet formulations and improved animal performance. Unlike pelleting, it is uncommon for a producer to have access to an extruder, let alone to have it on the site of feed manufacturing. This fact is an important consideration when investigating the applicability of extrusion into the swine feed industry.

Extrusion may be a more effective tool for reducing ANF than pelleting. Extrusion temperatures can often rise to 90-150°C and above, which could easily reduce ANF such as tannins, trypsin, chymotrypsin and  $\alpha$ -amylase inhibitors, and haemagglutinating activity (Alonso et al., 1998; Singh et al., 2007). Oftentimes, the reduction in ANF and increase in starch gelatinization and soluble fibre and decrease in lipid oxidation is achieved without modifying protein levels in food products (Singh et al., 2007; Soetan and Otewole, 2009). As Alonso et al (2000) found, extrusion was best at reducing ANF without affecting protein content, even in comparison to other methods such as dehulling, soaking, and germination. The reduction of ANF due to heat treatment is reasonably consistent across the research on human and plant feeds (Soetan and Oyewole, 2009). Regardless of plant and the types of ANF the plant contained, cooking at higher temperatures like that of extrusion was highly successful in reducing ANF and more often than not, protein and starch digestibility were either increased or unaffected (Soetan and Oyewole, 2009). To conclude, the high temperatures used in extrusion results in a highly successful reduction of ANF and increased nutrient digestibility.

Importantly, ANF reduction is typically dependent on the proteinaceous nature of the ANF (van der Poel, 1988). Non-protein structures are more heat stable than protein structures due to differences in the molecular structure (Vogt et al., 1997). Many factors enhance thermo-stability (Vogt et al., 1997); however, tannins and phytate are complex structures with ring-like formations, contributing the increased thermo-stability. Although the ANF compounds are more heat resistant,

there is still potential for a reduction in ANF, to varying degrees because of heat processing. However, heat exposure at temperatures over 100°C for a longer time is required (Khan et al., 1991; Yu et al., 1996; Rehman and Shah, 2005; Daneluti and Matos, 2013).

## **1.5 Conclusions**

Pulse grains are a non-oilseed legume, usually produced for human consumption. Of the pulse grains, lentil and field pea are examined and have been two of the most-produced pulse grains in Canada in recent years at 2.1 and 3.6 million mt, respectively (AAFC, 2019a). Excess production or non-feed grade pulse grains can be included in swine diets to partially replace SBM (Jezierny, 2010). Favourable agronomic conditions, improved soil quality via root rhizobia nitrogen fixation, and diversity in crop rotation aid in the reasons for high pulse grain production in Western Canada. The nutritional value of field pea and lentil is limited by: low levels of sulphur-containing AA (Peace et al., 1988); greater dietary fibre content that decreases nutrient digestibility, energy, and growth performance (Landerio et al., 2012); the presence of ANF such as protease inhibitors lectins and tannins that reduce nutrient availability and digestibility, growth, feed intake, feed efficiency, and gut health (Green and Lyman, 1972; Chubb, 1986; Huisman et al., 1989; Jansman et al., 1989). These limitations of pulse grains in swine diets can be reduced by formulating for based on NE and SID AA, and by processing of ingredients and diets using heat applications such as pelleting and extrusion to inactivate ANF.

Field pea and lentil have less starch, but higher levels of CP and lysine than that of cereal grains, though nutritional composition can be affected by environmental conditions, cultivar, harvest, and seed characteristics (Iliadis, 2001; Nikolopoulou et al., 2007; NRC, 2012). In

comparison to other legume crops, field pea has the greatest NE, likely due the high content of digestible starch and fermentable fibre (NRC, 2012). Feeding field pea or lentil could affect feed intake and growth performance and inclusion of field pea or lentil, particularly in young pigs, and may need to be limited (Stein et al., 2010; Landero et al., 2014). The ANF content in field pea and lentil may be inactivated by heat processing, such as pelleting and extrusion, allowing for greater inclusion levels than previously thought. Field pea has been successfully included into swine diets to replace SBM without affecting carcass quality or growth performance to reduce feed cost (Beltranena et al., 2006). Formulating for SID AA and NE along with heat processing of ingredients may further reduce the negative effects of feeding these products.

### **1.5.1 Knowledge gap**

Dietary inclusion of raw field pea and lentil reduces protein digestibility of diets and reduces growth performance in weaned pigs. These ingredients are appealing to add to swine diets to reduce feed cost to producers; however, their negative impacts on growth performance is counterintuitive to the goal of increasing feed efficiency and saving costs. Both field pea and lentil contain ANFs that reduce feed intake, digestibility, and growth; however, some of these ANF can be reduced via heat exposure. The effects of heat processing of feed ingredients are not well known in whether it can increase the nutrient digestibility and growth performance of pigs.

### **1.6 Thesis hypothesis and objectives**

The hypotheses of the present thesis were: a) heat-processing would decrease TIA and increase the digestibility of energy, CP, and AA and thus nutritive value of lentil and field pea in either

weaned or growing-finishing pigs; and b) dietary inclusion of 400 g field pea/kg, formulated to equivalent SID Lys and NE, would not affect growth performance of weaned pigs.

The objectives of the thesis were:

- a) To determine the CATTD of energy and CP of weaned pigs fed raw or heat-processed field pea and assess the effects on growth performance when fed as a partial substitution for SBM and wheat grain (Chapter 2);
- b) To evaluate differences in total tract and ileal digestibility of nutrients and energy of raw and heat-processed field pea in weaned pigs (Chapter 3); and
- c) To determine nutrient and energy digestibility of raw lentil and heat-processed lentil and assess how heat-processing may alter ileal or total tract digestibility in growing-finishing pigs (Chapter 4).

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## **Chapter 2. Growth performance of weaned pigs fed raw, cold-pelleted, steam-pelleted, or extruded field pea**

### **2.1 Introduction**

Annually, 4.8 million MT field pea (*Pisum sativum*) is produced in Canada, the largest production among pulse grains (AAFC, 2018). Field pea is grown in western Canada primarily as food for export and its favourable agronomic benefits that include atmospheric N fixation by root rhizobia, breaking crop disease cycle and diversifying soil nutrient use in crop rotations with cereal grains and oilseeds. Field pea is a good source of dietary starch (~300g/kg) and protein (~200 g/kg). Excess or non-food grade field pea can replace soybean meal (SBM) in swine diets to reduce feed cost (Landro et al, 2014). Field pea contains less crude protein (CP) and lysine (Lys) than SBM, but more than cereal grains. Field pea contains more fibre than SBM, corn or wheat (Woyengo et al., 2014). Among pulse grains, field pea has the greatest net energy (NE) value (NRC, 2012), likely due to its digestible starch and fermentable fibre content.

Young pigs fed diets containing 600 g raw field pea/kg to replace corn and SBM maintained feed intake but had reduced growth in the first two weeks and over the entire 28-day trial (Stein et al, 2010). The reduced growth might be due to the presence of anti-nutritional factors (ANF) in field pea, such as trypsin inhibitors, lectins and phytate (Jezierny et al., 2011). Heat treatment can destroy the heat-labile protease inhibitors and lectins thereby increasing nutrient digestibility of field pea and growth performance of pigs. For some pulse grains, e.g. faba bean, chickpea and lentil, thermal treatment increased *in vitro* protein and starch digestibility and deactivated ANF (Adamidou et al., 2011; Luo and Xie, 2013; Rathod and Annapure, 2016). Feeding steam-pelleted diets containing 400 g field pea/kg did not affect feed intake, growth and feed efficiency (G:F) in

weaned pigs (Landerio et al., 2014). However, feeding diets containing 490 g extruded field pea/kg reduced feed intake of weaned pigs starting from 2 weeks post-weaning (Stein et al, 2010). Literature on processing of field pea is scarce and the effect of feeding field pea is not consistent. Thus, effects of processing field pea on nutrient digestibility and growth performance of weaned pigs require investigation.

The hypotheses of the present study were: 1) 400 g field pea/kg, either raw or heat processed, to substitute SBM and wheat grain would not affect diet nutrient digestibility and growth performance in weaned pigs provided that diets are formulated to equal NE and standardised ileal digestible (SID) Lys; 2) heat processing of field pea would increase diet nutrient digestibility of weaned pigs. The objectives were to determine apparent total tract digestibility coefficients (CATTD) of dietary gross energy (GE) and CP and evaluate growth performance of weaned pigs fed 400 g raw or heat processed field pea/kg in substitution for up to 300 g SBM/kg and 100 g wheat/kg.

## **2.2 Materials and methods**

### **2.2.1 Experimental design and diets**

Animal use was approved and procedures were reviewed by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care (CCAC, 2009). The study was conducted at the Swine Research and Technology Centre, University of Alberta (Edmonton, AB, Canada).

One batch of field pea (*Pisum sativum*) was sourced from a commercial supplier (WA Grain and Pulse, Innisfail, AB, Canada) and ground entirely through a 4.0-mm screen in a hammer mill

(Model Jacobson 5550-113-01, Carter Day International, Minneapolis, MN, USA). After initial grinding, the batch of the field pea was divided into four parts that (1) remained ground (raw) or were (2) cold-pelleted, (3) steam-pelleted or (4) extruded (Table 1). Field pea was cold-pelleted at 70–75°C (Model PM 1230, Buskirk Engineering, Ossian, IN, USA). Field pea was steam-pelleted at 80–85°C (Model 1116-4, 70 hp; California Pellet Mill, Crawfordsville, IN, USA). Field pea was extruded at 115°C (Model X115, Wenger, Sabetha, KS, USA) at Agri-Food Discovery Place (Edmonton, AB, Canada). A constant feed rate of 400 kg/h and medium intensity were applied during extrusion. Steam and water were added at 14 and 5% in the preconditioner and at 1 and 3% in the extruder, respectively. A speed of 420 rpm was set up for extrusion with a 7-mm die. The extruder had 5 zones, each zone increasing in temperature by 5°C from 95°C to 115°C. Following heat processing, cold-pelleted, steam-pelleted and extruded field pea was re-ground through a 3.2-mm sieve in the hammer mill.

In total, 236 crossbred pigs (Duroc × Large White/Landrace F1; Hypor, Regina, SK, Canada) weaned in four groups at  $20 \pm 1$  days of age were selected based on average daily gain (ADG) and body weight (BW) on day 7 post-weaning. Pigs were fed a commercial phase-1 and phase-2 diets for 7 days each before being offered the test diets. Pigs were moved into the experimental pens 7 days before the start of the experiment. One heavy and one light BW pig of each gender were randomly assigned into pens, four pigs per pen.

Experimental diets were introduced 2 days prior to the start of test ( $34 \pm 2$  days of age) by adding 500–750 g into the trough of a feeder. A control diet containing 300 g SBM/kg and four diets containing 400 g field pea/kg replacing the SBM and 100 g of wheat grain/kg were formulated (Table 2). Diets were formulated to provide 10.0 MJ NE/kg, 12.1 g SID Lys/kg, meeting ideal AA ratios, 8.0 g calcium/kg and 4.0 g standardised total tract digestible phosphorus/kg (NRC, 2012).

The NE value and SID AA content information for field pea and other ingredients was based on table values (NRC, 2012). Diets were fed as mash. Acid-insoluble ash (Celite 281; World Minerals, Santa Barbara, CA, USA) was included at 8 g/kg in diets as an indigestible marker. Diets did not contain antibiotics or antimicrobial growth promoters. Premixes were added to meet mineral and vitamin requirements (NRC, 2012).

The study was conducted as a complete randomised block design with 12 blocks, 5 pens per block and pens within block randomly assigned to one of the 5 diets. Pigs began test 2 weeks after weaning (initial BW, 10.0 kg  $\pm$  0.85 kg) and were on test for 3 weeks. Pens (1.1  $\times$  1.5 m) were equipped with a self-feeder, a nipple drinker, polyvinyl chloride pen partitions and plastic slatted flooring. Rooms were ventilated using negative pressure and fluorescent lights provided a 12-h light (0700–1900 h) and 12-h dark cycle.

Pigs had free access to feed and water throughout the trial. Individual pigs, feed added and remaining feed were weighed weekly to calculate average daily feed intake (ADFI), ADG and G:F for each pen. Freshly-voided faeces were collected hourly from 0800 to 1600 h by grab-sampling from pen floors on day 19 and 20. Faeces were pooled by pen and frozen at  $-20^{\circ}\text{C}$ . Upon completion of the trial, faeces were thawed, homogenised, sub-sampled and freeze-dried.

### **2.2.2 Chemical analyses and calculations**

Ingredients (raw and processed field pea and SBM), diets and freeze-dried faeces were ground through a 1-mm screen in a centrifugal mill (Model ZM200, Retsch GmbH, Haan, Germany). Ingredient and diet samples were analysed for moisture (method 930.15; AOAC, 2006), crude fat (method 920.39A), CP ( $\text{N} \times 6.25$ ; method 990.03), neutral detergent fibre (NDF) assayed without a heat-stable amylase and expressed inclusive of residual ash (Holst, 1973), acid detergent fibre

(ADF) inclusive of residual ash (method 973.18), total dietary fibre (method 985.29), starch (assay kit STA-20; Sigma, St. Louis, MO, USA) and ash (method 942.05). Ingredient samples were analysed for calcium (method 968.08), phosphorus (method 946.06), amino acids [method 982.30E (a–c)] and chemically-available (reactive) Lys (method 975.44) as described by AOAC (2006). Field pea samples were analysed for trypsin inhibitor activity (TIA; method NEN-EN-ISO 14902:2001; NEN, 2001). Faeces were analysed for dry matter (DM; method 930.15; AOAC, 2006), CP ( $N \times 6.25$ ; method 990.03; AOAC, 2006). Diets and faeces were analysed for acid-insoluble ash (Vogtmann et al., 1975 modified by Newkirk et al., 2003) and GE using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany). Based on results of chemical analyses, the CATTD of DM, CP and GE of diets were calculated using the acid-insoluble ash concentration of faeces relative to feed using the index method (Adeola, 2001). The DE values of diets were calculated by multiplying GE by CATTD of GE. Diet NE values were calculated using Eq. (5) in Noblet et al. (1994) with the determined diet DE value and analysed content of ADF, starch, CP and crude fat, as adopted by NRC (2012). Gain:feed was calculated by dividing pen ADG by pen ADFI for each period and the entire trial.

### **2.2.3 Statistical analyses**

Data were analysed using the PROC MIXED procedure with SAS (2016) using pen as the experimental unit. Normality and homogeneity of variance of the residual of each variable were confirmed using the UNIVARIATE procedure with ‘Normal’ option and GLM procedure with ‘Hovtest = Levene’ option, respectively. Diet CATTD of nutrients and GE, DE and calculated NE values were analysed using the MIXED model with diet as fixed effect and block as random effect. Growth performance data, except for G:F for the entire trial, were analysed as repeated measures

using weekly pen data with the best variance-covariance structure based on the Bayesian information criterion (BIC) fit statistics and mean initial BW per pen as a covariate if significant. Single-degree of freedom contrasts were used to compare digestibility and growth performance of the 4 field pea diets combined vs. SBM diet for each week and the entire trial (Littell et al., 2006). To test hypotheses,  $P < 0.05$  was considered significant whereas  $0.05 \leq P < 0.10$  was considered a trend.

### 2.3 Results

Raw field pea contained 10 g/kg more NDF than heat-treated field pea (Table 1). Diets with field pea contained 71–77 g/kg less CP and 18–66 g/kg less starch than the SBM diet (Table 2).

The CATTD of DM, CP, GE and the DE and calculated NE values were greater ( $P < 0.001$ ; Table 3) for the SBM diet than field pea diets but did not differ among field pea diets. Predicted NE value was 0.70–0.76 MJ/kg lower ( $P < 0.001$ ) for field pea diets than the SBM diet.

Overall (day 1–21), ADFI, ADG and G:F did not differ among pigs fed field pea diets (Table 4). The ADFI was 40–69 g/d greater ( $P < 0.001$ ) for field pea diets than the SBM diet. The ADG did not differ between pigs fed the SBM diet and fed field pea diets. Consequently, overall G:F was lower ( $P < 0.001$ ) for pigs fed field pea diets than pigs fed the SBM diet. For individual weeks, ADFI was greater ( $P < 0.05$ ) for field pea diets than for SBM diet consistently across 3 weeks. The ADG tended to be lower ( $P = 0.066$ ) and G:F was lower ( $P < 0.001$ ) for field pea diets than for SBM diet for day 7–14. Final BW of pigs fed raw, cold-pelleted, steam-pelleted and extruded field pea or SBM diets were 21.0, 21.1, 21.3, 21.4 and 21.7 kg, respectively. Final BW was not affected by feeding raw or processed field pea.



## **2.4 Discussion**

Increasing production of field pea allows for excess or off-food-grade field pea to be included in swine diets to reduce feed cost (Woyengo et al., 2014). Field pea serves as source of starch and protein, and energy and amino acids are the most costly components in swine diets. Indeed, field pea containing 200 g CP/kg, 15 g Lys/kg and 300–400 g starch/kg (NRC, 2012) might be fed as both starch and protein substitute without reducing growth performance of pigs.

### **2.4.1 Heat treatment of field pea**

Physicochemical properties of field pea may hamper its heat processing. Low moisture and crude fat and high starch content in field pea caused it to bind and stick to the die of the cold-pellet mill thereby causing build-up that prevented material flow through the die. This build-up slowed output and increased friction thereby increasing field pea temperature during processing. During heat exposure, starch granules swell making starch more digestible yet difficult to process (Thomas et al., 1998). Low-fat ingredients such as field pea lack additional lubrication between feed and die wall (Thomas et al., 1998). At times, temperature differences between cold-pelleting and steam-pelleting field pea were as little as 5°C compared with the intended 10°C difference, possibly reducing the differences observed between cold-pelleted and steam-pelleted field pea.

Heat processing did not alter chemical composition of field pea much, as indicated by similar content of CP, starch, AA and TIA of the field peas across treatments. Heat processing may alter fibre properties, as heat processing reduced NDF content. Crude fat was not affected by extrusion or pelleting, although heat may form amylose-lipid complexes (Becker et al., 2001) that may

reduce digestibility of starch by partially preventing  $\alpha$ -amylase from hydrolysing the starch (Holm et al., 1983).

Overheating may cause Maillard reactions and reduce protein digestibility (Owusu-Asiedu et al., 2002). Extrusion may cause Maillard reactions between amino acids and reducing sugars that give a browning colour to feed (Singh et al., 2007). Indeed, increasing heat processing temperatures from 110 to an extremely high level of 165°C decreased Lys in field pea from 15.9 down to 8.7 g/kg (Van Barneveld et al., 1994). However, in the present study, the extrusion temperature reached 115°C with a throughput of 400 kg/h, but the concentration of total Lys or available Lys was not reduced in extruded field pea. Heat treatment did not affect concentrations of indispensable AA, total AA and available Lys of field pea in the present study, indicating that Maillard reactions were likely negligible.

Leguminous seed may contain trypsin inhibitors. Pigs may tolerate up to 3.0 trypsin inhibitor units/mg or 4.7 mg trypsin inhibitor/g in the diet (Batterham et al., 1993; Woyengo et al., 2017). In the present study, extrusion reduced TIA in field pea by 70%, in agreement with the reported TIA reduction from 2.35 to 0.35 g/kg field pea with extrusion (Tusnio et al., 2017). However, cold-pelleting or steam-pelleting did not reduce TIA in the present study, likely due to lower processing temperature and shorter exposure to heat. In the present study, the 1.6 g TIA/kg in raw field pea was less than the reported 4 g TIA/kg for field pea (Hickling, 2003). At 400 g field pea/kg inclusion, or 0.68 g TIA/kg diet, any reduction of TIA in field pea may not be sufficiently significant to affect nutrient digestibility. Instead, locally-sourced soy expeller contained 2.29 g TIA/kg (Smit et al., 2018). Similarly, SBM has a reported mean of 2.28 g TIA/kg (Fan et al., 1995; Valencia et al., 2008; Baker et al., 2010) and TIA averaged 1.82 g/kg and ranged from 0.50 to 5.44

g/kg in 30 SBM samples (Sueiro et al., 2015), slightly greater than that of raw field pea used in the present study.

#### **2.4.2 Nutrient digestibility**

In the present study, feeding field pea reduced diet CATTD of GE that might be explained by the greater fibre content in field pea than SBM. Field pea contains double the total dietary fibre than SBM and fibre may reduce CATTD of GE and CP in grower pigs (Zhang et al., 2013). For young pigs, fibre in diets is more difficult to digest because they have an immature microbiome in the gastrointestinal tract and initially require highly digestible feed to optimise performance (Agyekum and Nyachoti, 2017). This reduced gut capacity may explain the reduced G:F of pigs fed diets containing field pea in the present study. The lower CATTD of GE in field pea diets vs. the SBM diet might also be due to the lower dietary fat and starch content in field pea diets. Previously, co-extrusion of field pea increased CATTD of GE (Htoo et al., 2008). In the present study, we formulated diets to equal NE value based on table values; however, the obtained predicted NE values were 0.70–0.76 MJ/kg lower for the field pea diets than the SBM diet. This difference might be attributed to the lower DE value, indicating that fluctuation in nutrient content and energy value exists among field pea samples due to cultivar and growing and harvest conditions. Indeed, starch content of field pea was 130 g/kg less in the present study than in NRC (2012) that also contributed to the lower predicted NE values in the field pea diets.

Field pea diets had lower CATTD of CP than SBM diet that was consistent with the lower ileal digestibility of CP for field pea in weaned pigs (Petersen et al., 2014). However, heat processing of field pea did not increase CATTD of CP. Although TIA may reduce the CATTD of CP (Bell, 1984), the TIA in the raw field pea was already low in the present study thereby reducing the

chance to observe processing effects on CATTD of CP. Extrusion of field pea reduced TIA content; however, CATTD of CP did not increase in the present study and in grower-finisher pigs (Htoo et al., 2008). Extrusion of field pea may not increase CATTD of starch and fibre in growing pigs (Stein and Bohlke, 2007), indicating that extrusion effects are not consistent among pigs fed late nursery diets and require further investigation.

### **2.4.3 Growth performance**

In the present study, weaned pigs that were fed with diets containing field pea replacing SBM increased feed intake. The greater ADFI for field pea diets than the SBM diet may be explained by the lower predicted NE content of the field pea diets than the SBM diet (Beaulieu et al., 2006). In weaned pigs, decreasing diet energy value increased feed intake and decreased G:F (Beaulieu et al., 2006) to compensate for the lower diet NE values to meet energy requirements for maintenance and growth (Black et al., 1986). Feed intake of pigs that were fed the diet containing raw pea was not different from those fed diets containing pelleted or extruded field pea indicating that low level of ANF did not limit feed intake and raw field pea can be fed to weaned pigs without palatability issues.

In the present study, weaned pigs maintained growth performance when fed diets containing 400 g field pea/kg to replace SBM, similar to the maintained overall performance in weaned pigs fed 400 g field pea/kg (Landro et al., 2014). However, ADG of weaned pigs (9 to 26 kg BW) decreased by 3% when fed diets containing more than 360 g/kg raw field pea to replace SBM and corn and formulated to similar metabolisable energy and SID of AA (Stein et al., 2010). Likewise, ADG of weaned pigs decreased by 6% when fed a diet containing 300 g/kg raw field pea and formulated to similar DE and total amino acid content (Friesen et al., 2006). Instead, grower-

finisher pigs had little adverse reaction to feeding field pea (Stein et al., 2004; Stein et al., 2006; Gatta et al., 2013; Smith et al., 2013). As pigs age, the digestive tract matures and pigs adapt easier to diets with high field pea inclusion (Agyekum and Nyachoti, 2017). In contrast to grower-finisher pigs, young pigs have limited gut fill capacity, thereby limiting feed and energy intake and thus growth performance (Whittemore et al., 2001). However, heat processing of field pea did not increase ADG in the present study, likely explained by the failure to increase nutrient and energy digestibility of field pea diets and to increase feed intake.

In the present study, weaned pigs fed field pea diets had lower G:F than pigs fed the SBM diet, similar to the reduced G:F in nursery pigs fed diets containing 190–400 g field pea/kg diet (Landblom and Polard, 1996; Owusu-Asiedu et al., 2002; Brooks et al., 2009), but contrasting to the maintained overall (day 1–35) G:F in a previous nursery pig trial (Landro et al., 2014). The lower predicted NE value of field pea diets than the SBM diet in the present study may explain the lower G:F. However, the previously-reported sharp drop of G:F in weaned pigs fed field pea diets in the first week (Landro et al., 2014) did not occur in the present study that might be explained by pigs being one week older in the present trial than our previous trial. Heat processing did not increase G:F, similar to young pigs fed 245–490 g extruded field pea/kg diet (Stein et al., 2010), indicating heat processing did not substantially increase nutritive value of field pea.

## **2.5 Conclusion**

Feeding 400 g field pea/kg diet to weaned pigs by replacing 300 g soybean meal/kg and 100 g wheat/kg reduced diet apparent total tract digestibility coefficients of gross energy and crude protein and predicted net energy value. Heat treatment of field pea did not increase apparent total

tract digestibility coefficients of crude protein and gross energy. Pigs fed field pea diets had increased average daily feed intake, but not increased average daily gain and thus reduced feed efficiency. Heat processing did not ameliorate reduced feed efficiency. Under these conditions, nursery pigs weighing 10 to 21 kg fed 400 g field pea/kg diet did not require heat processing for optimal growth. Field pea can be fed as a cost-saving alternative in late nursery diets as a source of both starch and AA maintaining growth performance of weaned pigs.

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**Table 2.1** Analysed nutrient and trypsin inhibitor content (g/kg, as fed) of soybean meal (SBM) and 4 field pea ingredients included in experimental diets.

Item	SBM	Field pea <sup>a</sup>			
		Raw	Cold-pelleted	Steam-pelleted	Extruded
Dry matter	872.0	882.1	874.4	893.4	891.1
Crude protein	477.6	202.0	204.7	198.2	205.9
Crude fat	14.3	5.3	14.6	8.9	5.2
Starch	0.0	282.5	273.5	290.4	271.9
Total dietary fibre	79.4	173.0	161.9	151.5	152.6
Insoluble dietary fibre	74.6	162.2	152.9	143.4	143.5
Soluble dietary fibre	4.9	10.6	8.9	8.7	8.3
Neutral detergent fibre	71.0	98.1	72.9	82.2	77.8
Acid detergent fibre	36.0	68.9	52.9	61.5	59.0
Ash	67.7	28.7	32.0	28.8	29.8
Calcium	6.4	0.7	1.9	0.6	0.7
Phosphorus	6.2	3.8	4.4	4.1	4.2
Indispensable amino acids					
Arginine	34.1	12.7	12.1	12.6	16.3
Histidine	12.3	2.0	4.9	2.0	5.2
Isoleucine	23.3	9.0	9.0	8.8	9.1
Leucine	36.7	14.4	14.5	14.2	14.6
Lysine	30.5	15.0	14.6	14.8	15.4
Methionine	6.5	1.9	2.1	1.8	2.0

Phenylalanine	25.0	9.9	9.9	9.8	9.9
Threonine	18.0	7.2	7.1	7.2	7.6
Tryptophan	6.6	1.8	1.9	1.7	2.1
Valine	23.9	9.9	9.9	9.8	10.1
Total amino acids	464.1	189.0	189.5	187.0	195.5
Chemically-available lysine	29.8	14.7	14.3	14.4	15.2
Trypsin inhibitor activity <sup>b</sup>	N/A	1.6	1.4	1.7	0.5

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<sup>a</sup> Raw, cold-pelleted, steam-pelleted and extruded field pea, contained the following dispensable amino acids (g/kg, as fed;): alanine: 8.5, 8.6, 8.5, 8.9; aspartic acid: 22.0, 21.0, 21.8, 22.8; cysteine: 2.9, 2.7, 2.9, 3.0; glutamic acid: 32.1, 33.7, 31.7, 33.2; glycine: 8.6, 8.8, 8.6, 9.0; proline: 8.2, 9.0, 8.1, 8.5; serine: 7.6, 7.6, 7.5, 8.1; tyrosine: 5.7, 5.5, 5.7, 6.1, respectively.

<sup>b</sup> N/A, not analysed.

**Table 2.2** Ingredient composition and analysed nutrient content (g/kg, as fed) of experimental diets<sup>a</sup>.

Item	SBM	Field pea			
		Raw	Cold-pelleted	Steam-pelleted	Extruded
Wheat (ground)	567.7	475.0	475.0	475.0	475.0
Soybean meal (SBM, 460 g CP/kg)	300.0	-	-	-	-
Field pea, raw <sup>b</sup>	-	400.0	-	-	-
Field pea, cold-pelleted	-	-	400.0	-	-
Field pea, steam-pelleted	-	-	-	400.0	-
Field pea, extruded	-	-	-	-	400.0
Menhaden fish meal (620 g CP/kg)	30.0	30.0	30.0	30.0	30.0
Soy protein concentrate (560 g CP/kg) <sup>c</sup>	30.0	30.0	30.0	30.0	30.0
Canola oil	29.5	10.0	10.0	10.0	10.0
Limestone	10.0	12.5	12.5	12.5	12.5
Mono/dicalcium phosphate	8.5	9.0	9.0	9.0	9.0
Celite <sup>d</sup>	8.0	8.0	8.0	8.0	8.0
Salt	5.5	5.5	5.5	5.5	5.5
Vitamin premix <sup>e</sup>	5.0	5.0	5.0	5.0	5.0
Mineral premix <sup>f</sup>	5.0	5.0	5.0	5.0	5.0
L-Lysine HCl (780 g/kg)	-	3.5	3.5	3.5	3.5
DL-Methionine (990 g/kg)	0.4	2.5	2.5	2.5	2.5
L-Threonine (990 g/kg)	0.1	2.2	2.2	2.2	2.2
L-Tryptophan (990 g/kg)	-	1.5	1.5	1.5	1.5

Choline chloride (600 g/kg)	0.3	0.3	0.3	0.3	0.3
Analysed nutrient content <sup>g</sup>					
Dry matter	891.1	882.2	881.2	885.3	891.1
Crude protein	264.5	194.0	187.4	192.5	192.7
Crude fat	34.5	25.5	22.6	25.0	23.0
Starch	338.9	304.8	272.8	290.3	320.8
Neutral detergent fibre	95.3	94.9	96.8	94.6	105.9
Acid detergent fibre	46.1	42.9	52.5	48.5	59.0
Ash	59.3	58.3	55.5	56.0	56.9
Gross energy (MJ/kg)	17.09	16.18	16.09	16.19	16.28

<sup>a</sup> CP, crude protein.

<sup>b</sup> *Pisum sativum*; W.A. Grain and Pulse Solution, Innisfail, AB, Canada.

<sup>c</sup> HP300, Hamlet Protein Inc., Findlay, OH, USA.

<sup>d</sup> Celite 281, World Minerals Inc., Santa Barbara, CA, USA was used as acid insoluble ash.

<sup>e</sup> Supplied per kilogram of diet: 7500 IU of vitamin A, 750 IU of vitamin D, 50 IU of vitamin E, 37.5 mg of niacin, 15 mg of pantothenic acid, 2.5 mg of folacin, 5 mg of riboflavin, 1.5 mg of pyridoxine, 2.5 mg of thiamine, 2000 mg of choline, 4 mg of vitamin K, 0.25 mg of biotin and 0.02 mg of vitamin B<sub>12</sub>.

<sup>f</sup> Supplied per kilogram of diet: 125 mg of Zn as ZnSO<sub>4</sub>, 50 mg of Cu as CuSO<sub>4</sub>, 75 mg of Fe as FeSO<sub>4</sub>, 25 mg of Mn as MnSO<sub>4</sub>, 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub> and 0.3 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>.

<sup>g</sup> Diets were formulated to provide (as fed): 10.02 MJ net energy (NE)/kg and 1.21 g standardised ileal digestible (SID) lysine/MJ NE, 7.3 g SID threonine/kg, 3.9 g SID methionine/kg, 3.1 g SID tryptophan/kg, 6.9 g SID valine/kg and 5.9 g SID isoleucine/kg.

**Table 2.3** Apparent total tract digestibility coefficients (CATTD) of nutrients and gross energy and digestible energy (DE) and calculated net energy (NE) values of diets containing raw, cold-pelleted, steam-pelleted and extruded field pea in substitution for soybean meal (SBM) and wheat<sup>a</sup>.

Variable	SBM	Field pea				SEM <sup>b</sup>	P-value	
		Raw	Cold-pelleted	Steam-pelleted	Extruded		SBM vs. field pea	Field pea processing
CATTD								
Dry matter	0.845	0.825	0.827	0.823	0.814	0.0060	< 0.001	0.211
Gross energy	0.841	0.812	0.823	0.817	0.806	0.0073	< 0.001	0.189
Crude protein	0.851	0.771	0.791	0.770	0.773	0.0102	< 0.001	0.219
DE (MJ/kg as fed)	14.38	13.14	13.24	13.23	13.12	0.119	< 0.001	0.725
NE <sup>c</sup> (MJ/kg as fed)	9.80	9.09	9.06	9.10	9.04	0.083	< 0.001	0.898

<sup>a</sup> Least-squares means based on 12 pen observations per diet.

<sup>b</sup> SEM, standard error of the mean.

<sup>c</sup> Diet NE values were calculated using Eq. (5) in Noblet et al. (1994).



**Table 2.4** Average daily feed intake (ADFI), average daily gain (ADG), final body weight (BW) and feed efficiency (ADG/ADFI) of weaned pigs fed diets with raw, cold-pelleted, steam-pelleted and extruded field pea in substitution for soybean meal (SBM) and wheat starting 2 weeks post-weaning<sup>a,b</sup>.

Variable	SBM	Field pea				SEM <sup>c</sup>	P-value	
		Raw	Cold-pelleted	Steam-pelleted	Extruded		SBM vs. field pea	Field pea processing
ADFI (g/d)								
Day 1–7	528	562	567	562	598	31.7	0.033	0.614
Day 7–14	792	843	876	817	868	31.7	0.003	0.246
Day 14–21	1119	1153	1203	1193	1162	31.7	0.020	0.336
Day 1–21	813	853	882	857	876	20.2	0.001	0.416
ADG (g/d)								
Day 1–7	331	339	333	357	401	32.9	0.141	0.177
Day 7–14	594	506	538	499	526	42.9	0.066	0.790
Day 14–21	738	738	721	764	710	53.8	0.805	0.758
Day 1–21	554	528	531	540	545	15.6	0.357	0.648
Final BW (kg)	21.7	21.0	21.1	21.3	21.4	0.34	0.333	0.683

Feed efficiency								
Day 1–7	0.62	0.61	0.58	0.63	0.68	0.05	0.773	0.322
						1		
Day 7–14	0.74	0.60	0.61	0.61	0.59	0.04	< 0.001	0.905
						4		
Day 14–21	0.66	0.64	0.61	0.64	0.61	0.04	0.200	0.803
						5		
Day 1–21	0.68	0.62	0.60	0.63	0.62	0.01	< 0.001	0.253
						4		

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<sup>a</sup> Least-squares means based on 12 pen observations per diet.

<sup>b</sup> For ADFI and ADG, but not feed efficiency, a week effect was observed ( $P < 0.001$ ). Interactions between diet and week were not observed ( $P > 0.05$ ) for ADFI, ADG and feed efficiency.

<sup>c</sup> SEM, standard error of the mean.

## **Chapter 3. Nutrient digestibility of heat-processed field pea in weaned pigs**

### **3.1 Introduction**

Because of rising feed cost, alternative feedstuffs such as pulse grains are increasingly included in swine diets (Woyengo et al., 2014). Soybean meal is worldwide the most commonly fed protein source in swine diets but has become increasingly expensive, particularly following biosecurity concerns for feeding animal by-products such as meat and bone meal, plasma or blood meal as protein sources (Kim et al., 2007; Jezierny et al., 2010). Field pea is among the top major pulse grains and Canada is a leading pulse producer in the world. Field pea is an alternative starch (~450 g/kg) and protein (~210 g/kg) source (Bell and Wilson, 1970) and more than 300 g/kg may be included in grower-finisher pig diets (Stein et al., 2004). However, field pea grain has 3.3% lower net energy (NE) than soybean meal (NRC, 2012) and the presence of protease inhibitors may limit its inclusion in young pig diets (Hickling, 2003; Stein et al., 2004). Feed processing of field pea may increase its feeding value by increasing both energy and protein digestibility and reducing trypsin inhibitor activity (TIA; Hickling, 2003).

Reported effects of heat-treatment on field pea have been inconsistent. For example, cold-pelleting field pea grain did not increase the coefficient of apparent ileal digestibility (CAID) of gross energy (GE) in grower pigs (Stein and Bohlke, 2007). Extrusion increased the CAID of dry matter (DM), the coefficient of standardised ileal digestibility (CSID) of crude protein (CP) and amino acids (AA) of field pea grain (Mariscal-Landín et al., 2002) and increased the CAID of starch and CP, and coefficients of apparent total tract digestibility (CATTD) of DM and CP of field pea in grower-finisher pigs (Sun et al., 2006). In growing-finishing pigs, heat processing of field pea grain did not consistently increase the CAID of DM, CP and AA in subsequent years

despite using equivalent heat processing (Canibe and Eggum, 1997). Therefore, whether different heat processing treatments can increase energy and protein digestibility of field pea grain in weaned pig diets remains unclear.

The null hypothesis of the present study was that heat processing (cold-pelleting, steam-pelleting or single-screw extrusion) would not affect the energy and AA digestibility of field pea grain in weaned pigs. The objective was to determine and compare the CATTD and CAID of GE and CP and the CSID of CP and AA of raw, cold-pelleted, steam-pelleted and single-screw extruded field pea in ileal-cannulated weaned pigs.

## **3.2 Materials and Methods**

Trial procedures were reviewed and animal use was approved and by the University of Alberta Animal Care and Use Committee for Livestock. Procedures followed principles established by the Canadian Council on Animal Care (CCAC, 2009). The animal study was conducted at the Swine Research and Technology Centre, University of Alberta (Edmonton, AB, Canada).

### **3.2.1 Test materials, grinding and heat processing**

Yellow field pea grain (*Pisum sativum*) was sourced from W. A. Grain and Pulse Solution, Innisfail, AB, Canada. Field pea grain was ground using a hammer mill through a 4.0-mm screen (Model Jacobson 5550-113-01, Carter Day International, Minneapolis, MN, USA). The ground field pea was divided into 4 batches after grinding. One batch was not processed further (raw), one batch was cold-pelleted (70–75°C, Model PM 1230, Buskirk Engineering, Ossian, IN, USA), another was steam-pelleted at 80–85°C (Model 1116-4, 70 hp; California Pellet Mill,

Crawfordsville, IN, USA) and the fourth batch was extruded at 115°C using a single-screw extruder (Model X115, Wenger, Sabetha, KS, USA). Extrusion conditions were set at a constant feed rate of 400 kg/hr. Steam was added at 14 and 1% in the preconditioner and extruder barrel, and water was added at 5 and 3% in the preconditioner and extruder, respectively. The extrusion screw speed was set to 420 rpm with a 7-mm die. The extruder had 5 incremental temperature zones from 95 to 115°C, increasing by 5°C for each zone. Following heat-processing, cold-pelleted, steam-pelleted and extruded field pea was re-ground using a hammer mill through a 3.2-mm sieve prior to mixing the mash diets (Hugman et al., 2020).

### **3.2.2 Experimental diets and design**

Field pea grain diets were formulated to contain 956 g field pea/kg as the sole source of starch and protein (Table 1). Test diets and the N-free diet included 5 and 8.3 g Cr<sub>2</sub>O<sub>3</sub>/kg, respectively, as an indigestible marker.

Eight crossbred barrows (initial BW: 11.6 ± 1.2 kg; Duroc x Large white/Landrace F1; Genex Hybrid; Hypor, Regina, SK, Canada) were housed in individual metabolic pens (0.81 m wide, 1.12 m long, 0.76 m high) for the first 14 days of the experiment. From day 15 and until completion of the experiment, pigs were housed in larger individual metabolic pens (1.2 m wide × 1.2 m long × 0.9 m high). Pens in the first room had a plastic self-feeder on the side of the pen, and in the second room the pens had stainless-steel feeders on the front of the pen in relation to the pen door. Pens in both rooms were also equipped with a cup drinker next to the feeder, polyvinyl chloride walls with windows, and plastic grated flooring. Temperature in both rooms was set at 25 ± 1 °C with a photoperiod from 0700 to 1900 h using automated thermostats connected to a negative pressure

ventilation system and timed fluorescent light controls. Pigs had freedom of movement and free access to water throughout the experiment.

Pigs were surgically fitted with a simple T-cannula at the distal ileum. After surgery, pigs recovered for 12–14 days, with a gradual increase in feed allowance. Three days prior to the start of the experiment, cannulated pigs were introduced to the test diets by gradual one-third increases in inclusion until 100% test ration was supplied at the start of the first acclimation period. Daily feed allowance was adjusted to 3.0 times the maintenance requirement for digestible energy (DE;  $3.0 \times 110 \text{ kcal DE/kg BW}^{0.75}$ , NRC, 1998), which was fed in 2 equal meals at approximately 0800 and 1500 h. The 4 test diets were fed to 8 pigs for 4 periods in a double  $4 \times 4$  Latin square. Each 9-day period comprised of 5-day diet adaptation, a 2-day faeces collection, and a 2-day digesta collection. Following the fourth period, pigs were fed the N-free diet for an additional period to measure basal endogenous losses of CP and AA. Freshly-voided faeces were collected for 2 days using plastic bags attached to Velcro patches glued on the skin around the anus (van Kleef et al., 1994). Sequentially, digesta samples were collected for 2 days from 0800 h to 1700 h using small plastic bags containing 15 mL of 5% formic acid that were attached to the opened cannula barrel with a rubber band (Li et al., 1993). Collected faeces and digesta were separately pooled for each pig, for each period, and were frozen at  $-20^{\circ}\text{C}$ . At the end of trial, frozen samples were thawed, homogenised, subsampled, and freeze dried.

### **3.2.3 Chemical analyses**

Raw and processed field pea grain samples, diets, and lyophilised digesta and faeces were ground through a 1-mm screen in a centrifugal mill (Retsch GmbH, Haan, Germany) and were analysed for moisture (method 930.15; AOAC, 2006), CP (method 990.03;  $\text{N} \times 6.25$ ) and GE using

an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany). The field pea grain samples and diets were also analysed for ash (method 942.05), crude fat (method 920.39A), starch (assay kit STA-20; Sigma, St. Louis, MO, USA), acid detergent fibre (ADF; method 973.18) and neutral detergent fibre (NDF; Holst, 1973). Field pea grain samples were analysed for calcium (method 968.08), phosphorus (method 946.06), total dietary fibre (method 985.29), AA [method 982.30E (a–c)] and chemically-available lysine (method 975.44) as per AOAC (2006) and TIA (method NEN-EN-ISO 14902:2001; NEN, 2001) at Nutrilab (Giessen, The Netherlands). Diets, digesta and faeces were analysed for Cr<sub>2</sub>O<sub>3</sub> by spectrophotometry (model 80–2097-62, KBULtraspec III, Pharmacia, Cambridge, UK) at 440 nm after ashing at 450°C overnight (Fenton and Fenton, 1979).

### 3.2.4 Calculations

The diet CAID and CATTD of nutrients and GE were calculated using the index method (Adeola, 2001):

$$\text{CAID/CATTD} = 1 - \frac{\text{Concentration Cr2O3}_{\text{feed}} \times \text{Concentration Component}_{\text{digesta/faeces}}}{\text{Concentration Cr2O3}_{\text{digesta/faeces}} \times \text{Concentration Component}_{\text{feed}}}$$

The basal ileal endogenous loss ( $I_{\text{end}}$ ) of AA and CP (g/kg DM intake) was calculated as per Stein et al., 2007:

$$\text{Basal } I_{\text{end}} = \text{Component}_{\text{digesta}} \times \frac{\text{Concentration Cr2O3}_{\text{feed}}}{\text{Concentration Cr2O3}_{\text{digesta}}}$$

The standardised ileal digestibility coefficients (CSID) for indispensable AA in the diets were calculated by correcting for basal ileal endogenous losses of AA using CAID (Stein et al., 2007):

$$\text{CSID} = \text{CAID} + \frac{I_{\text{AAend}}}{\text{AA}_{\text{diet}}}$$

The CSID of AA in field pea diets was considered identical to the CSID in field pea.

The DE values were calculated by multiplying GE by CATTD of GE. The NE values were calculated using Eq. (5) in Noblet et al. (1994) with the determined DE value and analysed content of ADF, starch, CP and crude fat, as adopted by NRC (2012).

### 3.2.5 Statistical Analysis

Digestibility coefficients and the DE and calculated NE values were analysed using the MIXED procedure of SAS (2016). Pig was the experimental unit. Normality and homogeneity of variance of the residual of each variable were confirmed using the UNIVARIATE procedure with 'Normal' option and GLM procedure with 'Hovtest = Levene' option, respectively. Carryover effect was checked prior to ANOVA analysis and was not significant. In the statistical model, diet or ingredient was the fixed effect, and pig and period were random factors. For multiple pair-wise comparisons, Tukey option was used in the model to adjust  $P$  values. To test the hypothesis,  $P < 0.05$  was considered significant whereas  $0.05 \leq P < 0.10$  was considered a trend.

### 3.3 Results

Cold-pelleted field pea diet contained 27 g ADF/kg and 31 g NDF/kg less than the raw field pea diet (Table 2). Heat processing reduced analysed ADF by 17–26 g/kg, NDF by 17–27 g/kg and total dietary fibre by 29 g/kg in field pea grain (Table 3). About 90% dietary fibre in field pea grain was insoluble. Heat processing did not reduce lysine or chemically available lysine content in field pea grain. Cold-pelleting or extrusion reduced TIA by 0.8–1.1 mg/g in field pea grain.

Cold-pelleting of field pea grain increased ( $P < 0.05$ ; Table 4) diet CATTD of DM by 0.041 and CATTD of GE by 0.052 and tended to increase ( $P < 0.10$ ) CATTD of CP. Cold-pelleting of



field pea grain increased ( $P < 0.05$ ) diet and field pea grain DE value by 0.92 and 0.94 MJ/kg and calculated NE values by 0.83 and 0.65 MJ/kg (as fed), respectively, whereas other heat-processing did not.

Cold-pelleting of field pea grain increased ( $P < 0.05$ ; Table 5) diet CAID of DM, GE and starch. Extruded field pea grain had lower ( $P < 0.05$ ) diet CAID of DM and GE than cold- or steam-pelleted field pea grain. Heat processing did not increase diet CAID of CP and indispensable AA, except that extrusion increased ( $P < 0.05$ ) diet CAID of threonine, tryptophan and valine. Extruded field pea grain had greater ( $P < 0.05$ ) CAID diet of arginine, leucine, phenylalanine, cystine, proline and tyrosine than cold-pelleted field pea grain and greater ( $P < 0.05$ ) diet CAID of arginine, phenylalanine, proline, serine and tyrosine than steam-pelleted field pea grain.

Heat processing did not increase CSID of CP and AA in field pea grain (Table 6) except that extrusion increased ( $P < 0.05$ ) CSID of leucine, threonine, tryptophan and valine. Extruded field pea grain had greater ( $P < 0.05$ ) CSID of arginine, leucine, phenylalanine, cystine, proline, serine and tyrosine than cold-pelleted field pea grain, and greater ( $P < 0.05$ ) CSID of leucine, phenylalanine, proline, serine and tyrosine than steam-pelleted field pea grain.

### **3.4 Discussion**

Field pea grain can be fed as an alternative feedstuff to reduce the feed cost in swine. In Canada, the temperate climate is suitable for growing field pea as a rotational crop to cereals and canola in the Prairie provinces; however, excess rain and snow in early fall may delay harvest thereby compromising grain quality (Alberta Agriculture and Forestry, 2018). Low moisture at harvest may increase the proportion of split seeds thus reducing field pea grade from human food to feed

quality. Consequently, more off-grade field pea might then be available at lower costs for inclusion in swine feed.

### **3.4.1 Trypsin inhibitor activity**

In the present study, extrusion was most effective in reducing TIA in field pea grain followed by cold-pelleting. Pigs react more strongly to anti-nutritional factors in field pea grain than rats or chickens (Huisman, 1989; Jansman et al., 1989). The negative effects of TIA are well documented, such as decreasing growth and feed efficiency (Chubb, 1986), causing pancreatic hypertrophy (Green and Lyman, 1972), and increasing endogenous protein loss with decreased exogenous protein absorption (Barth et al., 1993). Trypsin inhibitor is protein-based and thus susceptible to denaturation by heat and catalytic decomposition. Raw soybean contains protease inhibitors (Woyengo et al., 2017), but most are destroyed during processing with the application of moist heat to produce soybean meal, the worldwide standard for plant-based protein in swine diets (Yasoithai, 2016). In the present study, steam-pelleting was less effective in reducing TIA of field pea likely because the pass through the steam conditioner was too short. Trypsin inhibitor inactivation might be biphasic and can be explained by multiple forms of inhibitors, including Kunitz and Bowman-Birk inhibitors, as seen in other legumes (Tsukamoto et al., 1983; van der Poel et al., 1990). In general, more trypsin inhibitor in field bean (*Phaseolus vulgaris*) was destroyed using higher temperature and moisture and longer processing time (Buera et al., 1984; van der Poel et al., 1990).

### 3.4.2 Energy digestibility

In the present study, cold-pelleting increased CATTD of GE in field pea diet that was associated with the lowest ADF and NDF content. Heat processing can alter structural properties of fibre and may lower ADF and NDF content; consequently, nutrient and energy digestibility may increase (Björck et al., 1984; Nasri et al., 2008; de Vries et al., 2012). Moisture and cooking, with and without pressure, reduced NDF, ADF and insoluble dietary fibre content in other pulse grains (Valverde and Frias, 1991; Rehman and Shah, 2004). Extrusion appeared not to affect digestibility of non-starch polysaccharides but increased CATTD of GE in field pea grain (Stein and Bohlke, 2007). In parallel with the increased CATTD of GE, cold-pelleting increased DE and calculated NE values of field pea grain. The lowest ADF and NDF content in cold-pelleted field pea supported the greater DE value of field pea grain, because increased fibre content is associated with reduced diet DE values (King and Taverner, 1975; Fairbairn et al., 1999). Increasing energy digestibility using cold-pelleting may increase the potential to include field pea grain as alternative energy source in swine diets.

In the present study, cold-pelleting increased the CAID of GE in field pea grain that is associated with the increased CAID of starch. Pelleting can induce partial gelatinization of starch associated with disruption of endosperm cell wall, as observed in pelleted barley (Graham et al., 1989). The temperature of cold-pelleting was within the range for starch gelatinization to occur that may coincide with the increased diet CAID of starch observed in the present study. The increased CAID of GE in cold-pelleted field pea grain and lack thereof in extruded field pea grain was opposite to a previous report that indicated that extrusion, but not pelleting, increased CAID of GE of field pea in grower-finisher pigs (Stein and Bohlke, 2007). We can assume that some degree of starch gelatinization occurred during all forms of heat processing as temperatures for

gelatinization of pea starch can range from 49–70°C and up with peak temperatures around 65°C (Ratnayake et al., 2002). Extrusion at lower feed moisture levels increased soluble starch in cereal grains (Mercier and Feillet, 1975) and increased the degree of starch gelatinization in corn grain (Gomez and Aguilera, 1983). Extrusion can alter proportions of rapidly-digestible, slowly-digestible and resistant starch and increase digestion rate and CAID of starch in field pea grain (Sun et al., 2006). Extrusion reduced crystallinity of starch in field pea grain and increased CAID of starch in young broilers (Al-Marzooqi and Wiseman, 2009), but not in the present study.

### **3.4.3 Protein and amino acids digestibility**

The CSID of AA in raw field pea fed to weaned pigs obtained in the present study are similar to CSID values previously reported for weaned pigs (Petersen et al., 2014). In the present study, cold-pelleting did not increase CAID of CP and all AA of field pea diet, in agreement with the unincreased CAID of nutrients in field pea grain pelleted at 75 °C in growing pigs (Stein and Bohlke, 2007). The absence of increased ileal digestibility of CP and AA by cold- or steam-pelleting may have a few possible explanations.

Pelleting of field pea grain with low levels of TIA at or below 1.7 mg/g has limited effects on ileal protein or AA digestibility. Despite TIA reducing CP and AA digestibility (Green and Lyman, 1972; Barth et al., 1993), and extrusion deactivating TIA in field pea grain, extrusion did not increase CAID of AA in broilers (Al-Marzooqi and Wiseman, 2009). Given the reduced TIA levels in cold-pelleted field pea grain yet the lack of increased ileal digestibility of all AA, the low level of TIA in field pea grain in the present study likely played an insignificant role in endogenous and exogenous protein loss or absorption in pigs.

Fibre may decrease digestibility of most macronutrients including protein. Heat processing reduced fibre content in field pea grain; however, fibre may have a limited effect on protein digestion, specifically in the ileum (Sauer et al., 1980). For the cold-pelleted field pea diet, the energy value was increased as expected with the simultaneous decrease in fibre; however, protein digestibility was not affected. Fibre source may play a role in effects of fibre level on protein digestion and utilisation; for example, insoluble fibre may not affect CAID of AA (Sauer et al., 1991).

Reported effects of heat processing on improving protein digestibility have not been consistent and might be affected by cultivar and agronomic conditions such as harvest year. Nutritional composition varies among years of harvest in many legume seeds; large differences are not common (Hlodversson, 1987; Gatel and Grosjean, 1990; Swanson, 1990; Petersen and Spencer, 2006). Highlighting the inconsistency, toasting at 130°C and 150 g/kg moisture increased CAID of CP and AA in field pea in the first but not second year of a study, despite equivalent experimental conditions and processing techniques (Canibe and Eggum, 1997).

Extrusion did not increase CAID of CP and most AA, in contrast to the consistent linear increase of CAID of CP in field pea grain extruded with temperature increasing from 75 to 155°C (Stein and Bohlke, 2007) and increased CSID of CP and AA in growing pigs (Mariscal-Landin et al., 2002). Nevertheless, extrusion increased CAID and CSID of threonine, tryptophan, valine, alanine, serine, alanine and tyrosine of field pea in the present study. Similarly, extrusion only increased CSID of some AA in field pea (Owusu-Asiedu et al., 2002). The increased CSID of AA by extrusion, but not by pelleting, might be explained by the partly-denatured dietary protein caused by extrusion, thereby making protein more easily digestible (Svihus and Zimonja, 2011).

### 3.5 Conclusions

Cold-pelleting or single-screw extrusion reduced TIA in field pea. Cold-pelleting increased energy digestibility and thus DE and calculated NE value, but did not affect the CAID or CSID of AA in field pea. The lack of increased energy value of field pea by steam-pelleting or extrusion requires clarification. Under the reported conditions, steam-pelleting did not increase ileal or total tract digestibility of protein, AA and GE in field pea grain. Extrusion increased ileal digestibility of some AA. However, considering only limited sample size, the lack of effect of heat-processing on protein digestibility of field pea in weaned pigs remains inconclusive.

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**Table 3.1** Ingredient composition (g/kg diet, as fed) of experimental diets.

Item	N-free	Field pea <sup>a</sup>
Field pea grain <sup>b</sup>	–	956.0
Cornstarch	679.9	–
Sugar	200.0	–
Solka floc <sup>c</sup>	30.0	–
Canola oil	20.0	–
Limestone	20.0	13.0
Mono/dicalcium phosphate	17.5	11.0
Salt	8.3	5.0
Vitamin premix <sup>d</sup>	5.0	5.0
Mineral premix <sup>e</sup>	5.0	5.0
K <sub>2</sub> CO <sub>3</sub> (55% K)	5.0	–
MgO (58% Mg)	1.0	–
Cr <sub>2</sub> O <sub>3</sub>	8.3	5.0

<sup>a</sup> Field pea grain was fed either raw, cold-pelleted, steam-pelleted or extruded.

<sup>b</sup> W.A. Grain and Pulse Solution, Innisfail, AB, Canada.

<sup>c</sup> Solka-floc, International Fiber Corp., North Tonawanda, NY, USA.

<sup>d</sup> Supplied per kilogram of diet: 7500 IU of vitamin A, 750 IU of vitamin D, 50 IU of vitamin E, 37.5 mg of niacin, 15 mg of pantothenic acid, 2.5 mg of folacin, 5 mg of riboflavin, 1.5 mg of pyridoxine, 2.5 mg of thiamine, 2000 mg of choline, 4 mg of vitamin K, 0.25 mg of biotin and 0.02 mg of vitamin B<sub>12</sub>.

<sup>e</sup> Supplied per kilogram of diet: 125 mg of Zn as ZnSO<sub>4</sub>, 50 mg of Cu as CuSO<sub>4</sub>, 75 mg of Fe as FeSO<sub>4</sub>, 25 mg of Mn as MnSO<sub>4</sub>, 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub> and 0.3 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>.

**Table 3.2** Analysed nutrient composition (g/kg) and gross energy (GE) value of experimental diets (as fed).

Item	Field pea				N-free
	Raw	Cold-pelleted	Steam-pelleted	Extruded	
Moisture	107.3	103.9	94.3	87.4	66.3
Starch	320.9	372.8	326.9	320.0	586.8
Crude protein	188.6	192.5	194.7	199.1	3.1
Crude fat	8.7	8.5	11.7	5.8	12.2
Acid detergent fibre	67.6	40.6	70.5	65.2	17.1
Neutral detergent fibre	96.3	65.1	95.5	81.9	21.8
Ash	56.3	55.0	57.7	55.8	43.0
GE (MJ/kg)	15.58	15.69	15.93	16.02	15.36

**Table 3.3** Analysed nutrient composition (g/kg, as fed) and trypsin inhibitor activity (TIA) of raw and processed field pea grain samples.

Item, %	Field pea			
	Raw	Cold-pelleted	Steam-pelleted	Extruded
Moisture	112.2	124.0	99.9	92.0
Starch	350.3	324.3	313.8	314.2
Crude protein (N × 6.25)	190.3	202.4	204.6	208.9
Total dietary fibre	127.0	96.4	97.9	97.3
Insoluble dietary fibre	117.1	89.2	90.0	89.0
Soluble dietary fibre	10.0	7.6	7.5	7.4
Neutral detergent fibre	105.4	78.6	88.4	87.7
Acid detergent fibre	81.3	55.2	63.6	64.4
Crude fat	3.2	3.8	5.7	6.5
Ash	27.3	28.3	31.7	29.1
Phosphorus	3.9	4.2	4.4	4.2
Calcium	0.9	0.8	1.9	0.8
Indispensable amino acids				

Arginine	14.7	15.9	15.5	16.3
Histidine	4.5	4.8	4.9	5.0
Isoleucine	8.3	8.9	8.8	9.2
Leucine	13.6	14.5	14.6	14.7
Lysine	14.5	15.3	15.0	15.6
Methionine	1.8	1.9	2.2	2.0
Phenylalanine	9.3	10.0	10.0	10.2
Threonine	7.2	7.6	7.7	7.9
Tryptophan	1.8	1.8	1.7	2.0
Valine	9.1	9.6	9.5	9.7
Dispensable amino acids				
Alanine	8.3	8.8	8.9	9.2
Aspartic acid	21.5	22.8	22.1	23.4
Cystine	3.0	3.1	3.2	3.4
Glutamic acid	31.3	33.3	34.7	34.3
Glycine	8.5	9.0	9.2	9.3



Proline	7.6	8.3	8.8	8.4
Serine	7.8	8.3	8.4	8.6
Tyrosine	5.7	6.1	5.9	6.5
Total amino acids	181.3	192.6	193.9	198.9
Available lysine	14.1	15.2	14.6	15.4
TIA (mg/g)	1.6	0.8	1.7	0.5

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**Table 3.4** Coefficient of apparent total tract digestibility (CATTD) of dry matter, gross energy, and crude protein, and digestible energy (DE) and calculated net energy (NE) values of experimental diets<sup>1</sup>, DE and NE values in field pea grain samples<sup>1</sup> (standardised to 100 g moisture/kg).

Variable	Field pea				SEM <sup>2</sup>	P-value
	Raw	Cold-pelleted	Steam-pelleted	Extruded		
CATTD						
Dry matter	0.873 <sup>b</sup>	0.914 <sup>a</sup>	0.894 <sup>ab</sup>	0.883 <sup>ab</sup>	0.012	0.037
Gross energy	0.863 <sup>b</sup>	0.915 <sup>a</sup>	0.892 <sup>ab</sup>	0.882 <sup>ab</sup>	0.013	0.020
Crude protein	0.812 <sup>B</sup>	0.866 <sup>A</sup>	0.851 <sup>AB</sup>	0.855 <sup>AB</sup>	0.016	0.064
Diet						
DE (MJ/kg, as fed)	13.79 <sup>b</sup>	14.71 <sup>a</sup>	14.43 <sup>ab</sup>	14.25 <sup>ab</sup>	0.221	0.019
Calculated NE <sup>3</sup> (MJ/kg, as fed)	9.39 <sup>b</sup>	10.22 <sup>a</sup>	9.84 <sup>ab</sup>	9.67 <sup>b</sup>	0.155	0.002
Field pea grain						
DE (MJ/kg, as fed)	14.31 <sup>b</sup>	15.25 <sup>a</sup>	15.02 <sup>ab</sup>	14.85 <sup>ab</sup>	0.229	0.022
Calculated NE (MJ/kg, as fed)	9.72 <sup>b</sup>	10.37 <sup>a</sup>	10.17 <sup>ab</sup>	10.04 <sup>ab</sup>	0.160	0.021

<sup>1</sup> Least square means based on 8 pig observations per diet

<sup>2</sup> SEM = Standard error of the mean.

<sup>3</sup> Diet NE values were calculated using Eq. (5) in Noblet et al. (1994).

<sup>a-b</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>A-B</sup> Within a row, means without a common superscript tend to differ ( $P < 0.10$ ).

**Table 3.5** Coefficient of apparent ileal digestibility (CAID) of dry matter, energy, crude protein and amino acids of experiments diets<sup>1</sup>.

Variable	Field pea				SEM <sup>2</sup>	P-value
	Raw	Cold-pelleted	Steam-pelleted	Extruded		
Dry matter	0.667 <sup>bc</sup>	0.757 <sup>a</sup>	0.719 <sup>ab</sup>	0.642 <sup>c</sup>	0.020	<0.001
Gross energy	0.663 <sup>bc</sup>	0.760 <sup>a</sup>	0.712 <sup>ab</sup>	0.637 <sup>c</sup>	0.021	<0.001
Crude protein	0.772	0.798	0.785	0.802	0.013	0.198
Starch	0.941 <sup>b</sup>	1.00 <sup>a</sup>	0.977 <sup>ab</sup>	0.950 <sup>b</sup>	0.016	0.012
Indispensable amino acids						
Arginine	0.888 <sup>ab</sup>	0.880 <sup>b</sup>	0.879 <sup>b</sup>	0.898 <sup>a</sup>	0.007	0.030
Histidine	0.842	0.853	0.845	0.860	0.011	0.316
Isoleucine	0.808	0.812	0.809	0.838	0.012	0.055
Leucine	0.813 <sup>ab</sup>	0.813 <sup>b</sup>	0.812 <sup>ab</sup>	0.843 <sup>a</sup>	0.012	0.025
Lysine	0.854	0.863	0.850	0.869	0.010	0.257
Methionine	0.814	0.847	0.825	0.823	0.016	0.242
Phenylalanine	0.815 <sup>ab</sup>	0.805 <sup>b</sup>	0.809 <sup>b</sup>	0.848 <sup>a</sup>	0.012	0.005
Threonine	0.752 <sup>b</sup>	0.791 <sup>ab</sup>	0.774 <sup>ab</sup>	0.807 <sup>a</sup>	0.016	0.037

Tryptophan	0.773 <sup>b</sup>	0.818 <sup>ab</sup>	0.799 <sup>ab</sup>	0.845 <sup>a</sup>	0.019	0.027
Valine	0.780 <sup>b</sup>	0.810 <sup>ab</sup>	0.806 <sup>ab</sup>	0.836 <sup>a</sup>	0.014	0.019
Dispensable amino acids						
Alanine	0.755 <sup>b</sup>	0.816 <sup>ab</sup>	0.788 <sup>ab</sup>	0.826 <sup>a</sup>	0.019	0.031
Aspartic acid	0.814	0.822	0.818	0.838	0.019	0.085
Cystine	0.686 <sup>ab</sup>	0.668 <sup>b</sup>	0.678 <sup>ab</sup>	0.738 <sup>a</sup>	0.025	0.033
Glutamic acid	0.856	0.849	0.860	0.848	0.011	0.589
Glycine	0.735	0.756	0.745	0.729	0.025	0.660
Proline	0.744 <sup>ab</sup>	0.710 <sup>b</sup>	0.721 <sup>b</sup>	0.782 <sup>a</sup>	0.024	0.024
Serine	0.772 <sup>b</sup>	0.798 <sup>b</sup>	0.795 <sup>b</sup>	0.829 <sup>a</sup>	0.012	0.004
Tyrosine	0.812 <sup>b</sup>	0.816 <sup>b</sup>	0.801 <sup>b</sup>	0.852 <sup>a</sup>	0.012	0.003
Total amino acids	0.814	0.818	0.812	0.834	0.010	0.136

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<sup>1</sup> Least square means based on 8 pig observations per diet.

<sup>2</sup> SEM = Standard error of the mean.

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

**Table 3.6** Coefficient of standardised ileal digestibility (CSID) of crude protein and amino acids of field pea grain samples<sup>1</sup>.

Variable	Field pea				SEM <sup>2</sup>	P-value
	Raw	Cold-pelleted	Steam-pelleted	Extruded		
Crude protein	0.820	0.845	0.832	0.850	0.013	0.199
Indispensable amino acids						
Arginine	0.900 <sup>ab</sup>	0.899 <sup>b</sup>	0.900 <sup>ab</sup>	0.918 <sup>a</sup>	0.007	0.030
Histidine	0.869	0.880	0.872	0.887	0.011	0.342
Isoleucine	0.835	0.838	0.835	0.864	0.012	0.061
Leucine	0.824 <sup>b</sup>	0.840 <sup>b</sup>	0.839 <sup>b</sup>	0.870 <sup>a</sup>	0.012	0.013
Lysine	0.875	0.884	0.871	0.889	0.010	0.284
Methionine	0.848	0.881	0.885	0.859	0.016	0.223
Phenylalanine	0.842 <sup>ab</sup>	0.832 <sup>b</sup>	0.836 <sup>b</sup>	0.874 <sup>a</sup>	0.012	0.006
Threonine	0.804 <sup>b</sup>	0.843 <sup>ab</sup>	0.826 <sup>ab</sup>	0.858 <sup>a</sup>	0.016	0.041
Tryptophan	0.773 <sup>b</sup>	0.818 <sup>ab</sup>	0.799 <sup>ab</sup>	0.845 <sup>a</sup>	0.020	0.027
Valine	0.809 <sup>b</sup>	0.839 <sup>ab</sup>	0.835 <sup>ab</sup>	0.865 <sup>a</sup>	0.014	0.020
Dispensable amino acids						

Alanine	0.815 <sup>b</sup>	0.855 <sup>ab</sup>	0.827 <sup>ab</sup>	0.865 <sup>a</sup>	0.017	0.050
Aspartic acid	0.838	0.847	0.843	0.862	0.010	0.101
Cystine	0.748 <sup>ab</sup>	0.731 <sup>b</sup>	0.738 <sup>ab</sup>	0.798 <sup>a</sup>	0.025	0.040
Glutamic acid	0.875	0.868	0.879	0.866	0.011	0.601
Glycine	0.828	0.848	0.834	0.819	0.025	0.628
Proline	0.919 <sup>ab</sup>	0.710 <sup>c</sup>	0.881 <sup>b</sup>	0.955 <sup>a</sup>	0.024	<0.001
Serine	0.810 <sup>b</sup>	0.835 <sup>b</sup>	0.832 <sup>b</sup>	0.867 <sup>a</sup>	0.012	0.004
Tyrosine	0.846 <sup>b</sup>	0.851 <sup>b</sup>	0.837 <sup>b</sup>	0.883 <sup>a</sup>	0.012	0.005
Total amino acids	0.852	0.856	0.849	0.870	0.010	0.158

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<sup>1</sup> Least square means based on 8 pig observations per diet.

<sup>2</sup> SEM = Standard error of the mean.

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

## **Chapter 4. Energy and nutrient digestibility of raw, steam-pelleted, or extruded red lentil in growing-finishing pigs**

### **4.1 Introduction**

Lentil (*Lens culinaris*) is a pulse crop, non-oilseed legume. In Western Canada, 2.09 million metric tonnes of lentil seed will be produced in 2020 with the majority intended for human consumption (AAFC, 2019). Lentil grows well in the cool, moist growing seasons in Western Canada (Castell, 1990). Lentil also benefits soil quality, nitrogen fixation and crop rotation making it a desirable crop to grow. Lentil grain contains 400 g starch/kg, 250 g crude protein (CP)/kg and 150 g neutral detergent fibre (NDF)/kg making it a nutritious ingredient for swine (Landro et al., 2012). Lentil not used for human consumption can be an alternative feedstuff substituting soybean meal (SBM) in swine diets to reduce feed cost (Landro et al., 2012; Woyengo et al. 2014a).

A major concern of feeding lentil to pigs is the presence of anti-nutritional factors (ANF) in raw lentil. Adverse effects of feeding lentil to swine include reduced protein utilization and meat quality and impaired taste; thus, inclusion of lentil may be limited and the use of lentil as sole protein source in swine feed was not advised historically (Castell, 1990). Dietary inclusion of above 225 g lentil/kg decreased average daily gain (ADG) and gain:feed ratio (G:F) in weaned pigs (Landro et al., 2012). However, dietary inclusion of up to 400 g lentil/kg to replace barley and SBM did not affect feed intake, growth, or feed efficiency in growing-finishing pigs (Bell and Keith, 1986; Castell and Cliplef, 1988). Protein and amino acid digestibility in lentil have rarely been investigated but were reported to be lower than that of SBM in pigs (NRC, 2012; Woyengo et al., 2014b). Heat processing can reduce ANF and may gelatinise starch of lentil, thereby increasing *in vitro* starch digestibility (de Vries et al., 2012; Dogan et al., 2013; Rathod and



Annapure, 2016). However, whether heat processing can increase nutrient and energy digestibility of lentil in pigs remains unknown.

The null hypothesis of the present study was that steam-pelleting or extrusion would not affect the digestible nutrient value of lentil in growing-finishing pigs. The objectives of the study were to determine and compare coefficients of standardised ileal digestibility (CSID) of amino acids and coefficients of total tract digestibility (CATTD) of gross energy (GE), digestible energy (DE) and calculated net energy (NE) values of raw, steam-pelleted or extruded lentil in ileal-cannulated growing-finishing pigs.

## **4.2 Materials and methods**

The animal use and procedures were reviewed by the University of Alberta Animal Care and Use Committee for Livestock and followed principles established by the Canadian Council on Animal Care (2009). The animal study was conducted at the Swine Research and Technology Centre, University of Alberta (Edmonton, AB, Canada).

### **4.2.1 Test article, grinding and heat processing**

The red lentil (*Lens culinaris*) was sourced from CorNine Commodities, Lacombe, AB, Canada. The entire lentil sample was ground using a hammer mill through a 4.0-mm screen (Model Jacobson 5550-113-01, Carter Day International, Minneapolis, MN, USA). Portions of ground lentil were either steam-pelleted (Model 1116-4, 70 hp; California Pellet Mill, Crawsfordville, IN, USA) at 80–85°C, extruded (Model X115, Wenger, Sabetha, KS, USA) at 115°C, or remained

ground (raw). Extruded lentil passed through a single screw extruder at 400 kg/h with preconditioner set to 1% water and 3% steam. The extruder barrel consisted of 5 zones, each with a 5°C increase from the previous zone starting at 95°C up to 115°C before passing through a 7-mm die. Screw speed of 420 rpm was used. Following heat processing, the raw, steam-pelleted, and extruded lentil samples were re-ground using a hammer mill through a 3.2-mm screen before mixing the mash diets.

#### **4.2.2 Experimental diets and design**

Lentil diets were formulated to include 956 g lentil/kg as sole source of energy and AA and 5 g Cr<sub>2</sub>O<sub>3</sub>/kg as an indigestible marker (Table 1). Nine crossbred barrows (initial BW 69.0 ± 6.7 kg; Duroc × Large white/Landrace F1; Genex Hybrid, Hypor, Regina, SK, Canada) were used in the trial for 4 periods. Each period was 9 days long and comprised of 5-day adaptation to a diet, 2-day faecal collection, and 2-day digesta collection. In the first period, pigs were fed the N-free diet to measure endogenous losses of CP and AA. For the remaining 3 periods, diets were fed in a triple 3 × 3 Latin square arrangement.

Pigs were housed in individual polyvinyl chloride metabolic pens (1.2 m wide, 1.2 m long, 0.9 m high) equipped with a stainless-steel feeder attached to polyvinyl chloride wall opposite the pen door. A stainless-steel cup drinker was installed next to the feeders. Clear plastic windows were installed on 3 walls of the pens and the floors were constructed of plastic grated flooring raised off the ground on steel frames. The room was temperature-controlled at 22 ± 1 °C using automated temperature controls with a negative pressure ventilation system. A photoperiod of 0700 h to 1900 h was set with fluorescent lighting and timed switch controls to provide a 12-h light and 12-h dark

cycle. Pigs had environmental enrichment through toys, as well as freedom of movement and free access to water throughout the experiment.

Pigs had a simple T-cannula surgically inserted at the distal ileum, and were involved in a previous experiment before. Daily feed allowance was adjusted to 2.8 times the maintenance requirement for DE ( $2.8 \times 110 \text{ kcal DE/kg BW}^{0.75}$ , NRC, 1998), fed in 2 equal meals at approximately 0800 h and 1500 h. Faeces were collected continuously for 48 hours using plastic bags attached to Velcro rings secured to the skin around the anus (van Kleef et al., 1994). Digesta samples were collected for 2 sequential days from approximately 0800 h to 1700 h using plastic bags containing 15 mL of 5% formic acid, attached to the opened cannula barrel with a rubber band (Li et al., 1993). Collected faeces and digesta were pooled for each pig for each period and were frozen at  $-20^{\circ}\text{C}$ , then thawed, homogenised, subsampled and freeze-dried.

#### **4.2.3 Chemical analyses**

Lentil grain, diets, and lyophilised digesta and faeces were ground through a 1-mm screen in a centrifugal mill (Retsch GmbH, Haan, Germany) and analysed for moisture (method 930.15; AOAC, 2006), CP (method 990.03;  $\text{N} \times 6.25$ ) and GE using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany). Lentil grain and diets were analysed for ash (method 942.05), crude fat (method 920.39A), starch (assay kit STA-20; Sigma, St. Louis, MO, USA), acid detergent fibre (ADF; method 973.18) and NDF (Holst, 1973). Lentil grain were also analysed for calcium (method 968.08), phosphorus (method 946.06), total dietary fibre (method 985.29), AA [method 982.30E (a–c)], chemically-available Lys (method 975.44) as per AOAC (2006) and TIA (method NEN-EN-ISO 14902:2001; NEN, 2001) at Nutrilab (Giessen, The Netherlands). Diets, digesta and faeces were analysed for  $\text{Cr}_2\text{O}_3$  by spectrophotometry (model 80–2097-62,

KBULtraspec III, Pharmacia, Cambridge, UK) at 440 nm after ashing at 450 °C overnight (Fenton and Fenton, 1979).

#### 4.2.4 Calculations

The diet CAID and CATTD of nutrients and GE were calculated using the index method with the following equation (Adeola, 2001):

$$\text{CAID or CATTD} = 1 - \frac{\text{Concentration Cr2O3}_{\text{feed}} \times \text{Concentration Component}_{\text{digesta/faeces}}}{\text{Concentration Cr2O3}_{\text{digesta/faeces}} \times \text{Concentration Component}_{\text{feed}}}$$

The CAID and CATTD of GE of lentil grain was considered identical to that of test diets because lentil grain was the major ingredient in diets.

The following equation was used to calculate basal ileal endogenous loss ( $I_{\text{end}}$ ) of AA and CP (g/kg DM intake; Stein et al., 2007):

$$\text{Basal } I_{\text{end}} = \text{Component}_{\text{digesta}} \times \frac{\text{Concentration Cr2O3}_{\text{feed}}}{\text{Concentration Cr2O3}_{\text{digesta}}}$$

The CSID of AA and CP in the diets was calculated by correcting CAID for basal ileal endogenous losses using the following equation (Stein et al., 2007):

$$\text{CSID} = \text{CAID} + \frac{\text{Basal } I_{\text{end}}}{\text{AA}_{\text{diet}}}$$

The CSID of amino acids in the lentil grain was considered identical to the CSID in lentil diets, because lentil grain was the sole ingredient providing protein and amino acids in diets.

#### 2.5 Statistical analyses

Data were analysed using the MIXED procedure of SAS (2016). Pig was the experimental unit. Each variable was confirmed for normality and homogeneity of variance of the residual using the UNIVARIATE procedure with ‘Normal’ option and GLM procedure with ‘Hovtest = Levene’ option, respectively. Carry-over effect was checked prior to the ANOVA analysis. Diet was the

fixed effect, whereas pig and period were random factors. For multiple pair-wise comparisons, Tukey option was used in the model to adjust  $P$  values. To test the hypothesis,  $P < 0.05$  was considered significant whereas  $0.05 \leq P < 0.10$  was considered a trend.

### 4.3 Results

Due to variation in moisture content among diets and ingredients, nutrient and ANF content and energy values were standardised to 100 g moisture/kg. Steam-pelleting or extrusion did not affect CP and ADF contents of lentil grain but rendered starch more measurable. Fat content in the extruded lentil was not detectable using the analytical method without acid digestion (Table 2). Extrusion reduced NDF content in lentil by 13.2 g/kg and TIA by 1.2 mg/g. Steam-pelleting or extrusion did not reduce total AA content, but reduced lysine and chemically-available lysine content by 0.4–0.7 g/kg.

Steam-pelleting or extrusion increased ( $P < 0.05$ ; Table 3) diet CAID of DM, GE, starch and CP, and diet CATTD of DM, GE and CP, DE and calculated NE values in lentil. The CAID of DM of diet and NE value were greater ( $P < 0.05$ ) for steam-pelleted lentil than extruded lentil.

Steam-pelleting or extrusion increased ( $P < 0.05$ ; Table 4) diet CAID of all AA except for cysteine and glutamic acid for extrusion. Steam-pelleting tended to increase ( $P < 0.10$ ) diet CAID of glutamic acid. Diet CAID of phenylalanine and tryptophan was greater ( $P < 0.05$ ) for extrusion than steam-pelleting. Steam-pelleting or extrusion increased ( $P < 0.05$ ; Table 5) the CSID of CP and all AA in lentil except for cysteine and glutamic acid for extrusion and tended to increase ( $P < 0.10$ ) CSID of glutamic acid for steam-pelleting. Extrusion further increased ( $P < 0.05$ ) the CSID of phenylalanine in lentil compared with steam-pelleting.

Steam-pelleting increased ( $P < 0.05$ ; Table 6) standardised ileal digestible (SID) content of CP and all AA in lentil, except for lysine and aspartic acid. Extrusion increased ( $P < 0.05$ ) SID content of CP and all AA in lentil, except cysteine and glutamic acid. Compared with steam-pelleting, extrusion increased ( $P < 0.05$ ) the SID content of methionine, tryptophan and aspartic acid in lentil.

#### **4.4 Discussion**

Limited data exists on nutrient and energy digestibility of pulse grain lentil or effects of processing on feeding values and nutrient digestibility in pigs. North American database on nutrient content and digestibility in lentil is restricted to a single raw sample (NRC, 2012). In the present study, heat processing increased nutrient digestibility and energy value in lentil; therefore, steam-pelleted or extruded lentil might be a good source of energy and AA for nursery or growing-finishing pigs.

##### **4.4.1 Heat processing and chemical composition of lentil**

The starch, CP and ash content of raw lentil grain sample tested in the present study are within range of reported values (Landro et al., 2012). Steam-pelleting or extrusion slightly reduced lysine and chemically-available lysine content in lentil, indicating that minor heat damage of protein, or more specifically Lys, might have occurred during heat processing involving both heat and moisture (Hendriks, 2018). Crude fat was low in lentil and undetectable in extruded lentil. Extrusion can form lipid and amylose complexes, making fat more difficult to extract (Mercier,

1980; Bhatnagar and Hanna, 1994). In addition, high temperatures inside the barrel of the extruder may evaporate volatile compounds, thereby resulting in fat losses (Brenes et al., 2008).

Heat processing may reduce content of heat-labile ANF in feedstuffs (Akande and Fabiyi, 2010). The TIA in pulse grains may reduce digestibility of nutrients in pigs (Barth et al., 1993; Li et al., 1999; Grosjean et al., 2000). Extrusion was effective in reducing TIA in lentil, but steam-pelleting was not. The TIA in the lentil sample tested in the present study was low, considering the reported range of 1.9–2.8 mg TIA/g in lentil (Wang et al., 2009). Steam-pelleted and raw lentil diet both exceeded tolerance limits of 1.23 g TIA/kg (Woyengo et al., 2012); however, TIA remained below previously-suggested limits of 4.7 mg TIA/g for growing-finishing pigs, whereas TIA following extrusion was well below both (Batterham et al., 1993). At 80°C pellet temperature and with high throughput, steam-pelleting may not provide sufficient temperature and exposure time to deactivate TIA (Manzoor et al., 2016). However, limited inclusion of raw or steam-pelleted lentil in commercial nursery or growing-finishing pig diets may allow for lentil use without exceeding recommended TIA limits or causing adverse negative effects (Landerio et al., 2012).

#### **4.4.2 Heat processing and nutrient and energy digestibility of lentil**

Heat processing may increase energy digestibility in feedstuffs (Marty and Chaves, 1993; Stein and Bohlke, 2007). In the present study, steam-pelleting or extrusion increased CAID of GE of lentil diet, supported by increased CAID of starch and CP. Similarly, steam-pelleting or extrusion increased CATTD of GE and CATTD of CP of lentil diet in the present study. In addition, steam-pelleting slightly increased soluble fibre content, which may render lentil fibre more degradable, thus increasing CATTD of GE (Noblet and Goff, 2001; Lindberg, 2014). The GE values were consistent among the processed lentil samples; therefore, the increased CATTD of GE in heat-

treated lentil samples increased DE and calculated NE values. The increase of measured starch content in steam-pelleted or extruded lentil samples would also contribute to the increase of calculated NE values of lentil (Noblet et al., 1994).

#### **4.4.3 Heat processing and amino acid digestibility of lentil**

Heat processing may increase protein digestibility in pulse grains such as lentil (Rehman and Shah, 2005; Rathod and Annapure, 2016). In the present study, the CAID and CSID of arginine, isoleucine, leucine, lysine, phenylalanine, and valine in raw lentil were similar to reported coefficients (NRC, 2012; Woyengo et al., 2014b). Both steam-pelleting and extrusion increased CAID of CP and all indispensable AA in lentil diets and CSID of CP and all indispensable AA in lentil grain samples. Increases in CAID and CSID of AA in extruded and steam-pelleted lentil may be because heat processing may partly denature dietary proteins, thereby unfolding them to increase access for porcine enzymes to proteins and AA (Hendriks and Sritharan, 2002). Trypsin inhibitors can increase ileal endogenous losses of AA and inhibit access of endogenous enzymes to pulse proteins, thereby reducing nutrient digestibility and growth in pigs (Brenes et al., 2004; Woyengo et al., 2017). However, TIA content in lentil seemed not to be a major factor to influence protein and AA digestibility of lentil in pigs.

Steam pelleting did not decrease TIA but increased CAID of CP in lentil in the present study. Previously, steam pelleting at 75-85°C was insufficient to deactivate ANF and increase protein digestibility of field pea in weaned pigs (Hugman et al., 2020). Instead, extrusion with greater heat, pressure, and retention time is a more suitable processing method to enhance nutrient digestibility in field pea (Owusu-Asiedu et al, 2002; Stein and Bohlke, 2007). However, in the present study, steam-pelleting increased CSID of CP and AA similarly to extrusion in lentil. Due to steam-



pelleting or extrusion increasing CSID of most AA in lentil, respective SID content in lentil was increased. Among the AA, SID content of methionine and tryptophan were increased the most due to increased digestibility of these AA. Sulphur-containing AA and tryptophan are typically the most limiting AA in pulse grains (Peace et al., 1988); therefore, heat processing made lentil a more valuable protein source in pig diets.

#### **4.5 Conclusions**

Steam-pelleting or extrusion increased ileal and total tract digestibility of CP, DM and GE, standardised ileal digestible AA, and DE and calculated NE values of lentil. Extrusion, but not steam-pelleting, reduced TIA in lentil, providing a potential of greater dietary inclusion of lentil as an alternative feedstuff in diets for young pigs. Despite increases in NE and further increase in some AA digestibility contents, our results indicate that extrusion, a more extreme form of heat-processing, may not be necessary to increasing nutritive value of lentil considering the additional associated cost of time, labour and machinery. Increased CP and AA digestibility for steam-pelleted lentil in the present study indicate that TIA present in lentil may not be sufficient to cause adverse effects on protein digestibility, and that lentil is sensitive to heat processing. Steam pelleting may thus make lentil a more versatile ingredient in swine diets.

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**Table 4.1** Ingredient composition (g/kg diet, as fed) and analysed nutrient content (standardised to 100 g moisture/kg) of experimental diets.

Item	Lentil			N-free
	Raw	Steam-pelleted	Extruded	
Lentil, raw <sup>a</sup>	956	–	–	–
Lentil, steam-pelleted	–	956	–	–
Lentil, extruded	–	–	956	–
Corn starch	–	–	–	846
Sugar	–	–	–	50
Solka floc <sup>b</sup>	–	–	–	30
Canola oil	–	–	–	20
Limestone	13	13	13	12
Mono/dicalcium phosphate	11	11	11	16
Salt	5	5	5	5
Vitamin premix <sup>c</sup>	5	5	5	5
Mineral premix <sup>d</sup>	5	5	5	5
K <sub>2</sub> CO <sub>3</sub> (560 g K/kg)	–	–	–	5
Cr <sub>2</sub> O <sub>3</sub>	5	5	5	5
MgO (580 g Mg/kg)	–	–	–	1
Analysed nutrient content				
Dry matter	900	900	900	900
Starch	301	392	390	758
Crude protein	234	228	233	4.7

Neutral detergent fibre	83.8	73.0	65.7	19.2
Acid detergent fibre	59.5	53.6	51.6	14.7
Ash	49.9	58.5	45.8	27.2
Crude fat	2.3	5.7	0.0	4.3
Gross energy (MJ/kg)	16.1	16.1	16.2	15.0

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<sup>a</sup> CorNine Commodities, Lacombe, AB, Canada.

<sup>b</sup> Solka-floc, International Fiber Corp., North Tonawanda, NY, USA.

<sup>c</sup> Supplied per kilogram of diet: 7,500 IU of vitamin A, 750 IU of vitamin D, 50 IU of vitamin E, 37.5 mg of niacin, 15 mg of pantothenic acid, 2.5 mg of folacin, 5 mg of riboflavin, 1.5 mg of pyridoxine, 2.5 mg of thiamine, 2,000 mg of choline, 4 mg of vitamin K, 0.25 mg of biotin and 0.02 mg of vitamin B<sub>12</sub>.

<sup>d</sup> Supplied per kilogram of diet: 125 mg of Zn as ZnSO<sub>4</sub>, 50 mg of Cu as CuSO<sub>4</sub>, 75 mg of Fe as FeSO<sub>4</sub>, 25 mg of Mn as MnSO<sub>4</sub>, 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub> and 0.3 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>.



**Table 4.2** Analysed nutrient content (g/kg) and gross energy (GE) value of raw, steam-pelleted, or extruded lentil grain (standardised to 100 g moisture/kg).

Item	Lentil		
	Raw	Steam-pelleted	Extruded
Dry matter	900	900	900
Starch	326	431	429
Crude protein (N × 6.25)	247	243	244
Total dietary fibre	93.5	96.5	81.2
Insoluble dietary fibre	79.5	80.1	69.7
Soluble dietary fibre	14.0	16.8	11.8
Neutral detergent fibre	85.0	87.1	71.8
Acid detergent fibre	54.3	56.1	52.7
Ash	24.3	27.6	24.1
Phosphorus	3.1	3.5	3.0
Calcium	0.7	1.2	0.7
Crude fat	1.1	6.7	0.0
GE (MJ/kg)	16.9	16.9	16.9
Indispensable amino acids			
Arginine	17.8	17.4	17.6
Histidine	5.9	5.9	5.9
Isoleucine	10.9	10.7	10.7
Leucine	17.6	17.6	17.4
Lysine	17.0	16.3	16.6

Methionine	1.9	2.0	1.8
Phenylalanine	12.2	12.2	12.1
Threonine	8.7	8.6	8.6
Tryptophan	1.4	1.4	1.5
Valine	12.0	11.9	11.9
Dispensable amino acids			
Alanine	10.1	10.1	10.0
Aspartic acid	26.7	25.6	26.5
Cysteine	2.6	2.7	2.6
Glutamic acid	37.7	38.7	37.4
Glycine	9.9	10.0	9.8
Proline	8.9	9.8	8.1
Serine	9.7	9.5	9.5
Tyrosine	7.2	7.2	7.1
Total amino acids	221	220	218
Chemically-available lysine	16.7	16.0	16.3
Trypsin inhibitor activity (mg/g)	1.7	2.1	<0.5

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**Table 4.3** Coefficients of apparent ileal digestibility (CAID) and coefficients of total tract digestibility (CATTD) of dry matter, gross energy and crude protein of experimental diets and digestible energy (DE) and calculated net energy (NE) values of lentil samples<sup>1</sup> (standardised to 100 g moisture/kg).

Variable	Lentil			SEM <sup>2</sup>	P-value
	Raw	Steam-pelleted	Extruded		
CAID of diet					
Dry matter	0.643 <sup>c</sup>	0.719 <sup>a</sup>	0.682 <sup>b</sup>	0.013	<0.001
Gross energy	0.647 <sup>b</sup>	0.728 <sup>a</sup>	0.699 <sup>a</sup>	0.012	<0.001
Starch	0.826 <sup>b</sup>	0.936 <sup>a</sup>	0.902 <sup>a</sup>	0.014	<0.001
Crude protein	0.730 <sup>b</sup>	0.794 <sup>a</sup>	0.801 <sup>a</sup>	0.012	<0.001
CATTD of diet					
Dry matter	0.818 <sup>b</sup>	0.840 <sup>a</sup>	0.834 <sup>a</sup>	0.004	<0.001
Gross energy	0.804 <sup>b</sup>	0.836 <sup>a</sup>	0.829 <sup>a</sup>	0.004	<0.001
Crude protein	0.768 <sup>b</sup>	0.818 <sup>a</sup>	0.810 <sup>a</sup>	0.006	<0.001
Lentil					
DE (MJ/kg, as fed)	13.62 <sup>b</sup>	14.14 <sup>a</sup>	14.01 <sup>a</sup>	0.075	<0.001
NE (MJ/kg, as fed)	9.16 <sup>c</sup>	9.79 <sup>a</sup>	9.62 <sup>b</sup>	0.053	<0.001

<sup>1</sup> Least square means based on 9 pig observations per diet.

<sup>2</sup> SEM = Standard error of the mean.

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

**Table 4.4** Coefficients of apparent ileal digestibility (CAID) of amino acids of experiments diets<sup>1</sup>.

Variable	Lentil			SEM <sup>2</sup>	P-value
	Raw	Steam-pelleted	Extruded		
Indispensable amino acids					
Arginine	0.817 <sup>b</sup>	0.879 <sup>a</sup>	0.886 <sup>a</sup>	0.011	<0.001
Histidine	0.764 <sup>b</sup>	0.828 <sup>a</sup>	0.840 <sup>a</sup>	0.011	<0.001
Isoleucine	0.749 <sup>b</sup>	0.826 <sup>a</sup>	0.848 <sup>a</sup>	0.012	<0.001
Leucine	0.761 <sup>b</sup>	0.837 <sup>a</sup>	0.855 <sup>a</sup>	0.011	<0.001
Lysine	0.780 <sup>b</sup>	0.845 <sup>a</sup>	0.853 <sup>a</sup>	0.012	<0.001
Methionine	0.717 <sup>b</sup>	0.812 <sup>a</sup>	0.818 <sup>a</sup>	0.017	<0.001
Phenylalanine	0.761 <sup>c</sup>	0.831 <sup>b</sup>	0.864 <sup>a</sup>	0.011	<0.001
Threonine	0.720 <sup>b</sup>	0.783 <sup>a</sup>	0.813 <sup>a</sup>	0.014	<0.001
Tryptophan	0.793 <sup>c</sup>	0.865 <sup>b</sup>	0.894 <sup>a</sup>	0.011	<0.001
Valine	0.740 <sup>b</sup>	0.814 <sup>a</sup>	0.838 <sup>a</sup>	0.012	<0.001
Dispensable amino acids					
Alanine	0.713 <sup>b</sup>	0.794 <sup>a</sup>	0.819 <sup>a</sup>	0.013	<0.001
Aspartic acid	0.785 <sup>b</sup>	0.845 <sup>a</sup>	0.856 <sup>a</sup>	0.011	<0.001
Cysteine	0.618 <sup>b</sup>	0.709 <sup>a</sup>	0.670 <sup>ab</sup>	0.024	0.008
Glutamic acid	0.798 <sup>B</sup>	0.839 <sup>A</sup>	0.831 <sup>AB</sup>	0.016	0.055
Glycine	0.627 <sup>b</sup>	0.701 <sup>a</sup>	0.732 <sup>a</sup>	0.016	<0.001
Serine	0.752 <sup>b</sup>	0.821 <sup>a</sup>	0.847 <sup>a</sup>	0.013	<0.001
Tyrosine	0.766 <sup>b</sup>	0.820 <sup>a</sup>	0.849 <sup>a</sup>	0.011	<0.001
Total amino acids	0.750 <sup>b</sup>	0.815 <sup>a</sup>	0.834 <sup>a</sup>	0.011	<0.001

- <sup>1</sup> Least square means based on 9 pig observations per diet.
- <sup>2</sup> SEM = Standard error of the mean.
- <sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).
- <sup>A-B</sup> Within a row, means without a common superscript tended to differ ( $P < 0.10$ ).

**Table 4.5** Coefficients of standardised ileal digestibility (CSID<sup>1</sup>) of crude protein and amino acids of raw, steam-pelleted or extruded lentil<sup>2</sup>.

Variable	Lentil			SEM <sup>3</sup>	P-value
	Raw	Steam-pelleted	Extruded		
Crude protein	0.803 <sup>b</sup>	0.868 <sup>a</sup>	0.875 <sup>a</sup>	0.012	<0.001
Indispensable amino acids					
Arginine	0.862 <sup>b</sup>	0.925 <sup>a</sup>	0.928 <sup>a</sup>	0.011	<0.001
Histidine	0.797 <sup>b</sup>	0.862 <sup>a</sup>	0.871 <sup>a</sup>	0.011	<0.001
Isoleucine	0.778 <sup>b</sup>	0.856 <sup>a</sup>	0.876 <sup>a</sup>	0.012	<0.001
Leucine	0.790 <sup>b</sup>	0.866 <sup>a</sup>	0.883 <sup>a</sup>	0.011	<0.001
Lysine	0.806 <sup>b</sup>	0.872 <sup>a</sup>	0.878 <sup>a</sup>	0.012	<0.001
Methionine	0.756 <sup>b</sup>	0.848 <sup>a</sup>	0.855 <sup>a</sup>	0.017	<0.001
Phenylalanine	0.787 <sup>c</sup>	0.858 <sup>b</sup>	0.890 <sup>a</sup>	0.011	<0.001
Threonine	0.790 <sup>b</sup>	0.854 <sup>a</sup>	0.879 <sup>a</sup>	0.014	<0.001
Tryptophan	0.864 <sup>b</sup>	0.924 <sup>a</sup>	0.951 <sup>a</sup>	0.011	<0.001
Valine	0.773 <sup>b</sup>	0.848 <sup>a</sup>	0.870 <sup>a</sup>	0.012	<0.001
Dispensable amino acids					
Alanine	0.775 <sup>b</sup>	0.857 <sup>a</sup>	0.879 <sup>a</sup>	0.013	<0.001
Aspartic acid	0.813 <sup>b</sup>	0.874 <sup>a</sup>	0.882 <sup>a</sup>	0.011	<0.001
Cysteine	0.717 <sup>b</sup>	0.802 <sup>a</sup>	0.763 <sup>ab</sup>	0.024	0.014
Glutamic acid	0.822 <sup>B</sup>	0.862 <sup>A</sup>	0.853 <sup>AB</sup>	0.016	0.061
Glycine	0.792 <sup>b</sup>	0.867 <sup>a</sup>	0.889 <sup>a</sup>	0.016	<0.001
Serine	0.806 <sup>b</sup>	0.876 <sup>a</sup>	0.898 <sup>a</sup>	0.013	<0.001

Tyrosine	0.802 <sup>b</sup>	0.858 <sup>a</sup>	0.883 <sup>a</sup>	0.011	<0.001
Total amino acids	0.820 <sup>b</sup>	0.885 <sup>a</sup>	0.900 <sup>a</sup>	0.011	<0.001

<sup>1</sup> The CSID for crude protein and amino acids were calculated by correcting the CAID for measured basal endogenous losses (g/kg dry matter intake): crude protein, 18.96; arginine, 0.80; histidine, 0.20; isoleucine, 0.32; leucine, 0.52; lysine, 0.05; methionine, 0.07; phenylalanine, 0.33; threonine, 0.63; tryptophan, 0.10; valine, 0.41; alanine, 0.07; aspartic acid, 0.77; cysteine, 0.26; glutamic acid, 0.91; glycine, 1.69; serine, 0.54 and tyrosine, 0.24.

<sup>2</sup> Least square means based on 9 pig observations per diet.

<sup>3</sup> SEM = Standard error of the mean.

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>A-B</sup> Within a row, means without a common superscript tended to differ ( $P < 0.10$ ).

**Table 4.6** Standardised ileal digestible (SID) content of amino acids of lentil (g/kg; standardised to 100 g moisture/kg)<sup>1</sup>.

Variable	Lentil			SEM <sup>2</sup>	P-value
	Raw	Steam-pelleted	Extruded		
Crude protein	198.0 <sup>b</sup>	211.2 <sup>a</sup>	213.4 <sup>a</sup>	2.95	<0.001
Indispensable amino acids					
Arginine	15.4 <sup>b</sup>	16.1 <sup>a</sup>	16.4 <sup>a</sup>	0.19	<0.001
Histidine	4.7 <sup>b</sup>	5.0 <sup>a</sup>	5.1 <sup>a</sup>	0.07	<0.001
Isoleucine	8.5 <sup>b</sup>	9.2 <sup>a</sup>	9.4 <sup>a</sup>	0.13	<0.001
Leucine	13.9 <sup>b</sup>	15.2 <sup>a</sup>	15.3 <sup>a</sup>	0.20	<0.001
Lysine	13.7 <sup>b</sup>	14.2 <sup>ab</sup>	14.5 <sup>a</sup>	0.20	0.005
Methionine	1.4 <sup>c</sup>	1.7 <sup>b</sup>	1.5 <sup>a</sup>	0.03	<0.001
Phenylalanine	9.6 <sup>b</sup>	10.4 <sup>a</sup>	10.7 <sup>a</sup>	0.13	<0.001
Threonine	6.9 <sup>b</sup>	7.3 <sup>a</sup>	7.5 <sup>a</sup>	0.12	0.001
Tryptophan	1.2 <sup>c</sup>	1.3 <sup>b</sup>	1.5 <sup>a</sup>	0.02	<0.001
Valine	9.3 <sup>b</sup>	10.1 <sup>a</sup>	10.3 <sup>a</sup>	0.15	<0.001
Dispensable amino acids					
Alanine	7.8 <sup>b</sup>	8.6 <sup>a</sup>	8.8 <sup>a</sup>	0.13	<0.001
Aspartic acid	21.7 <sup>b</sup>	22.3 <sup>b</sup>	23.4 <sup>a</sup>	0.29	<0.001
Cysteine	1.9 <sup>b</sup>	2.2 <sup>a</sup>	2.0 <sup>b</sup>	0.06	0.002
Glutamic acid	31.0 <sup>b</sup>	33.4 <sup>a</sup>	31.9 <sup>ab</sup>	0.60	0.006
Glycine	7.8 <sup>b</sup>	8.7 <sup>a</sup>	8.7 <sup>a</sup>	0.15	<0.001
Serine	7.8 <sup>b</sup>	8.4 <sup>a</sup>	8.6 <sup>a</sup>	0.13	<0.001



Tyrosine	5.8 <sup>b</sup>	6.2 <sup>a</sup>	6.3 <sup>a</sup>	0.08	<0.001
Total amino acids	181.5 <sup>b</sup>	195.0 <sup>a</sup>	196.3 <sup>a</sup>	2.42	<0.001

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<sup>1</sup> Least square means based on 9 pig observations per diet.

<sup>2</sup> SEM = Standard error of the mean.

<sup>a-c</sup> Within a row, means without a common superscript differ ( $P < 0.05$ ).

## **Chapter 5. General Discussion**

### **5.1 Main findings and conclusions**

Lentil and field pea grains are leguminous alternative starch and AA sources in swine diets. A large portion of the carbohydrates in pulse grains are slow-digestible starches that contribute to a shift in hindgut fermentation and lower glycemic index of the feed (Fledderus, 2004; Berrios et al., 2010). Though lower in sulfur-containing AA, lentil and field pea grains provide a good source of AA to the diet at a generally lower cost than SBM. In addition, the cultivation of pulse crops has important benefits for crop rotation to mitigate spread of crop disease and pest cycles, and nitrogen fixation to enhance soil quality and increase root rhizobia. These factors make lentil and field pea grains desirable feedstuffs for swine; however, acceptable inclusion rate of pulse grains in swine diets have been limited due to the presence of ANF, particularly for younger pigs (Jezierny et al., 2010). Trypsin inhibitors are particularly of concern due to their capability to bind, inhibit and inactivate trypsin, an enzyme that digests dietary protein (Hefnawy, 2011). Heat processing of pulse grains may ameliorate the negative effects of TIA and can therefore increase protein digestibility (Hefnawy, 2011). Heat processing may increase digestibility of field pea, increase starch gelatinization, and alter dietary fibre to increase the soluble fibre fraction (Singh et al., 2007; Stein and Bohlke, 2007; de Vries et al., 2012). We hypothesized that heat processing would increase nutrient digestibility of lentil and field pea grain in either weaned or growing-finishing pigs without affecting growth performance indicators.

In Chapter 2, data revealed that heat processing of field pea grain did not affect nursery pig growth performance or diet CATTD of field pea nutrients when fed 400 g field pea grain/kg diet. Although extrusion of field pea may increase pig performance, reports also exist of extruded field

pea not increasing pig performance (Myer and Froseth, 1993; Stein et al., 2010). Extrusion of field pea grain did decrease TIA, though energy and nutrient digestibility did not increase. Chapter 2 did not reveal decreased ADG for pigs fed a high dietary inclusion of raw or heat-processed field pea. The ADFI increased in pigs fed field pea, whether raw or heat-processed, reflecting non-isocaloric diet formulating; however, the increased ADFI also signified the absence of negative effects of TIA on feed intake, though TIA values were obtained only from a single field pea sample of unknown cultivar origin. Regardless, inclusion of field pea in nursery diets without reducing growth may be greater than previously thought but might not be optimal.

The hypothesis that feeding 400 g field pea/kg would not affect growth performance of nursery pigs was rejected. Diets were formulated to equal predicted NE value to that of the SBM control diet; however equal diet NE value was not achieved. Therefore, our hypothesis was not properly tested. Feeding raw or heat-processed field pea grain moderately increased feed intake, though impact of caloric deficit in that study cannot be ruled out. Heat processing was not required for equivalent growth in nursery pigs fed 400 g field pea grain/kg diet and implied that field pea is an alternative for starch and AA to maintain growth of young pigs.

Chapter 3 investigated the nutrient digestibility of heat-treated field pea grain in weaned pigs and found benefits to thermal processing. This digestibility study confirmed that cold-pelleting increased the energy digestibility of field pea and thereby field pea grain DE and NE values. The CATTD and CAID of DM and GE, as well as CAID of starch increased with cold-pelleting; however, CP and AA digestibility did not differ from that of raw field pea grain. Both cold-pelleting and extrusion of field pea grain reduced TIA, yet only extrusion increased ileal digestibility of some AA. As steam-pelleting affected neither TIA nor AA digestibility of field pea grain, the effects of heat processing on field pea protein digestibility in weaned pigs remain

inconclusive. It is also not clear why increased energy digestibility was observed in the low temperature processing method, but not in the medium and high temperature treatments. Consequently, we accept our hypothesis that heat processing would alter the energy and AA digestibility of field pea grain in weaned pigs, although some effects remain unclear.

Digestibility of nutrients was less in raw lentil grain than in steam-pelleted or extruded lentil grain (Chapter 4). The CATTD of GE, DM and CP and calculated NE value were greater in steam-pelleted and extruded than in raw lentil grain fed to growing-finishing pigs. The CAID and CSID of CP and all AA except glutamic acid were greater in steam-pelleted and extruded lentil grain, with greater increases in extruded lentil. Steam-pelleting, however, did not decrease TIA content. Implications are that heat-processed lentil is a more nutritious alternative feedstuff than raw lentil, but the high cost of extrusion may limit extruded lentil as a viable option over steam-pelleting to increase energy and AA digestibility. We therefore accept the thesis hypothesis that heat-processing would decrease TIA and increase the digestibility of energy, CP, and AA in lentil fed to growing-finishing pigs. Of particular interest is that in Chapter 4 heat processing of lentil grain clearly increased nutrient digestibility, yet field pea, under equivalent processing in Chapter 3 did not. This observation indicated that the level of TIA in field pea was insufficient to produce noticeable adverse effects on nutrient and energy digestibility in young pigs. However, differences in pig age between Chapter 3 and Chapter 4 might be another reason.

## **5.2 Limitations**

Overall, the studies were well designed and were conducted thoroughly to investigate how heat processing, whether cold-pelleted, steam-pelleted (of field pea only) or extrusion, of lentil or field

pea grain could affect energy digestibility, CP and AA digestibility, or growth performance (in the case of field pea) in swine. However, some difficulties and constraints did exist.

For all experiments, ingredient moisture content proved an important factor to address. In Chapters 2, 3 and 4, it was clear that moisture content needed to be addressed and mitigated to ensure that statistical analyses were detecting true variation from heat processing. This was done so by standardizing diet and ingredient values to 100 g moisture/kg prior to data analysis. Consequences of failing to do so were an increased prevalence in both type 1 and type 2 errors from dilution factors between diets. Ingredient instead of complete feed processing is a new concept in swine nutrition, and as such, addressing moisture along with other nutritional variations that arise from heat processing during formulation and data analysis became evident. In swine feed, it is uncommon to use ingredients that have a large moisture content difference between them because most ingredients are air-dried; therefore, variation due to moisture is low and not always considered. In the present experiments where moisture variation was evident, drawing from experiences from other industries that utilize ingredients with large moisture differences like pet food would be beneficial to learn how best to approach feed formulation. Pet food commonly uses fresh or raw animal meat that is high in moisture in combination with dry animal proteins and plant material. As such, pet food nutrition has adapted to account for this difference to ensure accurate formulation and testing.

In Chapter 2, analyses of the test ingredients prior to diet formulation were not performed. When formulating the diets, DE value, ADF, starch, CP, and EE were based on NRC (2012) and the equation by Noblet et al. (1994) was used to estimate formulating for equal NE values among test diets. Variation therefore existed between anticipated nutrient value and measured nutrient values. As a result, field pea diets had a lower measured predicted NE value than the SBM control

diet when NRC (2012) values were substituted for measured nutrient values obtained from lab analysis after the study was performed. Differences in feed intake were observed among field pea diets and compared with SBM control, a factor we attributed to the lower NE value of the diet. As diets were not isocaloric, it is likely pigs ate to meet energy requirements. Performing the digestibility trial on field pea (Chapter 3) prior to the growth performance could have been beneficial. Having nutrient content and digestibility information would have been better to formulate isocaloric diets.

Chapters 3 and 4 utilized ileal cannulation method to sample digesta to measure nutrient digestibility. To calculate basal endogenous losses in the animals, a N-free diet was fed as a single period at either the beginning of the study (Chapter 3), or at the end of the study (Chapter 4). Calculations to determine basal endogenous losses were from Stein et al., (2007) and included the concentration of  $\text{Cr}_2\text{O}_3$  in feed and digesta from the terminal ileum. However, limitations of this method include: placing the pigs under an AA deficit, assuming that basal ileal endogenous losses depend only on DM intake, and that feeding a N-free diet for a few days removes variation in endogenous losses as animal age, grow, or eat a diet with different feed composition (Adeola et al., 2016). Though feeding a N-free diet is a common practice, feeding a diet void of protein and AA might be a poor method to use in pigs immediately after weaning.

Chapter 4 was performed using growing-finishing pigs and Chapter 3 involved weaned pigs. After weaning, pigs transition from a liquid to solid diet. In addition, weaned pigs are rapidly growing muscle mass. Consequently, young pigs have a high requirement for dietary AA to support their fast growth rate; thus, feeding N-free diets might provide too severe of an AA deficit for young pigs. The weaned pigs in Chapter 3 were lighter than pigs used in Chapter 4. Light pigs caused difficulty during digesta and faeces collection because of the limited sample quantity that

we were able to collect, particularly with the N-free diet that is largely formulated using highly-digestible cornstarch. Pigs were feed restricted based on metabolic BW and thus low feed intake further reduced digesta and fecal output. Consequently, we were limited in the number of analyses and duplicates that we could perform on the samples. Increasing feed intake may mitigate these limitations but feed intake is also limited by stomach capacity of weaned pigs.

Throughout all experiments, cultivar of field pea and lentil grain samples was not known. Sourcing ingredient from a commercial supplier is beneficial for being representative of the type of ingredient swine producers may source for their feed; however, we lose important identifiers and information on the samples that are important for research. Commercial field pea and lentil are often pooled from multiple producers, which means we lose information such as location of production, agronomic conditions, and most importantly, cultivar. Even though pulse grain cultivars are generally not developed for nutritional traits, cultivar does have an effect and can may impact nutritional composition. Chemical composition of core nutrients (protein, fat, ash) varies in field pea depending on cultivar and whether or not field pea was wrinkled or smooth (Kosson et al., 1994). In regard to ANF, trypsin inhibitor content of peas depends on the cultivar of seed (Valdebouze et al., 1980; Deo, 1987, Johns, 1987). Similarly, lentil has variations in nutrient and major variation in ANF content with cultivar (Savage, 1988; Ciurescu et al., 2018) However, we referred to ingredients only by their higher classification or scientific name (*Pisum sativum* and *Lens culinaris*). Throughout Western Canada there are a plethora of field pea and lentil cultivars. As such, the absence of this information limited detailed knowledge base on specific cultivars that we could apply to our studies.

The use of a commercial lab for ingredient and diet analysis was another limitation. The third party commercial lab used for all 3 experiments used older methods for nutrient analysis,

particularly for fibre and starch. Consequently, results were not as accurate due to the outdated methods. It was observed in the studies that insoluble fibre decreased; however, we did not necessarily see a subsequent increase in soluble fibre as expected. Starch content of field pea was much lower than reported ranges (Hall et al., 2017). The method used (AOAC 965.29), or the so called Porsky method, does not capture a large number of short-chain fibre components. Another major shortcoming of the Porsky method is that most of resistant starch categories are excluded. If the sample does not contain any low molecular weight dietary fibre (such as inulin, fructose oligosaccharides, or resistant maltodextrin), this method may suffice. A more suitable method for analysis in chapter 2, 3, and 4 for dietary fibre may be the more recent AOAC 2011.25.

### **5.3 Future research**

Our studies aided in expanding knowledge of pulse crops as a feedstuff for swine and broadened the data base on the digestibility of both raw and heat-processed field pea or lentil grain. Future studies should address knowledge gaps remaining from the present studies. Firstly, addressing the existing unanswered conclusions by replicating Chapter 1 could be beneficial in determining the effect of heat processing field pea grain on nutrient digestibility and growth performance in weaned pigs. Addressing issues with the NE value of field pea when feeding weaned pigs in a herd or growth performance setting can help address this knowledge gap and better evaluate how weaned pigs react to this feedstuff. In addition, replicating a study to better determine increases in nutrient digestibility, or lack thereof, in heat-processed field pea grain would increase our understanding of field pea digestibility in swine.



Studies that further investigate ingredient processing and cultivar are also necessary. Unknown cultivar was a major limitation in the experiments despite cultivar having a large effect on nutritional composition and ANF concentration. Comparing heat processing among cultivars would expand knowledge on these ingredients and could provide insight into differences observed in chapters 2 and 3 compared to previously-published research.

Further studies to build on the key findings of our research is another area for further research. As confirmed in Chapter 4, heat processing can increase digestibility of CP and energy; however, how this knowledge may cost-effectively translate into a herd setting is not clear. It is not known whether the increased digestibility of nutrients in steam-pelleted and extruded lentil would translate into cost-savings benefit for the producer. In addition, processing variables such as specific extruder settings may greatly affect nutrient digestibility of lentil. Implementing a cold-pelleting process or adjusting single-screw extruder conditions such as screw speed, or pre-conditioner and barrel temperature or moisture settings can be used to further define the limits of ingredient processing.

The relation between animal and feed can be explored further. Clear benefits of heat-processed lentil were observed in older growing-finishing pigs; however, younger weaned pigs fed field pea did not respond similarly. Young pigs have a developing gut morphology throughout the transition to solid food post-weaning (Pluske et al., 2018). Feeding ANF to piglets following weaning may cause atrophied intestinal villi thereby decreasing growth performance, though heat-processing to remove these ANF might alleviate the problem (Mekbungwan and Yamauchi, 2004). In addition, young pigs have underdeveloped microbiome and may have difficulty digesting high fiber diets, yet fiber is considered essential for proper gastrointestinal tract development (Montagne et al., 2003; Agyekum and Nyachoti, 2017). Comparing ANF levels among lentil or field pea cultivars

or comparing pulse grain fibres to other high-fibre ingredients such as dried distiller grain with solubles is an area for further study. In addition to low ANF content in heat-processed lentil, further benefits of heat-processed lentil fed to newly weaned pigs are not well known, such as increased microbial gut diversity or intestinal villi growth when added to weaned pig diets in moderate proportions.

Lastly, heat-processing of ingredients is still a new topic. Although feeding co-products of human food processing to pigs is not uncommon, processing of ingredients specifically for inclusion in swine feed is underdeveloped. There are multiple ingredients for swine feed that are processed, but most often because processed ingredients are a co-product of another industry such as biofuel or food, or because the processing method is cheap, easy, and has benefits such as increasing density for transport that make processing worthwhile. It is not known if ingredient processing of feedstuff that is not normally processed prior to formulation would translate to equivalent benefits as whole mixed feed that is heat-processed after mixing. Comparisons between an extruded ingredient and an extruded diet can be determined for efficacy of observed benefits. Similar to co-products, processing of ingredients may find a niche in the swine feed industry as an untapped resource for low digestibility, high ANF ingredients.

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