Nutritional Value of Faba Bean Cultivars as Dietary Source of Energy and Protein for Weaned and Growing Pigs

by

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A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science

in

Animal Science

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ABSTRACT

Novel faba bean cultivars vary in nutrient content, digestibility and antinutritional factors that must be characterized to prioritize which faba bean cultivars to grow for pigs feeding. In chapter 2, five diets included (20%, phase 1; 30%, phase 2) 3 different, zero-tannin and high vicine/covicine faba bean cultivars (Snowbird, Snowdrop and Tabasco) and 2 medium tannin cultivars (Fabelle, low vicine/covicine; Malik, high vicine/covicine). Diets were fed to weaned pigs to investigate their effect on nutrient digestibility and growth performance. Fabelle contained the most condensed tannins (**CT**; 0.53%) but the least vicine (0.04%) and covicine (0.0 1%). Zero-tannin cultivars contained little CT (< 0.2%) but had the greatest vicine (averaged 0.5%) and covicine content (0.4%). For phase 1, energy and nutrient digestibility and growth performance did not differ among cultivar diets. For phase 2, diet apparent total digestibility (**ATTD**) of energy and CP was greatest ($P < 0.05$) for Snowdrop and Tabasco, and lowest in Malik and intermediate for Snowbird or Fabelle. Diet net energy (**NE**) values was greater ($P < 0.05$) for Tabasco than Snowbird, Fabelle and Malik and intermediate for Snowdrop. For the entire trial (day 0 to 28), the average daily feed disappearance (**ADFD**) was 10% greater ($P < 0.05$) in pigs fed Fabelle than Malik and intermediate for pigs fed Snowbird, Snowdrop or Tabasco. Consequently, pigs fed Fabelle diet had the greatest ($P < 0.05$) average daily gain (**ADG**) and final body weight (**BW**). In Chapter 3, two zero-tannin (Snowbird and Snowdrop) and 2 mid-tannin (Fabelle and Florent, high vicine/covicine) cultivars were included in 4 diets at 95%. Diets were fed to 8 ileal-cannulated barrows (37.5 kg) for four 9-day periods in a double 4 × 4 Latin square. A nitrogen-free diet was also fed to correct for basal endogenous losses of protein and amino acids. Analysed tannins content was double in mid-tannin cultivars than zero-tannin cultivars (14 vs. 6.9 g/kg). Vicine/covicine was lowest in Fabelle (0.6 g/kg vicine; 0.4 g/kg covicine) but greatest in Snowbird, Snowdrop and Florent (5.5 to 7.0 g/kg vicine; 3.4 g/kg covicine). Apparent total tract digestibility of dry matter and gross energy was greatest ($P < 0.05$) for pigs fed Snowbird, intermediate for Snowdrop and Fabelle, and lowest for Florent. Apparent ileal digestibility (**AID**) of starch was greater ($P < 0.05$) in zero- than mid-tannin cultivars. Ingredient NE value was greatest in pigs fed Snowbird and did not differ among Snowdrop and Fabelle or Florent. The standardised ileal digestibility coefficient **(CSID**) of crude protein was greater in Fabelle, Snowbird, Snowdrop than Florent, with similar differences for lysine and most essential amino acids.

In conclusion, Fabelle had greater growth performance than Malik in weaned pigs and greater energy, protein, and AA digestibility than Florent in growing pigs, likely associated with its lowest vicine and covicine. Furthermore, combined mid-tannin and, vicine and covicine possibly contributed to both lower energy and nutrient digestibility in pigs fed Malik and Florent than zero-tannin cultivars and should also be considered in prioritizing modern faba bean cultivars to feed to pigs. Across cultivars, variation in dietary total and resistant starch, crude protein and fibre contributed to difference in energy, protein and amino acid digestibility and should be considered in diet formulation.

PREFACE

This MSc thesis was written in manuscript format and is composed of a literature review, manuscripts 1 and 2 and a general discussion in four chapters. Chapters were written according to guidelines for Animal Feed Science and Technology except for Chapter 2 that was written according to guidelines for Translational Animal Science. Animal use in Chapters 2 and 3 was approved and procedures reviewed by the University of Alberta Animal Care and Use Committee for Livestock.

Manuscript 1 in Chapter 2 has been submitted for peer review to Translational Animal Science as Protus W. Nyende, Li Fang Wang, Ruurd T. Zijlstra, and Eduardo Beltranena, "Effect of feeding mid- or zero-tannin faba beans differing in vicine and covicine on nutrient digestibility and growth performance of weaned pigs". Protus. W. Nyende was responsible for investigation, processing, partial chemical analysis, and writing the original draft. Li Fang Wang performed statistical analysis, validation, data curation, manuscript review and editing. Ruurd T. Zijlstra was responsible for funding acquisition, project administration, conceptualization, manuscript review and editing, Eduardo Beltranena was responsible for resources acquisition, conceptualization, methodology, validation, visualization, manuscript review and editing.

Manuscript 2 in Chapter 3 is under preparation and is a collaborative work by the above-mentioned authors for manuscript 1 with similar contributions.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my immediate supervisor, Dr. Ruurd T. Zijlstra for giving me this opportunity and his trust in me throughout my MSc programme. His concerns, many insightful conversations, unwavering patience, and friendship that have given me the best professional and learning environment during this period are greatly appreciated. Many thanks to my committee members Dr. Eduardo Beltranena and Dr. Thava Vasanthan for their expertise, fruitful discussions, and suggestions during our meetings. Special thank you to Dr. Li Fang Wang for his assistance in statistical analyses.

Technical assistance I received from Zijlstra lab group members, especially Miladel Casano during laboratory analyses and graduate students: Felina, Joaquin, Janine, Whitney and Lingjun during the animal experiment is highly appreciated. Thank you to the former and present members of the Swine Research and Technology Centre for their support during the animal experiment.

Special thanks also extended to Swine Innovation Pork (Quebec City, QC, Canada) and Alberta Pork (Edmonton, AB, Canada) for funding these research projects, Brad Goudy from Faba Canada (Melfort, Saskatchewan, Canada) for helping identify and source the faba bean cultivars. Tom Thompson and Johan van den Heever from Organic Residue Laboratory, Alberta Agriculture and Forestry for conducting the vicine and convicine analysis.

Finally, I would like to thank my family members, including my mom Scholastic Olumi, my brothers: Josephat, Chrispinus and sister, Imelda. Your mental support, motivation, and love had no bounds and kept me going.

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AA	Amino acid
ADF	Acid detergent fibre
ADFD	Average daily feed disappearance
ADG	Average daily gain
ANF	Anti-nutritional factor
ATTD	Apparent total tract digestibility
BW	Body weight
CAID	Apparent ileal digestibility coefficient
CATTD	Apparent total tract digestibility coefficient
CP	Crude protein
CSID	Standardised ileal digestibility coefficient
CT	Condensed tannins
d	Day
DE	Digestible energy
DM	Dry matter
GE	Gross energy
G:F	Feed efficiency (ADG/ADFD)
Lys	Lysine
NDF	Neutral detergent fibre
NE	Net energy
RS	Resistant starch
NSP	Non-starch polysaccharides

SBM	Soybean meal
SID	Standardised ileal digestibility
TIA	Trypsin inhibitor activity

Chapter 1. Faba bean in swine nutrition: A review

1.1 Introduction.

Feed is the single largest variable cost affecting the profitability of pork production (Niemi et al., 2010). Primary feed cost components are starch as main source of dietary energy and protein as source of amino acids. Worldwide cereal grains are the main source of starch and soybean meal is the main source of protein in swine diets. Soybean meal (SBM) is the major co-product of solvent oil extraction from soybeans. Pricing of other protein commodities on the world market is strongly correlated with the cost and availability of SBM. World market price for SBM is largely dependent on factors such as variation in population and economic growth, changes in consumer's product preferences, weather conditions and agricultural commodity trading (Gill, 1997; Trostle, 2008). The price of SBM and availability on global market may thus change rapidly. Such rapid changes in SBM markets hence stimulate the swine industry to make use of locally produced feedstuffs such as legume grains as an alternative source of starch and protein. Grain legumes have been identified as alternative to cereal grains. Field pea and faba bean have a high starch content whereas lupin has a greater lipid content (Gatel, 1994; Bach Knudsen, 1997; Salgado et al., 2002). Field pea (*Pisum sativum*), faba bean (*Vicia faba* L.) and lupin (*Lupinus* spp.) are the major grain legumes cultivated in Europe and Canada (except lupin), whereas soybean dominates in Argentina, Brazil, China, India, and the United States (Karr-Lilienthal et al., 2004).

Corn and soybean are the major crop grains fed as main sources of dietary starch and protein, respectively, in the pig industry in North America. However, corn is a C_4 carbon fixation plant, in which CO_2 is fixed initially into the 4-carbon acid in the mesophyll during the photosynthetic processes, thus requires higher heat units of at least 10ºC to grow. Corn thrives growing under hot conditions, but it is not as competitive in cooler agronomic conditions as C_3 plants such as wheat, faba bean and field pea. Soybean plants differ in their tolerance to chilling (0-15ºC). Low spring and fall temperatures experienced in most parts of western Canada may affect several aspects of soybean crop growth, survival, cell division, photosynthesis, water transport, and ultimately crop yield (Hasanuzzaman et al., 2016).

Faba bean is a legume species in the Fabaceae family and the fourth most pulses grain widely grown in cooler agronomic conditions in grain-growing areas of western Canada (AAFC, 2021, Muehlbauer et al., 1997) after field pea, lentil, and chickpea. Faba bean is rich in starch (33 - 46%; Hoover and Sosulski, 1991, Ross and Davies, 1992), and crude protein (28 - 37%) and can replace imported corn and soybean meal as sources of both starch and protein in swine diets in western Canada. Moreover, growing faba bean has many benefits in crop rotation to cereal grains and oilseeds due to their high atmospheric nitrogen (N) fixation capacity (López-Bellido et al., 2003).

Coloured-flowered and white-flowered faba bean cultivars are available. However, the coloured-flowered cultivars can be desirable due to their agronomic characteristics such as tolerance to disease (Duc, 1999) and frost (Link et al., 2010) due to presence of condensed tannins. Unlike soybean or lupin, faba bean contains little oil thus are not processed for oil extraction before being used for feed. Low oil content in faba bean lowers the cost of fractionation given that starch and protein components separate readily without lipid interference as in lupin. Both low and high-tannin cultivars can be sources of starch and protein to ruminant and non-ruminant animal species (Jezierny, 2009; Sinha et al, 2018; Blair, 2007). Recently, faba bean has received increasing attention as a protein supplement in diets for pigs, particularly in Canada and Europe due to insufficient local production of protein feedstuffs (Blair, 2007).

Globally, faba bean is ranked as sixth in production among legumes after soybean, peanut, common bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum* L.), and chickpea (*Cicer* *arietinum* L.). In the 2018 - 2019 period in Canada, soybean production was the largest tonnage at 7.4 million MT, followed by 3.6 million MT of field pea, 2.1 million MT of lentil, and only 0.34 million MT of faba bean (AAFC, 2019).

A large part of pulses produced in Canada are intended for trade. Export of dry pea, lentil and chickpea accounted for almost 40% of annual production (2007 - 2011). During this period, Canada produced over one-third of the world's lentil (1.5 million tonnes) and was the largest dry pea grower producing 2.1 MT, equivalent to 21.7% of the total global production (Statistics Canada 2011). Western Canada is an increasingly important source of faba bean production in North America and supplier to the world market. Faba bean has been traded globally for many years, and even though it has been grown on the Canadian prairies for a couple of decades, it still seems to be in early days for the Western Canadian market. Egypt has been a dominant export opportunity. However, the pulse grain market is highly competitive and crowded. Traditionally, the market was evenly divided among Australia, the United Kingdom, and France, but new entrants such as Canada and Baltic countries have emerged. Recently, Canadian export volumes have been increasing with an estimated 15,000 tonnes exported in the year 2017-2018. Furthermore, requests from Egypt for Canadian white-flowered cultivars have been increasing. However, reliance on a single market outlet is risky and therefore the need to explore other potential uses of faba bean such as livestock feed.

1.2 Chemical composition of faba bean

Faba bean seed contains 51 to 68% of carbohydrates in total with starch as the major proportion (Cerning et al., 1975). The protein content of faba bean depends on cultivar (Chavan et al., 1989). In comparison with cereals, faba bean contains more lysine and arginine and can therefore complement the low levels of these amino acids in cereal grains (Kumar et al., 2014). In addition to proteins and carbohydrates, faba bean presents a good alternative source of dietary minerals, such as phosphorus, calcium, potassium, sulphur, and iron. However, the nutritional value of faba bean can be reduced by the presence of anti-nutritional factors (ANF) such as tannins, , vicine and covicine, trypsin inhibitors, and phytic acid (Carnovale et al., 2000).

1.2.1 Starch

Starch is the largest carbohydrate component in legumes. Faba bean and field pea have a high starch content with a range of 42.2 – 45.1% and 47.8 – 53.4 g/kg DM, respectively (DLG, 1999; Jezierny, 2009), whereas lupin has comparatively a low level of starch of 4.2 – 10.1% (DLG, 1999; Jezierny, 2009). However, it should be noted that determination and profiling of starch in legume grains may be confounded by the analytical method used (Hall et al., 2000). Amylose and amylopectin are the two main starch components that determine the functionality and digestibility of starch (Jane, 2006). In comparison with cereals, legume seed starch has a greater amylose concentration varying from 10.2% in Great Northern beans to 65.8% in wrinkled field peas (Reddy et al, 19984). The gelatinization temperature of various legume starches generally ranges from 60^0C to 90^0C, which is comparable to the gelatinization temperature of corn starch but greater than that of waxy maize starch. Gelatinization temperature is determined by the structure and composition of starch as a substantial amount of amylopectin promotes gelatinization whereas the extent of amylopectin branching in the starch affects gelatinization temperature. Other factors such as presence of bound lipids, protein, and phosphate content, and starch granule size may also affect gelatinization temperature.

Faba bean starch is made up of 96.6% isolated starch, 40.0% amylose, 0.3% protein, 0.4% fat, and 0.1% ash, (Table 1.2). The amylopectin of isolated faba bean starch is made up of smaller proportion (21.5%) of DP 6 - 12 short branch chain but a larger proportion of 56.0% of DP 13 - 24 branched chain than those of maize starches (27.0 to 34.3%) (Li et al., 2019). Morphological characteristics of faba bean starch examined by Sofi et al., (2013) using scanning electron microscope indicated that starch granules are oval, round, elliptical, and irregular in shape and possess cavity on starch surface. As for other pulses starch, faba bean starch granules have smooth surface. However, scratches and damaged starch granules can be observed in faba bean starch isolated by air classification indicating damages caused by pin milling (Li et al., 2019; Pelgrom et al., 2013) can be observed on the surface of some granules. The length and width of starch granules range from 11 – 48 μm and 9 – 24 μm, respectively (Sofi et al., 2013). The solubility of starch in faba bean is as low as 9.92% at 90°C due to the integration of starch granules where restricted solubility and swelling power are present (Zhang et al., 2019). The swelling power is 12.67 g/g in faba bean starch (Zhang et al., 2019). Swelling power can be used to determine binding forces by measuring the ability of starch hydration under specific temperature and water content. The greater the swelling power the weaker the binding forces (Hoover and Manuel, 1996).

1.2.2 Protein and amino acids

Most of the protein in legume grains is in the cotyledon and embryonic axis with the seed coat containing little protein (Singh et al.,1968). Classification of protein in legume seeds is based on functionality, structural, and storage proteins. Structural proteins are made up of protease inhibitors, lectins, lipoxygenases, and amylase inhibitors found in the cotyledon and are primarily responsible for cell metabolism (Duranti and Gius, 1997). Legume proteins are known to present high resistance to proteolysis in the gastrointestinal tract due to their structural stability. Structural stability in legume protein has been reported to affect both in vivo digestibility and availability of essential amino acids. In addition, structural traits of legumes proteins are highly associated with allergenicity and toxicity (Smits et al., 2021). However, the beneficial effect of proteins and peptides from legume seeds can be exploited by carefully considering the adverse effects and defining their amino acid profile, digestibility, and bioavailability. Legume proteins are rich in lysine but low in sulphur-containing amino acid such as methionine. Tryptophan is the second most limiting amino acid in legume proteins, but it is the most limiting amino acid in cowpeas, lentil, and green peas (Iqbal et al., 2006).

Protein in faba bean ranges from 20 to 41% of DM. Even though sulphur-containing AA (cysteine and methionine) are deficient, there is an opportunity to overcome this deficiency by complementing diets with other proteins that their sulphur-containing AA that are not a limiting factor (Vincenzo et al., 2006). Faba bean accumulates large amounts of proteins during seed development (Chavan et al., 1989; Duranti, 2006). Proteins in faba bean seed are mainly stored in the form of globulins (comprise 69 to 78% of total seed protein) found in membrane-bound organelles called protein bodies (Duranti and Gius, 1997). As defined by analytical centrifugation (Danielsson, 1949), globulins consist of 2 high-molecular-weight proteins: legumin and vicilin, respectively known as 11S and 7S. Faba bean proteins contain legumin and vicilin as major protein portion followed by albumins, prolamins, and glutelins (Nikokyris and Kandylis, 1997). Globulins are generally rich in aspartic and glutamic acid, leucine, and arginine (El Fiel et al., 2002), although the AA sequence and general structure may vary. Besides globulins, faba bean, as for other legumes contains proteins such as albumins that are relevant to the nutritional quality of the seed. Albumins are biologically active and water-soluble proteins with a higher amount of methionine and cysteine as compared with globulins (El Fiel et al., 2002). Prolamins are alcohol-soluble proteins and contain large amount of proline and glutamine but only small amount of lysine and histidine. In comparison with cereals, faba bean seeds have high lysine (19.8 g/kg DM) and low methionine, cysteine, and tryptophan content (2.6, 3.7 and 2.7 g/kg DM respectively; Mosse, 1990, Duc et al., 1999). In practice, mixtures of faba bean with other protein sources in diets allow to meet animal protein requirements.

1.2.3 Non-starch polysaccharide and dietary fibre

The content of non-starch polysaccharides (NSP) in faba bean ranges from 173 to 181 g/kg DM and it contain about 55% glucose (Gdala and Buraczewska, 1997). This high level of glucose residues indicates that cellulose is a major component (43%) of faba bean NSP. Uronic acids, arabinose and galactose have intermediate values, whereas mannose, rhamnose and fructose are minor sugar residues. Considering average proportions of faba bean seed constituent, hulls constitute about 130 g/kg of whole bean with dietary fibre (910 g/kg degree of polymerization (DP) containing 85% NSP and 15% lignin extracted by Klason method (Daveby and Aman, 1993). Water-soluble fraction of cotyledon NSP constitute arabinose, galactose, galacturonic acid, and glucose as major sugars whereas rhamnose, mannose and xylose occur in minor amount (Brillouet and Carre, 1983). Pectic substances represent about 55% of cell walls as determined by colorimetric analysis of acidic and neutral sugars (Aspinall, 1970).

Legume seeds are excellent sources of dietary fibre. Fibre content in legume seeds ranges from about 1.2% to 25.6% (Table 1.3). Total fibre content and composition may vary greatly in the same type of legume seed. Fibre in legume seed contains significant levels of cellulose, hemicellulose, and lignin, along with minor amounts of pectic substance, arabinogalactan, and xyloglucan (Reddy et al.,1984.

The crude fibre content in faba bean ranges from 5.0 to 8.5% (Chavan et al., 1989). Dietary fibre content ranges from 15-30%, which seem to depend on cultivar, and hemicellulose seems to be the major component (60%; Pritchard et al., 1973; Hove et al., 1978). Compared with other ingredients commonly fed as source of dietary energy and protein in western Canada, faba bean contains relatively more crude fibre than soybean meal and barley grain, but less than canola meal (Table 1). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) content in faba bean varies among cultivars and ranges from 57 to 203 g/kg and 48 to 137 g/kg, respectively (Gdala et al 1997, Ivarsson et al.,2018; Garrido et al., 1991; Duc et al.,1999).

1.2.4 Fat

Legume seed oils are generally good sources of polyunsaturated fatty acids, especially the essential fatty acids omega-6, linoleic acid and omega-3, linolenic acid. However, fat content in legumes appears to be inversely related to carbohydrate content. Legumes such as faba bean and field pea with high carbohydrates have low fat contents as compared with soybean seed which has a high fat content (17 to 25%) but relatively low carbohydrate content (USDA, 2008). The fatty acid composition in faba bean is characterized by a relatively high proportion of unsaturated fatty acids (21.6 – 23.4% of saturated fatty acids, 18 – 18.4% of monounsaturated fatty acids, and 31.9–35.1% of polyunsaturated fatty acids; Grela and Günter, 1995), especially linoleic acid. Faba bean can provide greater amount (88.6%) of essential polyunsaturated fatty acids (Duke, 1981). Fatty acid distribution patterns seemed to be similar among faba bean cultivars (Hiromi et al., 2009). Samples had high amount of total unsaturated fatty acids (consisted mainly of linolenic and oleic acids), representing 81.0–81.7% and 77.6–79.7% for total lipids and phospholipids, respectively (Hiromi et al., 2009).

1.2.5 Mineral composition (Ca and P)

The P compounds in legume seed can be classified into four groups: phytates, phosphatides, nucleic compounds, and inorganic P (Earle and Milner, 1938). Phytins, the mixed Ca, and Mg salts of myo-inositol 1,2,3,5,6-hexakis (dihydrogen phosphate), also known as phytic acid are widespread in nature. Inositol hexaphosphate is the major component of phytic acid and it accounts for 70-90% of the total phosphorus in faba bean (Sandberg and Ahderinne, 1986; Lehrfield and Morris, 1992). As for other legumes, more than 40–60% of the P present in faba bean is unavailable as phytates. Furthermore, mineral bioavailability and some enzyme activities in faba bean may be reduced by (Deshpande et al., 1984) and inhibiting the activity of several enzymes (Knuckles et al., 1989). Phytate strongly binds positively charged divalent ions, and the decreasing order of stability of mineral phytate complexes in vitro is Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{2+} (Cheryan and Rackis, 2009). The Ca content in faba bean ranges from 120 to 260 mg/100 g DM (Chavan et al., 1989). One study indicated a higher seed concentration Ca, Mn, Mg, and Cd content in low-tannin faba bean genotypes compared with tannin-containing genotypes when grown in soils of the eastern Canadian prairies (Khazaei et al., 2020).

1.2.6 Antinutritional factors

Inclusion of faba bean in animal diets might be restricted because of the presence of several ANF that may impair nutrient availability, digestibility and feed intake if included at too high level. Condensed tannins, vicine and covicine, protease inhibitors, phytic acid and haemagglutinins are the most common ANF in faba bean (Marquardt et al., 1974; Abbey et al., 1979; Griffiths, 1979). Among these, condensed tannins are mainly located in the hulls of coloured-flowering cultivars (Griffiths and Jones, 1977; Ward et al., 1977; Newton and Hill, 1983) and are the most crucial ANF in relation to nutritive value of faba bean (Griffiths, 1981; Marquardt and Bell, 1988).

1.2.6.1 Tannins

Tannins are water-soluble polyphenol compounds (molecular weight between 500 and 3000) that naturally occur in plant tissues. Tannins are chemically divided into hydrolysable and condensed tannins (Jansman, 1994). The central carbohydrate core in hydrolysable tannins have a hydroxyl group esterified to phenolic carboxylic acids such as garlic acid and hexahydroxydiphenic acid. Whereas condensed tannins are mainly polymerized products of flavan-3-ol and flavan-3, 4-diol (figure 1), or mixture of these majorly present in the testa of coloured seed of faba bean. Condensed tannins are also referred to as flavanol or procyanidins. The presence of condensed tannins in coloured-flowered faba bean cultivars indicate genetic variability for the content of condensed tannins in the testa of faba bean, which is mainly linked to genes controlling flower or grain colour available in natural populations. The pattern of tannin evolution during grain development indicates an increase content of condensed tannins (and monomers) in the seed coat over time. The degree of polymerization peaks around 30 days after flowering, followed by a decline due to a decrease in associated tannin content. The presence of high tannin content in seed at the earlier development stage provides a natural line of defence against herbivorous and insects due to their astringent flavour that reduces their appetite. However, the level of polymerization linked to genetic differences on colour pigment synthesis varies among faba bean lines (Cabrera and Martin et al 1989). Furthermore, the concentration of tannins in faba bean cultivars can vary due to factors such as relationships between tannin level and temperature, soil fertility, stage of plant maturity and analyses methods used (Berard et al 2011).

Condensed tannins range from 0.65 to 2.15% in coloured flowered cultivars (Griffiths, 1981; Cabrera and Martin, 1986) and 0.06 to 0.19% in white flowered cultivars (Bos and Jetten, 1989; Wang, 1990) of faba bean. Tannins forms complexes with proteins that may lead to coagulation or precipitation generally referred as tannin-protein complexes. The strength and degree of interaction between tannins and proteins is determined by both the nature of the tannins and that of the proteins (Hagerman and Butler, 1989). Tannins also interact with carbohydrates, particularly starch. However, their affinity seems to be less than that of proteins. The high affinity with protein is due to abundant number of phenolic groups that create many bonding points with carbonyl groups. Relatively large and hydrophobic protein molecules with open and flexible structure show more affinity for tannins. However, protein-tannin complexes are generally unstable as hydrogen bonds tend to breakdown under pH condition above 8 (Bunglavan and Dutta 2013).

1.2.6.2 Vicine and covicine

Despite many benefits associated with growing and feeding faba bean, its inclusion in human and animal diets can be limited by pyrimidine glycosides antinutritional factors such as vicine and covicine (v-c). Vicine and covicine are stored in the cotyledons of most faba bean seed at the level of about 1% of DM (Khamassi et al., 2013). Vicine and covicine are found in the embryo radicle at 10-fold more content than those in the seed coat (Griffiths and Ramsay, 1992; Burbano et al., 1995; Goyoaga et al., 2008). The presence of v-c is associated with favism, an acute haemolytic anaemia, in humans who have an X chromosome-inherited glucose-6-phosphate dehydrogenase (G6PD) deficiency (Luzzatto and Arese 2018). On ingestion of faba bean, vicine and covicine undergo hydrolysis by glucosidase present in both the beans and gastrointestinal tract releasing the respective aglycones (figure 2): divicine (2, 6-diamino-4,5-dihydroxypyrimidine) and isouramil (6-amino-2,4,5-trhydrxypyrimidine). Moreover, these compounds are highly reactive redox with antifungal and pesticide activity (Desroches, et al.,1995) that may prevent faba bean from rotting but are also capable of triggering a favism attack (Khazaei et al., 2019).

The content of vicine and covicine in faba bean ranges from 0.3–15 mg/g (Pitz et al 1980). Vicine and covicine appear to have harmful effects in monogastric animals in additional to humans with the 6GPD deficiency gene. Laying hens fed diets supplemented with faba bean extracts containing v-c demonstrated reduction in egg production and size, and increased erythrocyte hemolysis (Olaboro et al., 1981; Muduli et al., 1981; Oryschak and Beltranena, 2018) whereas no effect has been observed in pigs (Jezierny et al., 2011).

1.2.6.3 Phytic acid

About 65-85% of total P is stored in legume and cereal seeds as phytate commonly referred to as phytic acid (myo-inositol-1,2,3,4,5,6, -hexakisphosphate, IP; (Raboy, 1997). The amount of phytic acid varies from 0.50% to 1.89% in cereals and from 0.20% to 2.06% in legumes (Reddy et al 1982). Although faba bean is a dietary source of several minerals, their bioavailability may be affected by phytate. Despite the high amount of phosphorous in this legume, sufficient to satisfy the requirement of growing animals, 40-60% of total phosphorous is present as phytate (Griffiths and Thomas 1981). This phosphate-rich compound can form insoluble chelates with several trace elements and macroelements, reducing the amount of phosphorous available for absorption. Faba bean seed contain a lower phytate content of 0.23% as compared with canola meal which contain up to 0.87% (NRC 2012). However, with the increasingly use of exogeneous enzyme to increase nutrient availability for swine, phytase enzyme can be supplemented to release P from the phytin compound in the swine diets.

1.2.6.4 Protease inhibitors and lectins

Protease inhibitors are proteins with specific antitrypsin and anti-chymotrypsin activities. Trypsin inhibitor (TI) is the most common protease inhibitor in legumes. Legume TI are classified in two families according to their molecular size (Kunitz and Bowman-Birk family). There are legumes containing both, for example soybean, and there are other legumes that only have one of them, for example common bean, lentil and faba bean, where their TI are part of the Bowman Birk family. Trypsin and chymotrypsin inhibitors are present in faba bean, but their activities are low compared with other legumes seeds (Thacker, 1990; Vidal-Valverde et al., 1998). The presence of interfering substances such as tannins in the hulls of faba beans prevents an accurate study of TI distribution in the grain (Valdebouze et al., 1980).

Lectins, otherwise referred to as phytohemagglutinins, are glycoprotein compounds that in vitro agglutinate red blood cells. In vivo they can bind to receptors of epithelial cells of the intestinal mucosa and disturb the digestive process. In faba bean, lectins are mostly found in the cotyledons (Marquardt et al 1974). The binding ability of lectins with sugars of glycoproteins in red blood plays a major role in their analysis. However, red blood cells from various animal species agglutinate to a different extent with lectins present in legumes (Marquardt et al.,1974).

Generally, lectin content in faba bean is lower than in field pea and soybean (1.4 to 5.4 mg/g DM).

1.3 Energy and nutrient digestibility of faba bean in pigs

Faba bean can be readily included in the swine diets for pigs of all ages and stage of production, if diets are formulated to meet dietary energy and AA requirements. High tannins levels in diets for non-ruminant species can reduce apparent protein digestibility (Salunkhe et al., 1990). Generally, white-flowering cultivars have a more digestible profile of starch and AA due to their lower hull tannin content compared with coloured cultivars. The following section describes the nutritional value of faba bean in swine diets with particular attention to nutrient and energy digestibility including starch digestion and fibre fermentation, protein, and AA, and mineral digestibility.

1.3.1 Energy digestibility

Faba bean can be a good source of complementary dietary energy for swine. As for other pulse grains, starches and NSP are the major carbohydrates in faba bean that constitute an important energy source to swine (Bach Knudsen, 1997). The rate of starch digestibility is an important aspect of swine nutrition. Starches can also be categorized based on the rate of release of glucose into the bloodstream (glycaemic index) and its gastrointestinal absorption, e.g., rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Faba bean starch generally contains less RDS and SDS at 15.3 and 34.5% and 46.7% RS (Bello-Pérez et al., 2007) indicating that faba bean starch is more resistant to enzymatic hydrolysis. Furthermore, in addition to its ability to bind carbohydrates, tannins in faba beans can also form complexes with enzymes such as α-amylase and lipase responsible for digestion of energy yielding molecules (starch and lipids, respectively) hence reducing energy digestibility (Deshpande and Salunkhe, 1982). Tannin-containing extracts from hulls of coloured-flowering cultivars of faba bean have a more significant reduction in trypsin, chymotrypsin, and α-amylase activities as compared with extract from white flowering faba bean (Griffiths, 1979).

The NSP, (major component of dietary fibre in pulses), can affect many processes along the entire gastro-intestinal tract in monogastric animals (Graham et al., 1990; Southgate, 1991). Some of these physiological effects of NSP may depend on their solubility, particle size, and fermentability. In general, viscous polysaccharides (gums, pectin substances) reduce the rate of nutrient absorption in the small intestine, whereas insoluble cell wall polysaccharides (cellulose) have little effect on this process. A high dietary NSP content increases the content of less digestible carbohydrates and decreases the amount of easily digestible substances. An increase of 1% in crude fibre may result in a 2.1-2.7% decrease in the content of starch and sugars in the nitrogen free extract (Fernandez and Jorgensen, 1986). Decrease in ileal and total tract digestibility has also been observed and is likely linked to increased intake of structural cell polysaccharides by pigs (Just et al, 1983; Chabeauti et al., 1991).

Zero-tannin faba bean (white flowered faba cultivars) contain similar digestible energy (DE) value as field pea that ranges between 3.10 and 3.74 Mcal/kg as fed (NRC, 1998; Zijlstra et al., 2008; Sauvant et al., 2004). A combination of higher DE value and starch content of zero-tannin faba bean can result in a net energy (NE) of 2.20 to 2.27 Mcal/kg (as fed; Sauvant et al., 2004; Zijlstra et al., 2008). Energy digestibility in zero-tannin faba bean is 60.2% at the ileum and 88.5% for the total tract (Zijlstra et al., 2008) indicating that, by difference, 29% of GE in zero-tannin faba bean is likely fermented in the large intestine. This difference reflects its high content of fermentable carbohydrates such as 2 to 4% oligosaccharides stachyose, raffinose and verbascose (Duc et al., 1999). Additionally, excessive consumption of such fermentable carbohydrates can produce loose feces or diarrhoea in young pigs (Jezierny et al., 2010). The DE value varies among faba bean cultivars. The effect of tannins seems important as the mean DE value of 10 references with low tannin faba bean cultivars was 16.2 MJ/kg DM, which was greater than the value of 12 references with high tannin faba bean was 14.9 for (Table 1.5). However, higher DE value of 15.6 MJ/kg DM for high tannin faba bean and a similar value of 16.1 MJ/kg DM for low tannin faba bean (Sauvant et al., 2004) indicates that variability may be probably due to interactions with other seed components such as insoluble cell walls, and variability of tanning effects (Katell et al., 2010).

1.3.2 Protein and AA digestibility

Faba bean can be included in diets for growing and finishing pigs up to 200 g/kg (Partanen, 2003). However, white flowered cultivars are preferred because of their low tannin content (Zijlstra et al., 2008). Compared with other legumes as source of protein and AA, faba bean contains the least SID of CP and essential AA (Table 1.4). The inclusion of faba bean as source of protein in pig diets can be constrained by their low level of sulphur-containing AA methionine and cystine (Jezierny, 2010). The SID of methionine and tryptophan in faba bean are relatively low when compared with SID of the other AA (Table 1.4). The SID of essential AA in faba bean is 0.85, 0.73, 0.78, and 0.64 for lysine, methionine, threonine, and tryptophan, respectively, compared with SBM measuring at 0.90, 0.85, 0.84, and 0.89 for the same AA, respectively (NRC, 2012). The AID and SID of CP and AA are greater in faba bean from white flowered cultivars than in coloured flowered cultivars (Mariscal-Landín et al., 2002; Kasprowicz et al., 2005; Jezierny 2010). Differences in SID of CP and AA within faba bean cultivars can be linked to variation in their ANF, particular to the varying content of condensed tannins (Salgado et al., 2002). Tannins in the intestinal tract bind proteins from both feed and endogenous proteins (Jansman et al., 1995). In addition, tannins enhance the turnover of mucus and glycocalyx endogenous secretions.

Glycocalyx contains digestive enzymes secreted by the absorptive cells that are essential for the final steps of digestion of proteins and sugars. The mucus layer and the glycocalyx are important factors in the protection of epithelial integrity of the small intestine and therefore tannin binding may change their biochemical and physical properties. Piglets fed diets containing up to 300 g/kg of high tannin faba bean cultivars had a lower AID of CP and most AA and increased endogenous N excretion than pigs fed diets with low tannin cultivars at same inclusion level (Jansman et al., 1993). Faba bean containing high vicine (10.4 g/kg DM) and covicine (4.3 g/kg DM) contents fed to pigs at a dietary inclusion of 500 g/kg had a small effect on protein and energy digestibility (Grosjean et al., 2001).

1.3.3 Mineral and vitamin digestibility

Despite the high content of minerals in legume grains (i.e., Fe, Zn, and P), their absorption and availability can be prevented by the presence of phytic acid. Phytic acid can undergo a complete dissociation during which, its six phosphate groups carry twelve negative charges that bind different di-and trivalent cations of minerals such as Ca, Mg, Fe and Zn into a stable but undigestible complex. This process happens under weak acidic to neutral pH conditions. Zinc appears to be the trace element whose bioavailability is most influenced by phytic acid (Davies and Olpin,1979). Monogastric animals such as pigs and poultry cannot digest phytic acid. These animals therefore need plant or feed phytase enzyme to release phosphate from the inositol ring in the phytate molecule. In pigs with liveweight ranging from 7 to 30kg, supplementation of 500U phytase per kg feed can replace approximately 1g from microbial phytase. Absorption of P in pigs occurs in the small intestine, and endogenous P is also secreted into the small intestine (Fan et al., 2001). The STTD of P in faba bean is 0.36 and that is higher than that of canola meal (0.32). The formation of insoluble complexes of tannins with divalent metal ions such as iron may also reduce the availability of these ions for absorption (Khazaei, and Vandenberg., 2020; Narasinga and Prabhavathi, 1982). Iron-binding capacity of extracts from seed coat of coloured-flowering cultivars of faba bean seems to be greater than that of white flowering cultivars (Griffiths, 1982). Dietary tannic acid may also reduce vitamin A absorption from the small intestine. The interaction of tannic acid with thiamin is known to reduce vitamin B absorption in rats (Carrera et al., 1973; Rungruangsak et al., 1977).

1.4 Effects of processing on the feeding value of faba bean

Increased demand for plant-based protein sources both locally and internationally has increased research focus on alternative vegetable protein sources. Advances in animal feed processing technology and the use of feed additives (such as exogenous enzymes) means that the presence of certain ANF can be managed effectively. Although plant breeding is the most feasible approach to reduce ANF, most ANF such as tannins have functions in growth and protection of plants (Bond and Smith, 1989). Therefore, any strategy to decrease these ANF levels must preserve their agronomic functions as well as improve the nutritional quality, which makes it necessary to consider other available approaches or feed processing technologies such as dehulling, particle size reduction and fractionation. As condensed tannins in faba bean are mainly located in the seed coat, dehulling would eliminate most of the condensed tannins as well as much of the insoluble fibre (Griffiths, 198l; Sosulski and Dabrowski, 1984; Van der Poel et al., 1991).

The introduction of dehulling by the animal feed industry would depend on cost (process and raw materials), benefits (price difference between whole and dehulled beans) and efficiency of dehulling. Thus, to make the process feasible, the hulls should also be used, for example as feed fibre source for ruminants. However, market price of the feed commodity products would also depend on their nutritional value; therefore, feeding studies are necessary to determine in vivo digestibility and energy values of the dehulled faba bean for non-ruminant animals and of the hulls for ruminants. In addition, faba bean hulls containing high tannin content can be investigated for further processing to extract tannins for the leather processing industry or nutraceutical applications. The in vitro digestibility (for pigs) of crude protein and organic matter was 4 and 13% greater, respectively, for dehulled faba bean than faba whole bean (Meijer et al., 1994). Nutrient digestibility in pigs was lower for diets containing hulled faba bean than diets containing pulse protein **(**Gunawardena et al., 2010). Furthermore, high indigestible fibre in the hull indicated greater NSP content in hulls than cotyledons of legume seed, hence may reduce AID and ATTD of DM in grower pigs fed 30% raw hulled vs. dehulled faba bean. (Longstaff and McNab, 1991). However, feeding broiler chicken faba bean cultivars dehulled to reduce tannin content showed no beneficial effects on growth performance (Cho et al. (2019).

Particle size reduction can also be of great benefit to feed efficiency in swine. Decreasing particle size by grinding ingredients improves digestibility of nutrients by increasing surface area, which allows digestive enzymes in the pig's digestive tract greater access to breakdown nutrients in feed (Laurinen et al., 2000; Kim et al., 2002) that should in turn result in improved feed efficiency. Moreover, particle size reduction can influence how uniformly feed is mixed and prevent separation during material handling. The benefit of providing a well-balanced diet with faba bean could be lost if adequate care is not taken during feed manufacturing. Extremely fine particle size and dusty feed may increase the incidence of gastric ulcers, reduce feed intake, or increase associated respiratory problems. Whereas very large particle size may reduce the surface area for enzymatic digestion thus lower digestibility. Variability in seed coat texture and tannin content in faba bean seed may result in variation in particle size despite a similar treatment during grinding. Very hard seed coat may produce larger particle size as compared with soft seed coat. Whereas very high tannin content in the faba bean seed may create some difficulties during seed grinding due its binding effect. However, there is a limited information on effects of tannin content on faba bean processing and particle size and therefore this can be an interesting subject for future investigation.

Fractionation technology can extract value-added or differentiated products from raw agricultural commodities with increased usefulness for food, feed, and industrial applications. Protein concentrates produced from faba bean and other legume pulses could have usefulness for younger classes of monogastric livestock such as pigs and poultry thus enhancing feed competitiveness in Western Canada, which is one of major pulse exporting regions. Furthermore, protein concentrates of plant origin could reduce reliance on animal and marine protein sources, which have perceived food safety and sustainability concerns. Despite it being the most efficient and conventional way for removing vicine and covicine and obtaining greater protein content ingredients, wet fractionation requires utilization of great amounts of water and chemicals (Schutyser et al., 2015). Furthermore, removal of vicine and covicine through combination of enzyme treatment with fermentation is possible but it can be expensive and energy intensive (Singhal et al., 2016) though this method removes more than 99% of vicine and covicine (Vioque et al., 2012). Dry fractionation is a continuous alternative process as compared with batch wet fractionation to avoid addition of water and chemicals. Dehulling, fine milling, and air classification to enrich the fractions are the three major steps involved in dry fractionation (Vose et al., 1976). The milling step of this process involves particle size reduction detaching the starch granules from the protein bodies thus allowing their subsequent air suspension and separation during the air classification step which separates the protein bodies that are smaller and lighter than the starch granules based on density, size, and shape (Boye et al., 2010). The CP and starch content of their respective concentrate fractions in faba bean can be increased from 28 to 63% and 50 to 69% respectively, by air classification (Gunawardena et al., 2010). Moreover, air classified pulse starch concentrates can be fed to pigs to achieve similar N retention as a percentage of N intake. However, dry fractionation for protein concentrates in faba bean on industrial scale should be performed carefully as up to four-fold of vicine and covicine may concentrate in the protein fraction during air classification of colour floured cultivars. (Tyler et al., 1981; Pitz et al., 1980).

Thermal processing such as roasting, can reduce vicine and covicine content in faba bean seed (Hussein et al., 1986; Ganzler and Salgó, 1987; Muzquiz et al., 2012; Cardador-Martinez et al., 2012). The best solution to reduce vicine and covicine is breeding for low vicine and covicine faba bean. The recent discovery of VC1 enzyme responsible for vicine and covicine biosynthesis (Björnsdotter et al 2021) presents a great opportunity to select and produce faba bean cultivars with up to 95% less vicine and covicine content.

1.5 Effect of feeding faba bean on growth performance in pigs

High inclusion of legume grains in pig diets have historically been associated with reduced performance (Crepon, 2010) due to the presence of ANF and a deficiency in indispensable AA, especially methionine and tryptophan (Gatel, 1994). The inclusion of faba bean in broiler diets can slightly increase feed conversion ratio because of an increase in feed intake and a decrease in weight gain (Katell, 2010). However, supplementing the diet with methionine dramatically improved performance (Marquardt and Fröhlich, 1981). One concern with feeding coloured flower faba bean cultivars with high condensed tannins in pigs is reduced feed intake because their bitter taste (astringency; Jansman, 1993). The physical basis for bitter taste may be that tannins bind and perhaps precipitate salivary mucoproteins. This binding of mucoproteins would reduce the lubricating property of saliva providing a mouth feeling of dryness that may affect the ability to swallow food and bind to taste receptor (Mole and Waterman, 1987). Moreover, considerable amounts of non-starch-polysaccharides (NSP) are present in several legume grains such as lupin (Bach Knudsen, 1997). High NSP content in the diets may also lead to decreased growth performance and feed intake in growing pigs by reducing digesta passage rate (Dunshea et al., 2001; Ferguson et al., 2003).

Early trials indicated that dietary inclusion of more than 20% faba bean reduced growth performance and voluntary feed intake in pigs (Castell, 1976; Aherne et al., 1977; Gatel and Grosjean, 1990). Consequently, plant breeding efforts have produced new cultivars of faba bean with decreased ANF content such as zero-tannin faba bean (Jezierny et al., 2010). Dietary inclusion rate of 30% of zero tannin faba bean did not affect voluntary feed intake on weaned pigs (Zijlstra et al., 2008). The presence of other ANF such as vicine and covicine did not hamper voluntary feed intake in pigs (Wang and Ueberscha, 1990; Duc et al., 1999). However, feed efficiency was reduced in pigs fed with diets containing >30% faba bean (Zijlstra et al., 2008) an observation which may be attributed to reduced nutrient digestibility (Wondra et al., 1995) as compared with pigs fed diets with SBM.

Furthermore, diets containing 20% of either white or coloured flower faba bean cultivars can be fed to weaned pigs without affecting performance. (Ivarsson and Neil, 2018). Pigs fed high tannin cultivars had the best feed efficiency and average daily gain (ADG) among the faba bean cultivars (Ivarsson and Neil, 2018). These results on feed efficiency were an indication that the level of CT in some faba bean cultivars may not be a major issue for weaned and growing pigs fed nutritionally well-balanced diets i.e., supplemented with AA (Ivarsson and Neil, 2018; Smith et al., 2013).

1.6 Faba bean and environmental sustainability

Pulses are rich in both starch and protein making them an excellent substitute for animal protein sources in swine nutrition. However, as the use of SBM in the pig industry increases, the demand for land for soybean production and land use change also increases. (Fearnside, 2001). Feeding of pulses such as faba bean and field peas to monogastric animals could therefore reduce the pressure put on soybean and safeguard the sustainable use of environmental resources such as forests. The contribution of pulses to total food/feed wastage including the carbon footprint and the blue water footprint is low making them an environmentally friendly source of key nutrients (FAOSTAT, 2018).

Furthermore, faba bean in association with rhizobia is an efficient N-fixing legume. It contributes to 270 kg/ha of N to the soil, increasing subsequent cereal crop yields by 31% (Pei-pei et al., 2012). Efficient nitrogen fixation in faba bean may play a crucial role in reducing the cost of crop production and improve yield not only due to N fixation where N-fertilizers are not required, but also due to improved disease and pest control when intercropped with cereal and oilseeds (Sprent and Bradford, 1977; Fan et al, 2006). Additionally, as climate change emerges to be a prominent global concern, focus on the study of CO_2 emission, carbon sequestration, and carbon cycling in the ecosystem has increased. However, rapid changes in land use to supply the increasing requirement for food production has led to holistic attention given to the greenhouse gas (GHG) budget of agricultural operation. Faba bean production as for other legume species, is therefore substantially increasing for the purpose of not only deriving food and energy but also soil amendments (Welbaum, 2004). Soil amendments provided by legumes is a result of their ability not only to fix nitrogen and convert it into a more plant available form through biological nitrogen fixation (BNF), but also carbon fixation. Carbon fixation by legumes into soils indirectly occurs from the process of BNF which produces H_2 as a by-product. The H_2 released in the soil acts as an energy source for chemoautotrophic bacteria which fixes atmospheric CO_2 into the microbial biomass through the process of H_2 oxidization (People et al., 2008). BNF carbon requirement ranges from 13% to 28%, which is approximately 6 to 7 grams of carbon as carbohydrate required to produce a gram of reduced nitrogen (Vance, 2008). Furthermore, this carbon consumption technique in legumes play a key role in offsetting N_2O emission associated with legume crops, making them near neutral in GHG emission.

1.7 Conclusion

The interest on alternative plant-based energy and protein sources has grown because of the concern on increasing cost of conventional sources of proteins such as SBM and health concerns such as porcine epidemic diarrhoea (PED) associated with inclusion of meat and bone meal, spray-dried blood, and plasma in swine diets. The need for alternative plant-based protein sources has led to an increasing interest in the use of grain legumes. Soybean meal is worldwide used in the diet of pigs. However, it could be profitably integrated or replaced with other protein-rich locally grown vegetable feedstuffs. Faba bean cultivated in western Canada, are a valuable source of plant-based energy and protein for monogastric animals, as principal or stubble crops that can partially or even totally replace imported SBM and wheat grain as a source of both starch and protein. Faba bean can be used as feed component due to their high starch (33-46%) and protein content (28-37%; Hoover and Sosulski, 1991, Ross and Davies, 1992). Moreover, growing faba bean can be of beneficial to crop rotation because of their high and consistent atmospheric N and C fixating capacity (López-Bellido et al., 2003). Nevertheless, their inclusion in monogastric diets is hampered due to their low level of sulphur-containing AA, greater content of dietary fibre and AFNs such as tannins, vicines and covicine, protease inhibitors, phytic acid and lectins which can additively reduce nutrient availability and digestibility, and growth performance (Jansman et al., 1994). However, with the introduction of standardized ileal digestibility (SID) for AA and the NE system, the true digestibility of each dietary AA and energy value in faba bean cultivars varying in nutrient and AFNs contents can be more accurately assessed (Stein et al., 2005), allowing the formulation of nutritionally balanced diets with faba bean that can meet the requirements of pigs. Furthermore, the newly improved cultivars containing low levels of tannins, vicine and covicine, and greater nutrient content can be evaluated for their inclusion in the swine diets.

1.7.1 Knowledge gap

The swine industry in Western Canada relies heavily on wheat as source of energy and imported SBM for protein because cultivation of soybean is challenging in northern latitudes given lower heat units to grow. However, there is an increased environmental concern with the continuous and increased demand for SBM in the swine industry due to the increased demand for more land for soybean cultivation hence increased demand for land use change (Fearnside, 2001). Faba bean can be a suitable alternative source of both energy and protein in swine diets thus increasing the profitability of swine production by reducing reliance on imported corn and soybean meal (SBM). Additionally, the presence of tannins in the outer faba bean hull improves frost tolerance despite their antinutritional effect. Furthermore, some faba beans cultivars with high vicine and covicine content may pose some problems associated with favism condition when included in human diets and thus their use in swine nutrition without reducing performance will provide an excellent alternative usage. Compared with coloured flowered faba bean, white-flowering cultivars have a much lower tannin content than the coloured flower cultivars and are therefore generally more digestible. Both white and coloured faba bean cultivars can be included at 20-30% in diets fed to weaned and growing pigs without reducing performance (Ivarsson and Neil, 2018). Low or zero tannin cultivars such as Snowbird, Snowdrop, and Tabasco and mid tannin cultivars such as Fabelle, Malik, and Florent are available in grain growing regions of Western Canada. However, it is not clear how these cultivars varying in condensed tannin, vicine and covicine, and nutrient content rank on nutrient and energy digestibility and growth performance fed to weaned and growing pigs.

1.8 Thesis hypothesis and objectives

The null hypothesis of this present thesis were: (a) dietary inclusion of 20-30% of faba bean cultivars differing in nutrients, condensed tannin, and vicine and covicine content, formulated on equivalent SID Lys to NE would not affect apparent total tract digestibility of CP and energy, and growth performance of weaned pigs; b) feeding diets including faba bean cultivars differing in nutrients, condensed tannin and vicine and covicine content would not affect energy, protein, and AA digestibility in the ileal-cannulated growing pigs.

The objectives of this thesis were therefore:

a) To evaluate the apparent total tract digestibility (ATTD)of dry matter, gross energy and crude protein, and the digestible energy and predicted net energy values, growth performance and faecal consistency of weaned pigs fed diets containing different faba bean cultivars varying in nutrients condensed tannins and vicine and covicine content.

b) To compare ileal apparent digestibility (AID) of starch, standard ileal digestibility (SID) of crude protein and AA, and ATTD of gross energy in ileal-cannulated growing pigs.

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Table 1.1. Nutrient content of faba bean as compared with other plant-based sources of starch and protein produced in western Canada (data on as is; NRC, 2012)

	Barley	Canola meal	Field peas	Faba bean
Dry matter (%)	89.90	91.33	88.10	88.12
Starch (%)	50.12	6.07	43.46	39.22
Crude protein (%)	11.33	37.50	22.17	27.16
NDF (%)	18.29	22.64	12.84	13.29
Ether extract (%)	2.10	3.22	1.20	1.30
NE[1] (kcal/kg, as is)	3150	3273	2419	3245

[1]NE, Net energy.

Table 1.2. Faba bean starch characteristics

	Mean	Reference
Yield, %	84.7	Li et al., 2019
Starch content, %	96.6	Zhang et al., 2019
Amylose, %	40.0	; Li et al., 2019
Protein, %	0.30	Zhang et al., 2019
Fat, %	0.38	Zhang et al., 2019
Ash, %	0.07	Zhang et al., 2019
Swelling power, g/g	12.7	Zhang et al., 2019
Solubility, %	9.92	Zhang et al., 2019
X-ray diffraction	C-type pattern	Hoover and Ratnayake, 2002
In vitro digestibility		
Rapidly digestible starch (RDS), %	15.30	Li et al., 2019
Slowly digestible starch (SDS), %	34.5	Li et al., 2019
Resistant starch (RS), %	46.7	Li et al., 2019

Table 1.3: Fibre composition of legume grains (Reddy et al 1984) Values reported in g/100g on dry weight basis.

	Soybean	Faba bean	Lentil	Cowpea	Lupine	Bengal gram	Field pea
Total fibre	2.4-5.5	8.0	2.6	1.7-4.0	3.0	1.2-25.6	4.6-7.0
Cellulose	_	1.0	4.1	_	_	1.1-13.7	0.9-4.9
Lignin	_	0.7-1.0	2.6	0.6-1.8	0.7-0.8	2.9-7.1	0.5-0.9
Hemicellulose	7.6	4.0-4.6	6.0	_	9.3-9.9	_	1.0-5.1

Table 1.4. Coefficients of standard ileal digestibility of crude protein and amino acids of legumes as compared with soybean meal (NRC, 2012).

	Faba bean	Field pea	Lupin	Soybean meal
Crude protein	0.79	0.80	0.86	0.87
Lysine	0.85	0.85	0.85	0.89
Methionine	0.73	0.77	0.81	0.90
Threonine	0.78	0.76	0.82	0.91
Tryptophan	0.64	0.69	0.82	0.85

Table 1.5. Nutrient digestibility values of faba bean cultivars with low- or high-tannin content for pigs (Katell et al., 2010)

	High tannin faba bean			Low tannin faba bean		
Characteristic	Mean	Min	Max	Mean	Min	Max
ATTD, CP (%)	79.6	71.9	82.9	84.6	73.9	90.7
ATTD, energy (%)	79.6	76.7	82	87.3	80.3	94.3
DE (MJ/kg DM	14.9	14.4	15.2	16.2	14.8	17.5
AID, CP (%)	81.1	76.7	84.2	80.6	75.8	85.7
AID, Lys (%)	85.9	79.8	90.7	87.5	84	91.6

CP, crude protein; ATTD, apparent total tract digestibility; DE, digestible energy; AID, apparent ileal digestibility; Lys, lysine; DM, dry matter.

Figure 1.1. Molecular structures of flavan-3-0l (a) and flavan-3, 4-diol (b), building blocks of procyanidins compounds of proanthocyanidins (or condensed tannins).

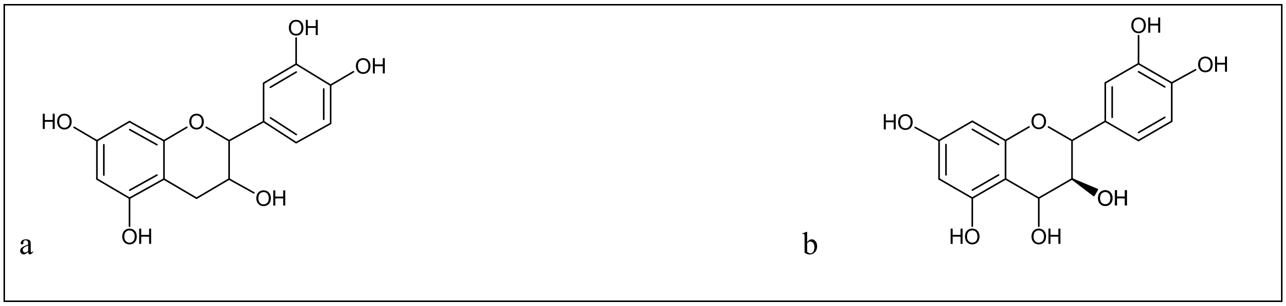
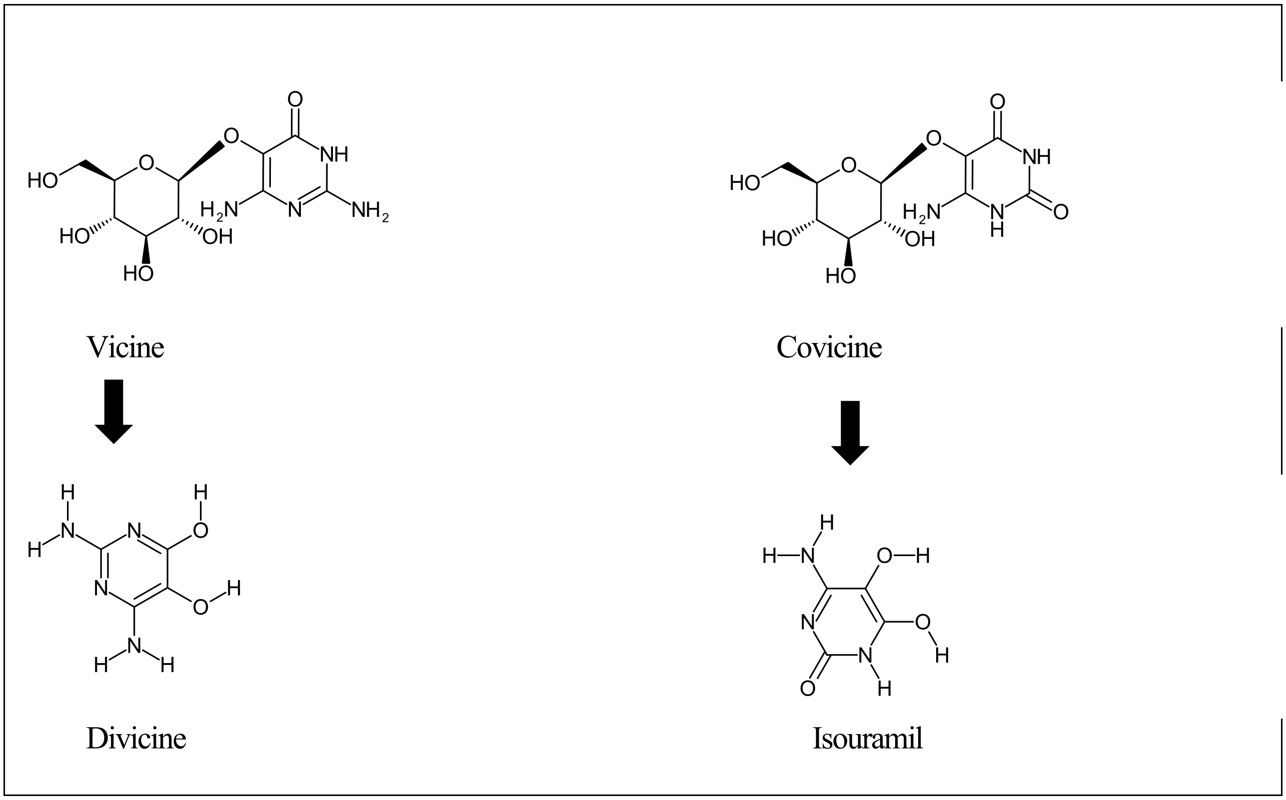


Figure 1.2. Molecular structures of vicine and covicine and their respective aglycones, divicine and isouramil.



Chapter 2. Effect of feeding mid- or zero-tannin faba bean cultivars differing in vicine and covicine on diet nutrient digestibility and growth performance of weaned pigs.

2.1 Introduction

Worldwide corn grain and soybean meal are the most common feedstuffs supplying starch and supplemental protein, respectively, in swine diets. However, in northern latitudes where heat units limit corn and soybean production, locally grown alternative ingredients like pulses can provide both starch and protein and present an opportunity to reduce feed cost (Woyengo et al., 2014). Faba bean (*Vicia faba* L.) contains 30 to 42% starch (dry matter basis; Cerning et al., 1975) and 25 to 37% crude protein (Duc et al., 1999). In contrast to field pea, lentil, and chickpea, faba bean fixes the most atmospheric nitrogen beyond blooming until the plant dries (López-Bellido et al., 2006); thus, it is an excellent rotational pulse crop to grow alternating with cereals and oilseeds.

White-flowered or zero-tannin faba bean cultivars contain less condensed tannin at 0.06 to 0.75% than color-flowered cultivars containing 0.40 to 2.15% (Cabrera and Martin, 1989). Condensed tannins form insoluble complexes with proline-rich proteins such as collagen, gelatin, salivary proteins, casein, and digestive enzymes that reduce diet nutrient digestibility (Hagerman and Butler, 1980). The astringent sensation of this complexation causing drying and puckering suppressed palatability (Butler et al., 1984). Other relevant antinutritional factors in faba bean are vicine and convicine that cause hemolytic anemia in humans (favism) with an erythrocyte genetic deficiency of glucose-6-phosphate dehydrogenase (Arese et al., 2012). Their aglycones, divicine and isouramil, react with blood oxygen forming reactive oxygen species that increase lipid peroxidation. Decreased liver function may result in insufficient bile acid production for micelle formation affecting lipid digestion thus reducing energy digestibility (Cho et al., 2019). Both white- and color-flowered faba bean cultivars can be included at up to 40% in diets fed to weaned and growing pigs without reducing growth performance (Ivarsson and Neil, 2018; Beltranena et al., 2009). Feeding low vicine and covicine and mid-tannin faba bean cultivars did not reduce nutrient digestibility or growth performance in broiler chickens (Cho et al., 2019). However, variation in content of nutrients, condensed tannins, vicine and convicine in faba bean cultivars and their effect on diet nutrient digestibility and growth performance of weaned pigs needs to be clarified to prioritize what cultivar to grow to feed pigs.

The null hypothesis of the present study was that feeding diets including faba bean cultivars differing in nutrients, condensed tannins, and vicine and covicine content would not affect nutrient digestibility and growth performance of weaned pigs. The objectives were to compare the apparent total tract digestibility of dry matter, gross energy, crude protein, digestible energy and net energy values, growth performance, and feces consistency of weaned pigs fed diets including 5 faba bean cultivars differing in nutrient, condensed tannins, and vicine and covicine content.

2.2 Materials and methods

Animal use and experimental procedure 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 in nursery rooms located at the Swine Research and Technology Centre, University of Alberta (Edmonton, Alberta, Canada).

In total, 260 pigs (Duroc × Large White/Landrace F1; Alliance Genetics Canada, Sand Ridge Farm Ltd., Barrhead, Alberta, Canada) weaned at 20 ± 1 day [**d**] of age, were selected based on post-weaning average daily weight gain (**ADG**) to day 5 after weaning. Pigs within sex were divided into heavy or light body weight (**BW**). One light- and one heavy-gilt and one light- and one heavy-barrow were randomly allocated to each pen within area block. Pigs were fed a common creep diet until 2 days after weaning and then a commercial starter diet for 5 days. Pigs were then fed the phase 1 diets for 2 weeks (d 0 to 14) and subsequently the phase 2 diets for 2 more weeks (d 14 to 28).

The 3 zero-tannin faba bean cultivars Snowbird, Snowdrop, and Tabasco were sourced from Galloway Seeds (Fort Saskatchewan, Alberta, Canada), Shewchuk Seeds (Blaine Lake, Saskatchewan, Canada), and Riddell Seed Company (Warren, Manitoba, Canada), respectively. The 2 mid-tannin faba bean cultivars Fabelle and Malik were both sourced from Stamp Seeds (Enchant, Alberta, Canada). Faba bean cultivars were ground through a 2.8-mm screen using a hammermill (model Jacobson 5550-113-01, Carter Day International, Minneapolis, MN).

2.2.1 ***Diets and Experimental Design***

Five experimental diets were formulated including 20% of 1 of the 5 faba bean cultivars in phase 1 (Table 1) and 30% in phase 2 (Table 2). Diets provided 10.2 and 10.1 MJ net energy (**NE**)/kg and 1.3 and 1.2 g standardized ileal digestible (**SID**) lysine (**Lys**) per MJ NE for phase 1 and phase 2 diets, respectively. Canola oil and L-lysine HCl were added to equalize NE value and SID Lys content, respectively. Other amino acids (**AA**) were formulated as an ideal ratio to Lys (NRC, 2012). The NE value for each faba bean cultivar was calculated using equation 5 of Noblet et al. (1994) including analyzed starch, crude fat, crude protein (**CP**), and acid detergent fiber (**ADF**) content for the same cultivars from a recent study (Cho et al., 2019). Tabulated NRC (2012) data were used to calculate the SID AA content for faba bean cultivars and main ingredients. Celite was included in diets as indigestible marker. Phase 1 diets were mixed in a 300-kg horizontal paddle mixer (model SPC2748, Marion Mixers Inc., Marion, IA) and cold-pelleted (model PM1230, Buskirk Engineering, Ossian, IN). Phase 2 diets were mixed in a 1000-

kg vertical mixer (model MFP-2100, Weigh-Tronix, Fairmont, MN) and steam-pelleted (model 1112-4, California Pellet Mill Co., San Francisco, CA).

The study was conducted as a randomized complete block design with 13 blocks. Pens of pigs within area block were randomly fed 1 of the 5 dietary regimens. Pens (1.1 × 1.5 m) were equipped with plastic slatted flooring, a concrete back wall, and pen partitions and front gate made of polyvinyl chloride. Pens were also equipped with a single 4-place adjustable self-feeder (model N4; Crystal Spring, Manitoba, Canada) and an adjustable-height nipple drinker. Rooms were ventilated using negative pressure and heated by convection from hot water pipes. Light tubes provided a 12-h light (0700 to 1900 h) and 12-h dark cycle. Pigs had free access to feed and water throughout the trial. Individual pigs, pen feed added, and remaining orts were weighed weekly to calculate pen ADG, average daily feed disappearance (**ADFD**), and gain-to-feed ratio (**G:F**). Freshly voided feces were scored for consistency twice daily (at approximately 0800h and 1600h) and collected hourly by grab-sampling from pen floors on day 12 and 13 for phase 1, and day 26 and 27 for phase 2. Fresh feces were pooled by pen and frozen at approximately −20°C. Upon completion of the trial, feces were thawed, homogenized, sub-sampled, and freeze-dried.

2.2.2 Chemical Analyses and Calculations

Faba bean cultivars, diets, and lyophilized feces samples were ground through a 1-mm screen in a centrifugal mill (Model ZM200, Retch GmbH, Haan, Germany) and were analyzed for dry matter (**DM**; method 930.15; AOAC, 2006), CP by Leco (nitrogen [**N**] × 6.25; method 990.03), and GE using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany) at the University of Alberta. Cultivar and diet samples were also analyzed for total (method 996.11) and resistant starch (method 2002.02) using Megazyme assay kits and crude fat using a Goldfisch fat extraction apparatus (method 945.16). Cultivars and diets were analyzed for neutral detergent fiber (**NDF**) without a heat-stable amylase and expressed inclusive of residual ash (Holst, 1973), ADF inclusive of residual ash (method 973.18), and ash (method 942.05) at the Agricultural Experiment Station Chemical Laboratories (**ESCL**), University of Missouri (Columbia, MO, USA). Faba bean cultivar samples were also analyzed for total dietary fiber (method 985.29), calcium (method 968.08), phosphorus (method 946.06), and AA [method 982.30E (a–c)] at ESCL. Vicine and covicine of faba bean cultivars were analyzed using a slight modification of the extraction procedure (Purves et al., 2018) at the Organic Residue Laboratory of Alberta Agriculture and Forestry (Edmonton, Alberta, Canada). Condensed tannins (**CT**) analysis was conducted at the Natural Resources Institute Finland using high performance liquid chromatography after thiolytic degradation as described by Ivarsson and Neil (2018). Diets and feces samples were analyzed for acid insoluble ash (Vogtmann et al., 1975 as modified by Newkirk et al., 2003) at the University of Alberta. Particle size of faba bean cultivars was established using a mechanical sieve shaker (Model RX-29, W.S. Tyler, ON, Canada) following the method of the American Society of Agricultural and Biological Engineers (2008).

Based on results of chemical analyses, the apparent total tract digestibility (**ATTD**) of DM, GE, and CP were calculated for each diet using the indicator method according to Adeola (2001):

$$ATTD = \left(1 - \frac{\text{Indigestible marker in feed} \times \text{content of component in feces}}{\text{Indigestible marker in feces} \times \text{content of component in feed}}\right) \times 100$$

The DE value of diets was calculated by multiplying GE by its digestibility coefficient (Adeola, 2001). The NE value of diets was calculated using equation (5) in Noblet et al. (1994) with the determined diet DE value and analyzed content of ADF, CP, crude fat, and starch.

Statistical Analyses

Nutrient digestibility data were analyzed using PROC GLIMMIX and growth performance data were analyzed using PROC MIXED (version 9.4; SAS Inst. Inc., Cary, NC) with diet, week or phase, and interactions as fixed effects, block as a random term, and pen as the experimental unit. Normality and homogeneity of residuals for each item were confirmed first using PROC UNIVARIATE with 'Normal' option and PROC GLM with 'Hovtest = Levene' option, respectively. Growth performance data were analyzed as repeated measures using weekly pen data with the best covariance structure based on fit statistics and initial BW as a covariate if significant. The *P* values were adjusted with the Tukey option for multiple comparisons among treatments. Feces consistency scores were averaged weekly from the daily greatest score of pen observations and were analyzed using PROC GLIMMIX with a Gaussian distribution and Identity link function options.

2.2.3 ***Statistical Analyses***

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2.3 Results

For phase 1 diets, analyzed starch content ranged from 29.4 to 31.4%, and CP from 25.7 to 26.8% (Table 1). The Snowbird diet had the lowest NDF content (8.7%) whereas the Fabelle diet had the greatest (15.1%); ADF content ranged from 5.0 to 5.3%. Gross energy values ranged from 16.5 to 16.8 MJ/kg. Lysine content ranged from 1.45 to 1.52%.

For phase 2 diets, analyzed starch content ranged from 37.2 to 41.2%, CP from 25.9 to 27.5%, NDF from 10.4 to 15.1%, and ADF from 6.1 to 6.7% (Table 2). Gross energy values ranged from 16.5 to 17.0 MJ/kg. Lysine content ranged from 1.40 to 1.53%.

For faba bean cultivars, starch content ranged from 30.7 to 36.1% with about 48% as resistant starch (Table 3). Crude protein content ranged from 29.4 to 31.9%, NDF from 13.1 to 17.1, ADF from 10.4 to 12.9%. Gross energy values ranged from 16.3 to 17.0 MJ/kg. Lysine content ranged from 1.87 to 2.05%. Between color-flowered cultivars, Fabelle contained twice as much CT as Malik, and both contained more CT than white-flowered cultivars (Snowbird, Snowdrop and Tabasco; < 0.2%). Between color-flowered cultivars, Fabelle contained less vicine and covicine than Malik, whereas white-flowered cultivars averaged 0.53% vicine and 0.40% covicine.

For phase 1 diets, ATTD of DM, GE, CP, and DE and NE values did not differ among cultivars (Table 4). For phase 2 diets, ATTD of DM and GE were greatest ($P < 0.05$) for Snowdrop and Tabasco, intermediate for Fabelle, and lowest for Malik; Snowbird was not different from Fabelle or Malik. Diet ATTD of CP was greatest ($P < 0.05$) for Tabasco, intermediate for Snowbird, and lowest for Malik; Snowdrop was not different from Tabasco or Snowbird, and Fabelle was not different from Snowbird or Malik. Diet DE and NE values were greatest ($P < 0.05$) for Tabasco, intermediate for Fabelle and Snowdrop, and lowest for Snowbird; Malik was not different from Fabelle or Snowbird.

For the entire trial (d 0 to 28), ADFD and ADG for pigs fed Fabelle were 10% greater ($P <$ 0.05) than those fed Malik; pigs fed zero-tannin cultivar diets were intermediate (Table 5). Gain-to-feed did not differ among pigs fed the 5 cultivar diets. Pigs fed Fabelle were 1.6 kg heavier (P < 0.05) than those fed Malik at the end of the trial; pigs fed zero-tannin cultivar diets were intermediate. Feces scores did not differ among pigs fed the five faba bean cultivar diets for the entire trial or each week (Table 6).

2.4 Discussion

2.4.2 Chemical Characteristics of Faba Bean Cultivars

Level of nutrients and antinutritional factors can differ among faba bean cultivars (Ivarsson and Neil., 2018). Therefore, it is of importance to screen for these compounds and evaluate their effect on nutrient digestibility and growth performance to prioritize cultivars that are best suited for swine production. Generally, chemical composition of faba bean cultivars in the present study was similar to previous reports (Duc et al., 1999; Sauvant et al., 2004). As expected, color-flowered cultivars (Fabelle and Malik) contained more condensed tannin than white-flowered cultivars (Snowbird, Snowdrop, and Tabasco; Cho et al., 2019). Variation in vicine and covicine content among cultivars is likely because of varying concentration or activity of guanosine triphosphate cyclohydrase II, a key enzyme modulating their synthesis (Björnsdotter et al., 2021).

Among macronutrients, starch was the major constituent that differed among faba bean cultivars irrespective of being color- or white-flowered. Starch content was slightly lower than 37 to 44% DM previously reported (Duc et al., 1999; Ivarsson and Neil, 2018). Variation in starch content may be attributed in part to laboratory assay variability despite similar analytical methods. Similar to other pulses, nearly half of total starch in faba bean grain fed in the present study was resistant starch. Greater resistant starch (46.7%) as compared with slower digestible starch (34.5%) and smaller amount of rapid digestible starch (15.3%) content in faba bean (Bello-Pérez et al., 2007) indicate that a large portion of starch is resistant to enzymatic hydrolysis in the small intestine.

Crude protein and lysine content were more consistent among faba bean cultivars. Crude protein content of cultivars in the present study was 3%-units greater than the 27% reported for the same cultivars previously (Cho et al., 2019) and was within the 24 to 37% range reported by Duc et al. (1999). In comparison with cereal grains, faba bean cultivars fed in the present study contained more lysine but less sulfur-containing amino acids (methionine + cystine) and tryptophan than soybean meal (Duc et al., 1999). Consequently, diets fed in the present study were supplemented with crystalline amino acids and included soybean meal, fish meal, and soy protein concentrate to complement their profile. Amino acid composition generally varies among faba bean cultivars (Jezierny et al., 2010).

The third constituent by mass in faba bean was fiber. Neutral detergent fiber was greater than 10.9 to 12.6% DM reported by Ivarsson and Neil (2018), but similar to 12.6 to 16.5% reported by Jezierny et al. (2010). On average, color-flowered cultivars (Fabelle and Malik) contained slightly more total dietary fiber than white-flowered cultivars (23.3 vs. 19.1%), matching their greater soluble fiber content, suggesting slightly greater fermentation potential (Tan et al., 2018).

2.4.3 Diet Nutrient and Energy Digestibility

Condensed tannins in faba bean are considered major antinutritional factors for monogastric species (Jezierny, 2009). In vitro digestibility of dry matter was 4.7% greater for white- than color-flowered faba bean cultivars presumably because lower grain tannin content (Bond, 1976). We fed diets including the same phase level of white- and color- flowered faba bean cultivars to weaned pigs so that effects of variation in tannin, vicine and covicine content, if contributing to differences in nutrient and energy digestibility, could be detected. Lack of extreme differences in condensed tannin content between zero- and mid-tannin cultivars in this study was not likely a large factor affecting total tract digestibility of nutrients or energy (Ivarsson and Neil, 2018).

Tannins interact with carbohydrates, particularly starch, thereby reducing energy digestibility (Jansman, 1993). However, their affinity for starch seems to be less than that for protein. Tannins may not only reduce energy digestibility by interacting with starch but also bind α-amylase and lipase thus reducing starch and fat hydrolysis, respectively (Griffiths, 1979). Pigs fed the color-flowered Malik diet in phase 2 had the lowest dry matter and energy digestibility but not different from zero-tannin Snowbird. Moreover, Malik contained less tannins than Fabelle, indicating that reasons other than tannin content in Malik should explain its low energy digestibility. Instead, the presence of vicine and covicine, greater total dietary fiber, and the greatest resistant starch content in Malik could have collectively reduced its energy digestibility. Following ingestion by pigs, vicine and covicine can be hydrolyzed by a ß-glycosidase-like enzyme produced by anaerobic bacteria in the colon (Hegazy and Marquardt, 1984). Vicine is converted into divicine and covicine into isouramil that are both absorbed and subsequently react with blood oxygen, thereby generating reactive oxygen species such as hydrogen peroxide (Chevion et al., 1982). Lipid peroxidation that may reduce energy digestibility due to insufficient bile acid production for lipid digestion (Losowsky and Walker, 1969).

Similar to other legume grains, carbohydrates in faba bean may pose digestibility concerns in young pigs. Faba bean starch is mainly type C with greater amylose content, which is more resistant than amylopectin to porcine pancreatic α-amylase hydrolysis (Hoover and Zhou, 2003). Greater inclusion rate of faba bean in phase 2 diets thus likely contributed to higher dietary resistant starch and non-fermentable non-starch polysaccharides that collectively reduced total tract digestibility of energy more than in Phase 1 diets. Furthermore, faba bean as legume grain contains more soluble oligosaccharides (α-galactosides) than cereal grains (Jezierny et al., 2010). Excessive consumption of α-galactosides may increase rate of passage and loosen feces or even stimulate diarrhea and flatulence in pigs due to excessive fermentation (Fleming et al., 1988). Still, dietary changes, presence of oligosaccharides, and variation in fiber content among faba bean cultivars in this experiment did not result in differences in fecal consistency scores. The greatest total tract digestibility of gross energy and digestible energy values in the Tabasco phase 2 diet might be because of its greater dietary starch and hemicellulose content (NDF minus ADF) (Noblet and Perez., 1993). Conversely, Malik phase 2 diet had the lowest total tract digestibility of gross energy, matching its greatest fiber content. Likewise, the greatest fiber content of the Malik diet likely contributed to increased non-fermentable fiber thereby reducing its energy digestibility. The greatest calculated net energy value in the Tabasco phase 2 diet was likely due to its digestible energy value (Noblet et al., 1994). Conversely, the lowest net energy value in Snowbird phase 2 diet was consistent with its lowest digestible energy value.

Feeding diets containing high tannin faba bean to broiler chickens and pigs may decrease digestibility of crude protein (Jansman et al., 1993). Tannins can bind both dietary protein and digestive enzymes, thereby reducing activity of trypsin and chymotrypsin (Griffiths, 1979), thus increase endogenous nitrogen losses. Despite lower tannin content than Fabelle, Malik phase 2 diet had the lowest digestibility of dietary protein. On the other hand, digestibility of protein of phase 2 diets did not differ between Fabelle with the greatest tannin content and zero-tannin Snowbird, indicating that tannin level in color-flowered faba bean did not affect protein digestibility greatly. Previously, a diet containing up to 3.3% of condensed tannins in faba bean hulls reduced apparent total tract digestibility of protein and amino acids (Jansman et al., 1993). In another study (Myrie et al., 2008), 1.5% condensed tannins in diets fed to weaned pigs did not reduce nitrogen retention despite reduced apparent ileal digestibility of threonine. The greatest fiber content in Malik phase 2 diet and total dietary fiber in Malik faba bean was likely associated with increased dietary fermentable fiber thereby reducing protein digestibility. Fermentation of resistant starch and fiber in the hindgut may shift nitrogen excretion from urine to feces (Younes et al., 1995) thereby reducing the digestibility of dietary protein and energy (Bach Knudsen et al., 1993). Lower digestibility of protein in phase 1 than phase 2 could be due to less developed proteolytic capacity of the digestive tract in pigs soon after weaning (Randy et al., 1982).

2.4.4 Growth Performance

One concern with tannin content in color-flowered faba bean cultivars is that voluntary feed intake might be reduced due to bitter or astringent taste that affects palatability (Jansman, 1993). In the present study, however, feed disappearance was greatest for pigs fed Fabelle diets despite its greater tannin content than Malik or zero-tannin faba bean cultivars. Similarly, the lowest feed disappearance for pigs fed the Malik diet in phase 2 was not likely due to tannin content. In addition, because diets were balanced to equal net energy value, greater feed disappearance of diets containing Fabelle to compensate for its low energy value was not likely the reason. Nonetheless, low dietary tannin content increased feed intake and thereby increased performance in monogastric animals (Huang et al., 2018). Given the astringent nature of tannins thereby reducing palatability, increased feed disappearance associated with low concentration of dietary tannins is not clear. Furthermore, pigs are more resistant to toxic effects of dietary tannins than chickens (Hassan et al., 2020). This ability to resist toxic effects of dietary tannins is likely due to parotid gland hypertrophy and salivary secretion of proline-rich proteins that bind and neutralize tannins (Cappai et al., 2010). Lowest feed disappearance for the Malik phase 2 diet might be explained by its greater fiber content. High dietary fermentable fiber and resistant starch can depress voluntary feed intake in pigs by stimulating satiety due to increased gastric retention time (Kyriazakis and Emmans, 1995). Between color-flowered cultivars, feed disappearance was greater for Fabelle (low vicine and covicine) than the Malik (high vicine and covicine) diet. Effects of vicine and covicine on feed intake of pigs are not clearly understood but might be linked to oxidative stress caused by divicine in red blood cells (Jollow and McMillan, 2001).

Tannins can reduce nitrogen retention due to increased endogenous losses (Mansoori and Acamovic, 2007) that would reduce whole-body protein retention thereby lowering weight gain. In the present study, pigs fed Fabelle diets that contained the most tannins had the greatest weight gain, indicating that tannin content did not affect weight gain. Instead, the greatest weight gain observed for Fabelle diets could be attributed to sustained greater feed disappearance (Zijlstra et al., 2009) despite lower nutrient digestibility than Snowbird, Snowdrop, and Tabasco diets. Furthermore, beneficial effects of tannins inhibiting pathogenic bacteria in weaned pigs might be associated with greater growth performance in pigs fed the Fabelle diet (Hassan et al., 2020). Lowest pig weight gain for Malik could be attributed to both low energy and protein digestibility and feed disappearance. Digestibility of essential amino acid, particularly lysine might be a concern for growth performance. However, we formulated diets to be equal in digestible amino acid content; thus, variations in feed disappearance and growth performance due to varying dietary amino acid content did not likely happen (Schiavon et al., 2018).

In conclusion, the level of condensed tannins in color-flowered faba bean cultivars fed in the present study did not prominently affect diet nutrient and energy digestibility nor growth performance. Pigs fed Fabelle had greater feed disappearance and weight gain than those fed Malik likely associated with its lowest vicine and covicine. Therefore, between mid-tannin cultivars, Fabelle should be prioritized to be grown to feed to pigs. Furthermore, vicine and covicine, and not tannin content should be of greater consideration in prioritizing modern faba bean cultivars to grow to feed to pigs. Across cultivars, variations in dietary fiber and starch content among faba bean cultivars contributed to differences in dry matter and energy digestibility and growth performance of pigs. Inclusion of up to 30% of either white- or color-flowered faba bean in nutritionally balanced weaned pig diets may reduce dry matter and energy digestibility but it is unlikely to affect overall growth performance and feces consistency.

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Table 2.1. Ingredient composition and analyzed nutrient content (as fed) of diets including five faba bean cultivars fed to weaned pigs during phase 1 (day 0 to 14)1

	Snowbird	Snowdrop	Tabasco	Fabelle	Malik
Ingredient, %					
Wheat, Hard Red Spring	40.86	40.73	39.68	40.28	39.75
Snowbird faba bean	20.00	–	–	–	–
Snowdrop faba bean	–	20.00	–	–	–
Tabasco faba bean	–	–	20.00	–	–
Fabelle faba bean	–	–	–	20.00	–
Malik faba bean	–	–	–	–	20.00
Soybean meal	15.00	15.00	15.00	15.00	15.00
Lactose	10.00	10.00	10.00	10.00	10.00
Menhaden fish meal	5.00	5.00	5.00	5.00	5.00
Soy protein concentrate[2]	2.50	2.50	2.50	2.50	2.50
Canola oil	1.50	1.60	2.70	2.10	2.60
Limestone	1.25	1.25	1.25	1.25	1.25
Salt	0.85	0.85	0.85	0.85	0.85
Celite[3]	0.80	0.80	0.80	0.80	0.80
Vitamin premix[4]	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50
L-Lysine HCl	0.40	0.43	0.37	0.37	0.40
L-Threonine	0.25	0.25	0.25	0.25	0.25
Mono-/di-calcium phosphate	0.20	0.20	0.20	0.20	0.20

DL-Methionine	0.15	0.15	0.15	0.15	0.15
Choline chloride, 60%	0.10	0.10	0.10	0.10	0.10
L-Valine	0.07	0.07	0.07	0.07	0.07
L-Tryptophan	0.06	0.06	0.06	0.06	0.06
Phytase[6]	0.02	0.02	0.02	0.02	0.02
Analyzed nutrient content, %					
Dry matter	88.13	88.18	88.24	88.63	88.74
Starch	31.13	31.36	29.56	30.98	29.40
Crude protein	26.76	25.72	26.38	26.36	26.26
Neutral detergent fiber	8.66	10.66	10.34	15.06	10.80
Acid detergent fiber	5.32	4.95	5.13	4.95	5.20
Crude fiber	3.07	3.13	3.42	3.49	3.45
Crude fat	3.12	3.19	3.96	3.81	4.09
Crude ash	7.10	6.81	7.12	6.63	7.04
Calcium	0.91	0.85	0.81	1.02	0.96
Phosphorus	0.64	0.59	0.67	0.62	0.68
Indispensable amino acid					
Arginine	1.44	1.43	1.54	1.47	1.42
Histidine	0.54	0.54	0.55	0.53	0.52
Isoleucine	0.98	0.96	0.96	0.94	0.91
Leucine	1.62	1.61	1.61	1.59	1.54
Lysine	1.52	1.52	1.51	1.45	1.48
Methionine	0.41	0.43	0.42	0.41	0.42

Phenylalanine	1.08	1.06	1.02	1.05	1.03
Threonine	0.97	0.97	1.11	1.10	1.11
Tryptophan	0.26	0.30	0.29	0.28	0.28
Valine	1.19	1.16	1.11	1.10	1.11
Dispensable amino acid					
Alanine	0.94	0.94	0.95	0.92	0.92
Aspartic acid	2.03	2.01	2.04	1.98	1.98
Cysteine	0.33	0.33	0.32	0.33	0.33
Glutamic acid	4.48	4.47	4.53	4.43	4.30
Glycine	1.02	1.01	1.03	1.00	1.00
Proline	1.30	1.34	1.28	1.29	1.30
Serine	0.85	0.86	0.99	0.86	0.84
Tyrosine	0.72	0.71	0.69	0.69	0.67
Gross energy, MJ/kg	16.47	16.63	16.68	16.59	16.77

[1]Diets were formulated to provide 10.2 MJ NE/kg, and 1.3 g SID Lys/MJ NE.

[2]HP300 (Hamlet Protein Inc., Findlay, OH).

[3]Celite 281 (World Minerals Inc., Santa Barbara, CA).

[4]Supplied per kilogram of diet: 7,500 IU of vitamin A, 750 IU of vitamin D, 76 IU of vitamin E, 60 mg of niacin, 21 mg of pantothenic acid, 2.8 mg of folacin, 6 mg of riboflavin, 4.8 mg of thiamine, 1,000 mg of choline, 4 mg of vitamin K, 0.33 mg of biotin, and 3 mg of vitamin B_{12}.

[5]Supplied per kilogram of diet: 149 mg of Zn as $ZnSO_4$, 53.9 mg of Cu as $CuSO_4$, 199 mg of Fe as $FeSO_4$, 49 mg of Mn as $MnSO_4$, 0.5 mg of I as $Ca(IO_3)_2$, and 0.3 mg of Se as Na_2SeO_3.

[6]Ronozyme HiPhos 2500 (DSM Ronozyme Hyphos–GT; North Dumfries, Ontario, Canada).

Table 2.2. Ingredient composition and analyzed nutrient content (as fed) of diets including five faba bean cultivars fed to weaned pigs during phase 2 (day 14 to 28)[1]

	Snowbird	Snowdrop	Tabasco	Fabelle	Malik
Ingredient, %					
Wheat, Hard Red Spring	49.63	49.48	47.96	48.86	48.12
Snowbird faba bean	30.00	–	–	–	–
Snowdrop faba bean	–	30.00	–	–	–
Tabasco faba bean	–	–	30.00	–	–
Fabelle faba bean	–	–	–	30.00	–
Malik faba bean	–	–	–	–	30.00
Soybean meal	10.00	10.00	10.00	10.00	10.00
Menhaden fish meal	2.50	2.50	2.50	2.50	2.50
Soy protein concentrate[2]	2.50	2.50	2.50	2.50	2.50
Canola oil	0.90	1.00	2.60	1.70	2.40
Limestone	1.30	1.30	1.30	1.30	1.30
Celite[3]	0.80	0.80	0.80	0.80	0.80
Salt	0.70	0.70	0.70	0.70	0.70
Vitamin premix[4]	0.40	0.40	0.40	0.40	0.40
Trace mineral premix[5]	0.40	0.40	0.40	0.40	0.40
L-Lysine HCl	0.36	0.40	0.32	0.32	0.36
L-Threonine	0.16	0.16	0.16	0.16	0.16
Mono-/di-calcium phosphate	0.15	0.15	0.15	0.15	0.15

DL-Methionine	0.11	0.11	0.11	0.11	0.11
Choline chloride, 60%	0.05	0.05	0.05	0.05	0.05
L-Tryptophan	0.03	0.03	0.03	0.03	0.03
Phytase[6]	0.02	0.02	0.02	0.02	0.02
Analyzed nutrient content, %					
Dry matter	89.19	88.83	89.22	89.01	89.31
Starch	38.30	40.46	37.87	41.22	37.20
Crude protein	26.63	26.33	27.46	26.98	25.92
Neutral detergent fiber	10.41	12.05	13.53	15.07	11.65
Acid detergent fiber	6.08	6.72	6.48	6.14	6.61
Crude fiber	3.98	4.30	4.09	4.19	4.34
Crude fat	3.10	4.04	3.81	2.81	3.97
Crude ash	6.33	6.40	6.50	6.33	6.22
Calcium	0.72	0.68	0.73	0.69	0.71
Phosphorus	0.57	0.58	0.63	0.59	0.64
Indispensable amino acid					
Arginine	1.52	1.47	1.58	1.58	1.54
Histidine	0.55	0.54	0.54	0.55	0.55
Isoleucine	0.95	0.94	0.95	0.95	0.95
Leucine	1.64	1.60	1.64	1.63	1.61
Lysine	1.53	1.49	1.43	1.40	1.45
Methionine	0.35	0.36	0.36	0.33	0.36
Phenylalanine	1.10	1.07	1.11	1.08	1.09

Threonine	0.89	0.88	0.86	0.86	0.88
Tryptophan	0.27	0.26	0.29	0.25	0.26
Valine	1.05	1.04	1.02	1.06	1.04
Dispensable amino acid					
Alanine	0.92	0.91	0.89	0.91	0.90
Aspartic acid	2.03	1.95	1.97	1.98	2.01
Cysteine	0.36	0.34	0.36	0.34	0.35
Glutamic acid	4.85	4.66	4.62	4.76	4.75
Glycine	1.01	1.00	1.00	1.01	0.97
Proline	1.43	1.39	1.33	1.40	1.39
Serine	0.95	0.90	0.87	0.87	0.88
Tyrosine	0.73	0.68	0.79	0.70	0.71
Gross energy, MJ/kg	16.66	16.54	16.87	16.78	17.03

[1]Diets were formulated to provide 10.1 MJ NE/kg, and 1.2 g SID Lys/MJ NE.

[2]HP300 (Hamlet Protein Inc., Findlay, OH).

[3]Celite 281 (World Minerals Inc., Santa Barbara, CA).

[4]Supplied per kilogram of diet: 6000 IU of vitamin A, 600 IU of vitamin D, 46 IU of vitamin E, 56 mg of niacin, 21 mg of pantothenic acid, 2.8 mg of folacin, 6 mg of riboflavin, 4.8 mg of thiamine, 1000 mg of choline, 3.2 mg of vitamin K, 0.28 mg of biotin, and 3 mg of vitamin B12.

[5]Supplied per kilogram of diet: 122 mg of Zn as $ZnSO_4$, 43.2 mg of Cu as $CuSO_4$, 173 mg of Fe as $FeSO_4$, 44 mg of Mn as $MnSO_4$, 0.4 mg of I as Ca $(IO_3)_2$, and 0.24 mg of Se as Na_2SeO_3.

[6]Ronozyme HiPhos 2500 (DSM Ronozyme Hyphos –GT; North Dumfries, Ontario, Canada).

Table 2.3. Analyzed chemical composition (as is) of five faba bean cultivars

Item, %	Snowbird	Snowdrop	Tabasco	Fabelle	Malik
Dry matter	89.50	87.80	89.38	90.40	89.72
Starch	30.70	36.09	33.29	33.36	31.83
Resistant Starch	12.77	16.64	16.05	17.50	16.92
Crude protein	31.84	28.97	30.95	31.86	29.39
Total dietary fiber	19.67	21.35	16.10	22.01	24.50
Soluble dietary fiber	1.22	1.53	0.33	1.82	1.71
Neutral detergent fiber	15.12	13.10	17.10	15.89	14.34
Acid detergent fiber	11.85	10.55	11.52	10.36	12.91
Crude fiber	10.64	8.01	9.59	9.91	10.55
Crude fat	1.03	0.75	0.71	0.57	0.47
Crude ash	3.96	3.34	3.47	3.39	4.04
Calcium	0.13	0.11	0.07	0.10	0.12
Phosphorus	0.56	0.46	0.57	0.46	0.65
Indispensable amino acids					
Arginine	2.48	2.49	3.05	3.06	2.61
Histidine	0.75	0.73	0.76	0.81	0.73
Isoleucine	1.33	1.28	1.32	1.40	1.23
Leucine	2.27	2.13	2.23	2.39	2.07
Lysine	2.00	1.87	1.90	2.05	1.90
Methionine	0.20	0.19	0.19	0.20	0.19
Phenylalanine	1.38	1.26	1.28	1.39	1.23

Threonine	1.02	0.99	0.99	1.08	0.97
Tryptophan	0.22	0.21	0.23	0.18	0.16
Valine	1.41	1.37	1.41	1.53	1.36
Dispensable amino acids					
Alanine	1.20	1.18	1.20	1.31	1.17
Aspartic acid	3.26	3.06	3.13	3.47	3.07
Cysteine	0.38	0.38	0.37	0.39	0.36
Glutamic acid	4.89	4.63	4.83	5.31	4.58
Glycine	1.29	1.25	1.27	1.34	1.21
Proline	1.16	1.16	1.23	1.23	1.13
Serine	1.20	1.14	1.17	1.30	1.11
Tyrosine	1.02	0.89	0.97	1.01	0.84
Total amino acids	27.86	26.51	27.83	29.76	26.21
Anti-nutritional factors					
Vicine, %	0.60	0.58	0.42	0.04	0.43
Covicine, %	0.39	0.40	0.42	0.01	0.42
Condensed tannins[1], %	ND[2]	ND	ND	0.53	0.27
Average particle size, µm	596	447	594	519	625
Standard deviation, µm	2.49	2.54	2.19	2.38	2.37
Gross energy, MJ/kg	16.34	16.62	16.85	16.97	16.83

[1]Detection limit for condensed tannins was 0.20%.

[2]ND, not detected.

Table 2.4. Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE) and crude protein (CP), and digestible energy (DE), and net energy (NE) values of diets including five faba bean cultivars fed to weaned pigs in phase 1 (day 0 to 14) and phase 2 (day 14 -28)[1]

	Diet						
Variable	Snowbird	Snowdrop	Tabasco	Fabelle	Malik	SEM[2]	*P*-value
ATTD of DM, %							
Phase 1	84.6	84.9	84.3	84.2	84.1	0.36	0.168
Phase 2	83.1[bc]	84.3[a]	84.3[a]	83.3[b]	82.1[c]	0.34	< 0.001
ATTD of GE, %							
Phase 1	85.5	85.6	85.4	85.2	85.1	0.41	0.155
Phase 2	83.5[bc]	84.7[a]	85.0[a]	83.7[b]	82.6[c]	0.34	< 0.001
ATTD of CP, %							
Phase 1	81.7	81.3	81.7	80.9	80.1	0.69	0.636
Phase 2	83.7[bc]	84.5[ab]	85.3[a]	82.5[cd]	81.3[d]	0.55	< 0.001
DE, MJ/kg, DM							
Phase 1	16.15	16.15	16.28	16.11	16.19	0.079	0.412
Phase 2	15.65[c]	15.86[b]	16.11[a]	15.86[b]	15.73[bc]	0.063	< 0.001
NE[2], MJ/kg, DM							
Phase 1	10.92	10.96	11.04	10.97	11.00	0.056	0.289
Phase 2	10.73[d]	10.97[ab]	11.04[a]	10.90[bc]	10.81[cd]	0.045	< 0.001

[a to d]Means within a row without a common superscript differ ($P < 0.05$).

[1]Least-squares means based on 13 pen observations per diet.

[2]Diet NE values calculated using Eq. (5) from Noblet et al. (1994).

Table 2.5. Average daily feed disappearance (ADFD), average daily weight gain (ADG), gain-to-feed (G: F), and final body weight (BW) of weaned pigs fed diets including five faba bean cultivars starting one-week post-weaning[1]

Variable	Snowbird	Snowdrop	Tabasco	Fabelle	Malik	SEM[3]	*P*-value
ADFD[2], g							
d 0 to 7	422	409	429	435	407	16.8	0.393
d 7 to 14	702	703	727	779	697	30.9	0.057
d 14 to 21	926	938	933	990	916	33.9	0.226
d 21 to 28	1254	1254	1251	1307	1182	40.3	0.058
d 0 to 28	826[ab]	826[ab]	835[ab]	878[a]	801[b]	22.2	0.020
ADG[2], g							
d 0 to 7	362	350	370	377	336	20.9	0.328
d 7 to 14	533	527	538	584	526	31.9	0.351
d 14 to 21	630	652	663	681	623	27.6	0.218
d 21 to 28	823[ab]	789[ab]	781[ab]	835[a]	753[b]	27.8	0.033
d 0 to 28	587[ab]	579[b]	588[ab]	619[a]	559[b]	14.1	0.002
G:F[2], g:g							
d 0 to 7	0.849	0.851	0.866	0.864	0.822	0.035	0.723
d 7 to 14	0.763	0.748	0.738	0.749	0.756	0.027	0.911
d 14 to 21	0.678	0.697	0.712	0.687	0.677	0.022	0.484
d 21 to 28	0.661	0.632	0.623	0.639	0.640	0.021	0.458
d 0 to 28	0.706	0.704	0.705	0.704	0.697	0.008	0.817
Final BW, kg							

d 28	24.96[ab]	24.77[ab]	24.92[ab]	25.60[a]	24.01[b]	0.48	0.038

[a to c]Means within a row without a common superscript differ ($P < 0.05$).

[1]Least-squares means based on 13 pen observations per diet.

[2]Week effect for ADFD, ADG, and G:F ($P < 0.05$).

Table 2.6. Feces consistency scores[1] from pigs fed five faba beans cultivar diets[2]

Period[3]	Snowbird	Snowdrop	Tabasco	Fabelle	Malik	SEM	*P*-value
d 0 to 7	3.91	3.97	3.88	3.92	3.88	0.103	0.908
d 7 to 14	3.86	3.85	3.88	3.91	3.78	0.103	0.778
d 14 to 21	3.39	3.43	3.42	3.38	3.45	0.103	0.956
d 21 to 28	3.12	3.16	3.12	3.16	3.12	0.103	0.989
d 0 to 28	3.57	3.60	3.57	3.59	3.56	0.052	0.912

[1]Score 1 = hard, dry, and pellet-like; 2 = firm but not hard, segmented appearance; 3 = log-like, moist surface; 4 = moist/soggy, distinct log shape; 5 = moist, distinct shape, present in piles rather than logs; 6 = has texture, but not defined shape; 7 = watery, no texture, flat, occurs as puddles; 8 = liquid, with a slight brown or yellow tinge.

[2]Least-squares means based on 13 pen observations per diet.

[3]Week effect ($P < 0.05$).

Chapter 3: Energy, protein and amino acids digestibility of mid- or zero-tannin faba bean differing in vicine and covicine content fed to growing pigs

3.1 Introduction

In temperate regions, to reduce reliance on imported sources of both dietary energy and protein from tropical zones given their transportation footprint, pig producers could increase inclusion of locally grown pulses in feed. Agronomic benefits of growing pulses include atmospheric nitrogen fixation by root rhizobia, diversifying soil microbial populations and nutrient uptake, and breaking pest and disease cycles in yearly crop rotation with cereal grains and oilseeds (Neugschwandtner et al., 2015). Of particular interest among pulses is faba bean (*Vicia faba L.*) due to its greater yield compared to field pea, lower crop inputs compared to other crops and lower carbon footprint than producing animal protein sources (Heusala et al., 2020). Faba bean grain contains 330 to 460 g starch/kg and 280 to 370 g protein/kg (Hoover and Sosuski, 1991). Predominantly white flowered, zero-tannin faba bean cultivars are grown because of their high energy and protein digestibility for monogastric animals (Crépon et al., 2010). However, zero-tannin cultivars have poor spring emergence in cold soil and greater susceptibility to fall frost damage compared with tannin cultivars (Kantar et al., 1994; Braaten, 2012). Tannins mostly concentrated on the bean hull protect against frost by promoting cooling of cellular molecules below their freezing point without producing solidification and crystallization acting as anti-ice nucleating agent that prevents intracellular ice formation (Koyama et al., 2014).

Inclusion of faba bean grain in swine diets might be limited by their content of antinutritional factors. The main adverse effects of tannins include their astringent taste affecting feed intake, irreversible binding with digestive enzymes and exogeneous proteins as well as precipitation of salivary protein in monogastric animals (Hagerman and Butler, 1980). Vicine and covicine that are present in most faba bean cultivars cause haemolytic anaemia in humans (favism) with an erythrocyte genetic deficiency of glucose-6-phosphate dehydrogenase (Arese et al., 2012) and can also reduce energy digestibility and growth performance in monogastric animals such as rats and chickens (Blair et al., 1970; Marquardt et al., 1974). Presence of resistant starch and non-starch polysaccharides can also reduce energy and nutrient digestibility despite having benefits to pig's gut health as prebiotics. Inclusion of faba bean in young pig diets may also be limited by the presence of soluble oligosaccharides (α-galactosides) that may stimulate diarrhoea due to excessive fermentation. As for other pulses, the presence of phytic acid and trypsin inhibitors in faba bean grain can reduce digestibility of dietary phosphorus and other essential mineral, as well as protein, respectively. Previously, pigs fed zero tannin faba bean (cultivar Snowbird) instead of soybean meal did not have reduced growth performance (Zijlstra et al., 2008; Beltranena et al., 2009). Notwithstanding, novel faba bean cultivars with and without tannins that prevent frost damage are available but vary in nutrient content, digestibility and other antinutritional factors that must be characterized to realize predictable growth performance in growing pigs.

The null hypothesis of the present study was that feeding diets including faba bean cultivars differing in tannin and vicine and covicine content would not affect energy, protein, and amino acid digestibility. The objective was to compare the coefficient of apparent ileal digestibility of gross energy, starch and crude protein, the coefficient of standardised ileal digestibility of crude protein and amino acids, the coefficient of apparent total tract digestibility of dry matter and gross energy, and digestible energy and calculated net energy values in ileal-cannulated growing pigs fed four faba bean cultivars diets differing in nutrient, tannin and vicine and covicine content.

3.2 Materials and methods

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, Alberta, Canada).

3.2.1 Test articles, diets and processing

Snowbird faba bean (low tannin, high vicine and covicine cultivar) was sourced from Millhouse Farms Inc. (Cutknife, Saskatchewan, Canada) from the 2020 crop. Snowdrop (low tannin, high vicine and covicine cultivar) was grown at the Crop Diversification Centre North (Edmonton, Alberta, Canada) in 2019. Fabelle (mid tannin, low vicine and covicine cultivar) was sourced from Stamp Seeds (Enchant, Alberta, Canada) from the 2020 crop. Florent (mid tannin, high vicine and convicine) was grown by CAS Grain Farm Partnership (Tisdale, Saskatchewan, Canada) in 2019. Four experimental diets were mixed each including 950 g/kg of one of the four faba bean cultivars (Table 1). Faba bean grains were ground through a 3.6-mm screen using a hammer mill (Model Jacobson 5550-113-01, Carter Day International, Minneapolis, Minnesota, USA). Macro minerals, salt, vitamins, trace minerals and marker were added at the same levels to all test diets to meet requirement (NRC, 2012). Ingredient composition of the nitrogen-free diet was a per Stein et al. (2007) with minor changes. Diets were mixed (6 minutes) using a horizontal, paddle mixer (model SPC2748, Marion Mixers Inc., Marion, Iowa, USA), hand-bagged and sampled.

3.2.2 Experimental design and management

The study was designed as a double 4 × 4 Latin square for a total of 8 pig observations per dietary treatment. The 4 dietary treatments were allotted to each individual pig in each square, so that each 4 pigs consumed one of 4 test diets over 4 experimental periods and the nitrogen-free diet for an additional period at the end of the experiment to measure basal endogenous losses of protein and amino acids. Each experimental period (9 days) consisted of a 5-day adaptation and a 4-day specimen collection (2 days for faecal samples and subsequently 2 days for digesta samples). Individual pig was both the sampling and experimental unit. Pigs were individually weighed using standardized scales at the beginning of each period. Individual metabolism pens measured 1.2 m width × 1.5 m length × 0.95 m height (1.8 m^2). Pen sides were made of polyvinyl plastic planking with front (0.3 m width × 0.4 m height) and side (0.4 m × 0.4 m) plexiglass windows. Metabolism pens were raised on a galvanized steel frame 0.4 m from the concrete floor of the room. Each pen was equipped with a stainless-steel feeder attached to the front of the pen (trough was 0.35 m width × 0.4 m height, 0.12 m off pen floor). A single adjustable-height cup drinker was also attached to the front wall (0.25 m above pen floor) near the feeder. There were two 0.56 m wide × 0.61 m deep slurry gutters in the room, one located under each row of pens. The room used negative pressure to ventilate, created by a single exhaust fan chimney located in the ceiling. Throughout the entire experiment, pigs had free access to water from the pen cup drinker. Temperature of the room was maintained at 22 ± 2.5ºC. Room lights were controlled by a timer set to come on at 07:00 and off at 19:00.

Eight crossbred, Duroc × Large White/Landrace barrows (F1), weighing approximately 37.5 kg were involved in a previous experiment and had a simple T-cannula surgically inserted at the distal ileum at approximately 28 days of age. Pigs had a 5-day diet adaptation period prior to specimen collections. Test diets were offered at 3.0 × maintenance (460 KJ DE/kg of $BW^{0.75}$; NRC, 2012) divided in two daily meals (~08:00, ~15:00) of approximately equal size. During the diet adaptation and specimen collection phases of each period, amount and consumption of each meal were confirmed and recorded. Velcro rings for colostomy bags were glued around the anus of all pigs on the last day of the adaptation period. Early in the morning (~8:00) on the days of faecal collections, colostomy bags were snapped between the inner Velcro and the leather ring for collection of faeces. Faeces were collected for 2 days prior to digesta collection. Early in the morning of ileal digesta collection days (~8:00), plastic bags containing approximately 15 mL of 5% formic acid were attached to the open T-cannula using rubber bands. Digesta was collected continuously from approximately 8:00 am until 16:00 pm for 2 days. Faeces lying on the pen floor during digesta collection were promptly cleaned to prevent coprophagy and recycling of marker. At the end of each period, digesta and faeces were homogenized, subsampled and freeze-dried.

3.2.3 Laboratory analyses

Test ingredients (faba bean cultivars), diets and lyophilized digesta and faeces were ground through a 1-mm screen in a centrifugal mill (Model ZM200, Retch GmbH, Haan, Germany) and were analysed for dry matter (**DM**; method 930.15; AOAC, 2006), crude protein (**CP**) by Leco (nitrogen [**N**] × 6.25; method 990.03) and gross energy (**GE**) using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany) at the University of Alberta. Test ingredients, diets and digesta samples were also analysed for total (K-TSTA-100A; method 996.11) and resistant starch (K-RSTAR; method 2002.02) using assay kits (Megazyme; Bray, Ireland) at the University of Alberta. Test ingredients and diets were analysed for crude fat (**CF**; method 920.39A), 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) and ash (method 942.05) at the Agricultural Experiment Station Chemical Laboratories (**ESCL**), University of Missouri (Columbia, Missouri, USA). Test ingredients were analysed for total dietary fibre (method 985.29), soluble and insoluble dietary fibre (method 991.43) and crude fibre (method 934.01), calcium (method 968.08), phosphorus (method 946.06), amino acids (**AA**) [method 982.30E (a–c)] at ESCL. Total tannins (method 952.03) and trypsin inhibitor activity

(**TIA**; method NEN-EN-ISO 14902:2001; NEN, 2001) were analysed at Nutrilab B.V. (Burgstraat, Giessen, the Netherlands). Vicine and covicine were analysed using a slight modification of the extraction procedure (Purves et al., 2018) at the Organic Residue Laboratory of Alberta Agriculture and Forestry (Edmonton, Alberta, Canada). Diets, faeces and digesta were analysed for TiO_2 content (Myers et al., 2004). Particle size of test ingredients and diets was measured using a mechanical sieve shaker (Model RX-29, W.S. Tyler, Ontario, Canada) following the method of the American Society of Agricultural and Biological Engineers (2008) at the University of Alberta.

3.4.1 Calculations and statistical analyses

The coefficient of apparent ileal digestibility (**CAID**) of DM, GE, starch, CP, and coefficient of apparent total tract digestibility (**CATTD**) of DM, GE, and CP in test diets were calculated using the index method (Adeola, 2001):

$$CAID \text{ or } CATTD = 1 - \frac{Concentration\ TiO2\ \textit{in feed} \times \textit{content of component in digesta or feces}}{Concentration\ TiO2\ in\ digesta\ or\ feces \times \textit{content of component in feed}}$$

Basal ileal endogenous loss (**I_{end}**) of AA and CP (g/kg DM intake) was determined and standardised ileal digestibility coefficient (**CSID**) for AA and CP in diets calculated by correcting for basal I_{end} of AA and CP using CAID (Stein et al., 2007) as follows.

Basal $I_{end} = AA_{digesta} \times (M_{diet} / M_{digesta})$,

$CSID = CAID + (basal\ IAA_{end} / AA_{diet})$

Where M_{diet} was the concentration of marker in the diet and $M_{digesta}$ was the marker concentration in digesta.

Diet and ingredient digestible energy (**DE**) values were calculated by multiplying diet GE by CATTD of GE. Diet and test ingredient NE values were calculated as per equation (5) of Noblet et al. (1994) adopted by NRC (2012) using determined DE value and analysed content of starch, CF, CP, and ADF.

Data were analysed using the GLIMMIX procedure of SAS (2016). Diet was the fixed effect; square, period nested within square, and pig nested within square were random terms. Normality and homogeneity of variance of residuals of each variable and carry-over effect were tested prior to analysis. The P values were adjusted by applying the Tukey option for multiple comparisons among treatments. Differences between treatments were considered significant if $P < 0.05$.

3.3 Results

Zero-tannin faba bean cultivars (Snowbird and Snowdrop) contained 21.9 g/kg less CP and 11.8 g/kg more starch than mid-tannin cultivars (Fabelle and Florent; Table 3). Approximately 40% of starch in faba bean cultivars was resistant starch. The NDF and ADF ranged from 112 to 134 g/kg and 79 to 103 g/kg, respectively, and were greatest in Snowdrop cultivar. Approximately 95% of dietary fibre in faba bean grain was insoluble. The GE value of faba bean cultivars averaged 16.4 MJ/kg and lysine content ranged from 16 to 19 g/kg. Mid-tannin cultivars contained double the tannin content than zero-tannin counterparts. Zero-tannin cultivars contained 0.6 g/kg more vicine and 1.5 g/kg more covicine than mid-tannin cultivars. Between mid-tannin cultivars, Fabelle contained less vicine and covicine than Florent, whereas vicine and covicine content of the latter was similar to zero-tannin cultivars.

Diet CAID of DM and GE were greatest ($P < 0.05$) for pigs fed Snowbird and Snowdrop, lowest ($P < 0.05$) for Florent and intermediate for Fabelle (Table 4). Diet CAID of starch was greater ($P < 0.05$) for pigs fed zero- than mid-tannin cultivars. Diet CAID of CP was greater ($P < 0.05$) for pigs fed Snowbird, Snowdrop and Fabelle than Florent. Diet CATTD of DM and GE was greatest ($P < 0.05$) for pigs fed Snowbird, lowest for Florent whereas Snowdrop and Fabelle were intermediate. Diet CATTD of CP was greater ($P < 0.05$) for pigs fed zero- than mid-tannin cultivars. Diet and ingredient DE values were greater (P < 0.05) for pigs fed Snowbird than Snowdrop within zero-tannin cultivars and greater (P < 0.05) for Fabelle than Florent within mid-tannin cultivars. Diet and ingredient NE values were greatest (P < 0.05) for pigs fed Snowbird but did not differ among Snowdrop, Fabelle and Florent cultivars.

Diet CAID of most essential AA were greater (P < 0.05) for pigs fed Snowbird, Snowdrop and Fabelle than Florent (Table 5). Diet CAID of tryptophan was greater (P < 0.05) for pigs fed Fabelle than Snowbird, Snowdrop and Florent cultivars. Ingredient CSID of CP, lysine, threonine, and methionine were generally greater (P < 0.05) for pigs fed Snowbird, Snowdrop and Fabelle than Florent. The CSID of tryptophan was greater (P < 0.05) for Fabelle than Snowbird, Snowdrop and Florent.

3.4 Discussion

To feed locally grown pulses, pig producers need to be convinced that pulse grains are cost effective and content of antinutritional factors has no major detrimental effects on nutrient digestibility that could affect growth performance. In addition to genotype, location and agronomic conditions, cultivar is a major source of variation that should be characterized to prioritize which faba bean cultivars to grow for animal feeding. This information is becoming available (Ivarsson and Neil, 2018; Mayer Labba et al. 2021), but cultivar availability differs among countries or global regions and evolves with genetic selection over time. Thus, current regional cultivar information is of economic relevance to both pulse growers and pig producers.

3.4.2 Chemical characteristics

Starch is the dominant constituent in faba bean grain followed by crude protein (Gdala and Buraczewska, 1997). Starch content of faba bean cultivars in the present study was lower than previously reported (Cho et al., 2019) but greater in zero- than mid-tannin cultivars. This finding contrasts with similar starch and crude protein content among 16 white- and colour-flowered faba bean cultivars reported by Ivarsson and Neil (2018). The nutrient content differences between zero- and mid-tannin cultivars observed in the present study relate in part to genotype, seed characteristics and agronomic conditions (Gatel and Grosjean, 1990). The inverse relationship between starch and crude protein in faba bean cultivars fed in the present study is consistent with earlier reports of strong negative correlations in faba bean (Duc et al., 1999) and field pea (Bastianelli et al., 1998) between these two grain components. Similar to other pulse grains, faba bean contain more resistant starch than most cereal grains. More resistant starch than cereals indicates that a greater portion of total starch is resistant to enzymatic hydrolysis and thus has a lower energetic efficiency in pigs (Bello-Pérez et al., 2007; Tan et al., 2021a). Compared with cereal grains, faba bean cultivars fed in the present study contained greater lysine but insufficient sulphur-containing amino acids (methionine and cysteine) and tryptophan (NRC, 2012). The third most relevant constituent in faba bean grain was dietary fibre (Duc et al., 1999); 96% of it being insoluble (Jezierny et al., 2010). Slightly greater ADF and less NDF in colour- than white-flowered faba bean suggests that enteric microbial fermentation in pigs might be greater for white-flowered faba bean (Jha and Berrocoso, 2015).

As expected, colour-flowered cultivars (Fabelle and Florent) contained more tannins than white-flowered cultivars (Snowbird and Snowdrop; Cabrera and Martin, 1986). Variation in vicine and covicine content among cultivars did match previous studies (Ivarsson and Neil, 2018; Cho et al., 2019) with greater content in white- than colour-flowered faba bean cultivars. Greater vicine and covicine content in Florent, Snowbird and Snowdrop in the present study was likely due to activity of guanosine triphosphate cyclohydrase II, a key enzyme involved in their synthesis (Björnsdotter et al., 2021). In the present study, trypsin inhibitors activity did not vary considerably among faba bean cultivars (Gdala and Buraczewska, 1997). In contrast, previous studies (Duc et al., 1999; Valdebouze et al., 1980) using similar method of analysis reported greater variability in trypsin inhibitors activity (3.3 to 6.2 mg/g DM) among faba bean cultivars. Laboratory assay variability despite similar analytical methods may restrict accurate comparisons of trypsin inhibitors activity distribution in faba bean cultivars (Valdebouze et al., 1980).

3.4.3 Energy digestibility

The range of chemical characteristics among faba bean cultivars in particular total and resistant starch, crude protein, dietary fibre, and antinutritional factors including tannins and vicine and covicine indicate that variation in energy digestibility exists. In the present study, CAID of starch was slightly greater (1.2%) than previously reported for Snowbird (Tan et al., 2021b). Greater CAID of starch in zero-than mid-tannins faba bean cultivars matched their lower tannin content. Indeed, tannins is a major group of antinutritional factors present primarily in the hull of faba bean grain that can reduce nutrient digestibility. Tannins can bind to starch and pancreatic α-amylase and consequently inhibit starch digestibility (Deshpande and Salunkhe, 1982). In addition to tannins, lower CAID of starch in Florent than Snowbird and Snowdrop diets was consistent with its greater resistant starch content. Compared with wheat and corn starch, faba bean contains more resistant starch in the form of amylose (Tan et al., 2021b) that is more resistant to enzymatic hydrolysis than amylopectin (Bello-Pérez et al., 2007). Furthermore, greater CAID of starch in Snowdrop than Fabelle and Florent diets might be due to lower total dietary fibre. Similar to other pulse grains, faba bean starch is trapped within cell walls, thereby presenting a physical barrier for enzymatic hydrolysis (Würsch et al., 1986). Insoluble portion of dietary fibre is typically associated with increased rate of passage thus reducing time for enzymatic digestion of nutrients in the small intestine (Hooda et al., 2011). Consequently, the lowest CAID of energy in the Florent diet matched its lower CAID of starch than the Snowbird and Snowdrop diets.

Diet CAID and CATTD of energy and DE value in the present study matched those from previous studies (Crépon et al., 2010) with lower values for mid- than zero-tannin cultivars. The effect of tannins seems thus to be important by reducing energy digestibility (Jansman et al., 1993). Lower CATTD of energy in Florent than Fabelle diets was consistent with previously reported lower CATTD of energy in high tannin faba bean cultivars with high vicine and covicine than with low vicine and covicine, possibly due to the additive effect of tannins and vicine and covicine (Grosjean et al., 2001). Effects of vicine and covicine on reduced energy digestibility was previously linked to reduced fat digestibility. Divicine and isouramil are metabolised from vicine and covicine, respectively, that react with blood oxygen forming reactive oxygen species such as lipid peroxides (Hegazy and Marquardt, 1984). Lipid peroxides may result in excessive β-oxidation of fatty acids due to reduced redox homeostasis thereby causing liver damage and oxidative stress (Losowsky and Walker, 1969). Liver damage may impair bile secretion thereby lowering dietary fat digestibility. However, fat content in faba bean grain and diets in the present study was low. Instead, carbohydrates, especially starch, was the major source of energy in faba bean. Thus, clarification on mechanisms for vicine and covicine to reduce energy digestibility in faba bean in pigs is needed. By difference (CATTD minus CAID of GE), the proportion of dietary energy digested in the hindgut was 5% greater in Florent than Snowdrop and Fabelle matching its greater resistant starch and soluble fibre content. The calculated NE value of faba bean cultivars in the present study was 0.53 MJ/kg greater than reported by NRC (2012). The greatest calculated NE value of Snowbird matched its greater DE value, total starch, lower CP and ADF content. However, because 12 to 17% of starch in faba bean grain was fermented and not hydrolysed into glucose, applying equations by Noblet et al. (1994) as adopted by NRC (2012), that use total starch content may overestimate NE value (Fouhse and Zijlstra, 2017). Resistant starch may need to be considered fermentable fibre for accurate prediction of NE values in feedstuffs (Tan et al., 2021b).

3.4.4 Protein and amino acid digestibility

In addition to starch, faba bean grain provides protein and amino acids. In the present study, average CSID of CP was 4.1% less than reported by NRC (2012). The SID content of lysine was consistent with reported values in the French database (Sauvant et al., 2004) for zero-tannin faba bean, but was 0.35 g/kg less than the reported 16.6 g/kg for colour-flowered faba bean. Between mid-tannin cultivars, combination of tannins with other factors such as resistant starch, amino acid profile and interactions between protein and carbohydrates within grain likely contributed to lower CSID of crude protein and essential amino acids including lysine, threonine, and tryptophan in Florent than Fabelle. The CSID of tryptophan was greater in mid- than zero-tannin faba bean, in contrast to the French database (Sauvant et al., 2004) reporting 11% greater CSID of tryptophan in white- than colour-flowered faba bean cultivars. Condensed tannins have great affinity for hydrophobic AA and proline-rich proteins (Hagerman and Butler, 1980). Indeed, CAID of tryptophan, histidine, glycine, and proline was lower in high- than low-tannin sorghum cultivars (Cousins et al., 1981). Low CAID of proline, glycine and tryptophan may result from increased endogenous protein secretion and reduced digestibility due to increased dietary tannin content. These contradicting results might indicate different affinity of tannins of different botanical origins for proteins with different amino acid profile (Asquith and Butler, 1986). In addition to tannins, as for other pulse grains, the presence of fibre and resistant starch bound to protein in faba bean may interfere with proteolysis thereby lowering protein digestibility (Gdala, 1998). Thus, greater resistant starch and ADF in Florent might also explain its lowest CSID of protein and essential amino acids.

3.5 Conclusions

Tannin content seemed to reduce digestibility of starch in mid- versus zero-tannin faba bean cultivars. Florent with both mid-tannin and vicine and covicine content, had lower energy and amino acid digestibility than zero-tannin cultivars. Fabelle with mid-tannin but the least vicine and convicine content, was intermediate in energy but not different in protein or amino acid digestibility from zero-tannin cultivars. Cultivar variation in content of macronutrients including total and resistant starch, crude protein and dietary fibre contributed to difference in energy, protein and amino acid digestibility and should be considered in diet formulation.

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

Item	Faba bean cultivar Snowbird	Snowdrop	Fabelle	Florent	Nitrogen-free
Snowbird faba bean	950.0				
Snowdrop faba bean		950.0			
Fabelle faba bean			950.0		
Florent faba bean				950.0	
Corn starch	3.5	3.5	3.5	3.5	712.0
Sugar					150.0
Solka floc[1]					50.0
Canola oil					30.0
Limestone	14.0	14.0	14.0	14.0	12.5
Mono/di-calcium phosphate	13.0	13.0	13.0	13.0	21.0
Salt	5.0	5.0	5.0	5.0	5.0
Vitamin premix[2]	5.0	5.0	5.0	5.0	5.0
Trace mineral premix[3]	5.0	5.0	5.0	5.0	5.0
K_2CO_3, 550 g K/kg					4.0
TiO_2, 600 g Ti/kg	4.0	4.0	4.0	4.0	4.0
MgO, 550 g Mg/kg					1.0
Choline chloride, 600 g/kg	0.5	0.5	0.5	0.5	0.5

[1]Solka-floc, International Fiber Corp., North Tonawanda, NY, USA.

[2]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_{12}.

[3]Supplied per kilogram of diet: 125 mg of Zn as $ZnSO_4$, 50 mg of Cu as $CuSO_4$, 75 mg of Fe as $FeSO_4$, 25 mg of Mn as $MnSO_4$, 0.5 mg of I as Ca $(IO_3)_2$ and 0.3 mg of Se as Na_2SeO_3.

Table 3.2. Analysed nutrient content (g/kg, standardised to 880 g DM/kg), gross energy value and particle size of experimental diets.

Item	Faba bean cultivar				Nitrogen -free
	Snowbird	Snowdrop	Fabelle	Florent	
Starch	361.8	312.6	296.8	329.8	664.2
Crude protein	234.5	260.5	298.8	267.4	4.9
Neutral detergent fibre	115.2	135.7	118.1	129.7	20.2
Acid detergent fibre	82.4	106.7	95.3	101.8	11.8
Crude fat	6.1	4.3	1.6	2.4	5.4
Crude fibre	70.9	94.2	71.3	75.2	13.6
Crude ash	59.6	58.8	67.5	55.6	37.9
Indispensable amino acid					
Arginine	16.6	20.1	27.8	22.0	0.1
Histidine	5.4	5.8	6.9	6.1	0.1
Isoleucine	9.1	10.3	11.6	10.0	0.1
Leucine	15.5	17.0	19.8	17.2	0.5
Lysine	14.4	15.1	17.5	15.6	0.2
Methionine	1.7	1.6	1.9	1.9	0.1
Phenylalanine	9.2	10.1	11.5	9.9	0.2
Threonine	7.6	7.8	9.2	8.1	0.1
Tryptophan	1.7	1.6	1.5	1.4	< 0.1
Valine	10.0	11.4	13.0	11.3	0.2

Dispensable amino acid					
Alanine	8.7	11.6	11.0	9.7	0.2
Aspartic acid	23.4	24.8	29.5	25.5	0.3
Cysteine	3.2	2.9	3.5	3.2	0.1
Glutamic acid	3.4	3.8	4.5	3.9	0.1
Glycine	9.2	9.8	11.3	10.1	0.2
Proline	8.7	9.8	11.4	9.5	0.3
Serine	8.6	9.2	1.0	0.9	0.1
Tyrosine	6.7	7.6	8.7	7.3	0.2
Total amino acids	196.5	218.9	253.1	219.2	4.3
Average particle size, µm	562	467	540	603	n/a[1]
Standard deviation, µm	3.0	2.8	2.9	2.7	n/a
Gross energy, MJ/kg	15.7	15.7	15.9	15.8	14.7

[1]Not available.

Table 3.3. Analysed nutrient content (g/kg, standardised to 880 g DM/kg), gross energy value, anti-nutritional factors and particle size of 4 faba bean cultivars included in experimental diets.

Item	Snowbird	Snowdrop	Fabelle	Florent
Dry matter	863.3	903.1	874.5	877.0
Starch	363.2	335.5	331.2	344.0
Resistant starch	142.6	120.8	135.5	150.8
Crude protein	251.3	277.3	294.9	277.4
Total dietary fibre	166.5	154.3	161.1	157.1
Insoluble dietary fibre	160.1	148.8	155.4	150.9
Soluble dietary fibre	6.3	5.6	5.7	6.2
Neutral detergent fibre	118.8	134.2	112.4	123.4
Acid detergent fibre	79.3	102.6	92.9	101.1
Crude fibre	70.0	85.8	68.8	81.3
Crude fat	11.3	5.1	1.3	2.7
Crude ash	31.3	31.1	37.8	29.1
Phosphorus	4.0	4.1	5.3	3.9
Calcium	1.4	1.3	1.3	1.0
Indispensable amino acids				
Arginine	18.1	21.1	30.2	24.1
Histidine	5.9	6.1	7.4	6.6
Isoleucine	10.2	10.8	12.5	10.9
Leucine	16.7	18.0	21.4	19.0
Lysine	15.9	15.9	19.0	17.1

Methionine	1.9	1.9	2.3	2.1
Phenylalanine	10.1	10.6	12.5	10.9
Threonine	8.3	8.3	10.1	9.1
Tryptophan	1.8	1.9	1.6	1.5
Valine	11.1	11.9	13.9	12.1
Dispensable amino acids				
Alanine	9.6	11.7	11.9	10.6
Aspartic acid	25.6	26.5	32.2	28.0
Cysteine	3.5	3.3	3.9	3.5
Glutamic acid	36.9	39.5	48.2	42.1
Glycine	10.1	10.3	12.2	10.9
Proline	9.8	10.1	12.2	10.6
Serine	9.2	9.8	11.4	10.4
Tyrosine	7.3	8.0	9.5	8.1
Total amino acids	214.9	230.1	274.7	240.4
Anti-nutritional factors				
Vicine, g/kg	6.4	7.0	0.6	5.5
Covicine, g/kg	3.4	3.4	0.4	3.4
Tannins, /kg	6.0	7.7	14.2	13.8
Trypsin inhibitor activity, g/kg	2.1	1.9	2.1	2.0
Average particle size, µm	596	592	611	726
Standard deviation, µm	2.3	2.5	2.5	2.3
Gross energy, MJ/kg	16.5	16.4	16.6	16.4

Table 3.4. Coefficient of apparent ileal digestibility (CAID) and coefficient of 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 and faba bean cultivarss[1] (latter two standardised to 880 g DM/kg)

Variable	Snowbird	Snowdrop	Fabelle	Florent	SEM[2]	*P*-value
CAID of diet						
Dry matter	0.621^{a}	0.639^{a}	0.603^{ab}	0.534^{b}	0.026	0.020
Gross energy	0.659^{a}	0.661^{a}	0.637^{ab}	0.570^{b}	0.026	0.028
Starch	0.878^{a}	0.896^{a}	0.832^{b}	0.847^{b}	0.014	0.001
Crude protein	0.712^{a}	0.700^{a}	0.750^{a}	0.621^{b}	0.022	0.001
CATTD of diet						
Dry matter	0.807^{a}	0.781^{b}	0.758^{b}	0.736^{c}	0.010	< 0.001
Gross energy	0.816^{a}	0.784^{b}	0.764^{b}	0.740^{c}	0.010	< 0.001
Crude protein	0.821^{a}	0.821^{a}	0.762^{b}	0.737^{b}	0.011	< 0.001
Diet						
DE (MJ/kg)	14.63^{a}	13.57^{bc}	13.75^{b}	13.17^{c}	0.172	< 0.001
NE (MJ/kg)	9.82^{a}	8.78^{b}	8.75^{b}	8.51^{b}	0.120	< 0.001
Cultivar						
DE (MJ/kg)	15.55^{a}	14.26^{bc}	14.51^{b}	13.87^{c}	0.181	< 0.001
NE (MJ/kg)	10.45^{a}	9.27^{b}	9.28^{b}	8.99^{b}	0.127	< 0.001

[a-c]Means within a row without common superscripts differ ($P < 0.05$).

[1]Least square means based on 8 pig observations per diet.

[2]SEM, standard error of the mean.

Table 3.5. Coefficient of apparent ileal digestibility (CAID) of amino acids of experiments diets[1]

CAID	Snowbird	Snowdrop	Fabelle	Florent	SEM[2]	*P*-value
Indispensable amino acids						
Arginine	0.877[a]	0.893[a]	0.886[a]	0.836[b]	0.007	< 0.001
Histidine	0.821[a]	0.819[a]	0.804[a]	0.735[b]	0.013	< 0.001
Isoleucine	0.774[a]	0.794[a]	0.773[a]	0.701[b]	0.012	< 0.001
Leucine	0.798[a]	0.814[a]	0.794[a]	0.725[b]	0.013	< 0.001
Lysine	0.813[a]	0.816[a]	0.801[a]	0.729[b]	0.013	< 0.001
Methionine	0.707[a]	0.712[a]	0.672[a]	0.609[b]	0.019	0.002
Phenylalanine	0.793[a]	0.809[a]	0.789[a]	0.716[b]	0.013	< 0.001
Threonine	0.721[a]	0.725[a]	0.726[a]	0.6290[b]	0.021	0.002
Tryptophan	0.709[b]	0.719[b]	0.841[a]	0.743[b]	0.026	0.001
Valine	0.738[a]	0.769[a]	0.757[a]	0.666[b]	0.014	< 0.001
Dispensable amino acids						
Alanine	0.723[b]	0.788[a]	0.738[ab]	0.629[c]	0.019	< 0.001
Aspartic acid	0.813[a]	0.809[a]	0.797[a]	0.732[b]	0.016	0.001
Cysteine	0.601[a]	0.496[a]	0.560[a]	0.407[b]	0.040	0.004
Glutamic acid	0.853[a]	0.8384[ab]	0.821[ab]	0.772[b]	0.023	0.025
Glycine	0.682[a]	0.669[a]	0.682[a]	0.575[b]	0.032	0.021
Serine	0.777[a]	0.787[a]	0.772[a]	0.696[b]	0.015	< 0.001
Tyrosine	0.803[a]	0.786[a]	0.799[a]	0.729[b]	0.010	< 0.001
Total amino acids	0.790[a]	0.797[a]	0.785[a]	0.713[b]	0.016	0.001

[a-c]Means within a row without common superscripts differ ($P < 0.05$).

[1]Least square means based on 8 pig observations per diet.

[2]SEM, standard error of the mean.

Table 3.6. Coefficient of standardised ileal digestibility (CSID[1]) of crude protein and amino acids of faba bean cultivars[2].

CSID	Snowbird	Snowdrop	Fabelle	Florent	SEM[3]	*P*-value
Crude protein	0.781[a]	0.762[a]	0.805[a]	0.681[b]	0.022	0.001
Indispensable amino acids						
Arginine	0.915[a]	0.924[a]	0.909[a]	0.866[b]	0.007	< 0.001
Histidine	0.854[a]	0.850[a]	0.830[a]	0.764[b]	0.013	< 0.001
Isoleucine	0.805[a]	0.821[a]	0.797[a]	0.730[b]	0.012	< 0.001
Leucine	0.829[a]	0.842[a]	0.818[a]	0.753[b]	0.013	< 0.001
Lysine	0.845[a]	0.846[a]	0.828[a]	0.758[b]	0.013	< 0.001
Methionine	0.757[a]	0.764[a]	0.717[ab]	0.654[b]	0.019	0.001
Phenylalanine	0.822[a]	0.836[a]	0.812[a]	0.744[b]	0.013	< 0.001
Threonine	0.790[a]	0.792[a]	0.783[a]	0.684[b]	0.021	0.001
Tryptophan	0.779[b]	0.792[b]	0.922[a]	0.829[b]	0.026	< 0.001
Valine	0.782[a]	0.808[a]	0.791[a]	0.706[b]	0.014	<0.001
Dispensable amino acids						
Alanine	0.780[a]	0.831[a]	0.784[a]	0.680[b]	0.019	< 0.001
Aspartic acid	0.843[a]	0.837[a]	0.821[a]	0.760[b]	0.016	0.001
Cysteine	0.660[a]	0.561[ab]	0.615[a]	0.467[b]	0.040	0.004
Glutamic acid	0.876[a]	0.859[ab]	0.838[ab]	0.791[b]	0.023	0.021
Glycine	0.829[a]	0.808[a]	0.802[ab]	0.709[b]	0.032	0.016
Serine	0.827[a]	0.834[a]	0.814[a]	0.743[b]	0.015	< 0.001
Tyrosine	0.837[a]	0.816[a]	0.826[a]	0.761[b]	0.010	< 0.001

Total amino acids	0.855[a]	0.856[a]	0.836[a]	0.771[b]	0.016	0.001

[a-c]Means within a row without common superscripts differ ($P < 0.05$).

[1]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.

[2]Least square means based on 8 pig observations per diet.

[3]SEM, standard error of the mean.

Table 3.7. Standardised ileal digestible (SID) content of amino acids in faba bean cultivars (g/kg, standardised to 880 g DM/kg)[1]

Variable	Snowbird	Snowdrop	Fabelle	Florent	SEM[2]	*P*-value
Crude protein	196.2[bc]	211.6 [b]	256.2[a]	189.1[c]	6.0	< 0.001
Indispensable amino acids						
Arginine	16.5[d]	19.5[b]	27.3[a]	20.9[b]	0.2	< 0.001
Histidine	5.0[b]	5.2[b]	6.2[a]	5.0[b]	0.1	< 0.001
Isoleucine	8.1[c]	8.9[b]	9.9[a]	7.9[c]	0.1	< 0.001
Leucine	13.7[c]	15.2[b]	17.4[a]	14.3[c]	0.2	< 0.001
Lysine	13.3[b]	13.4[b]	15.7[a]	12.9[b]	0.2	< 0.001
Methionine	1.4[b]	1.5[b]	1.7[a]	1.4[b]	0.1	0.001
Phenylalanine	8.2[c]	8.8[b]	10.1[a]	8.1[c]	0.1	< 0.001
Threonine	6.4[b]	6.5[b]	7.8[a]	6.2[c]	0.2	< 0.001
Tryptophan	1.4[a]	1.5[b]	1.5[a]	1.3[b]	0.1	< 0.001
Valine	8.6[c]	9.6[a]	10.9[a]	8.5[c]	0.2	< 0.001
Dispensable amino acids						
Alanine	7.4[b]	9.7[b]	9.2[ab]	7.2[b]	0.2	< 0.001
Aspartic acid	21.4[b]	22.1[b]	26.3[a]	21.2[b]	0.5	< 0.001
Cysteine	2.3[a]	1.9[b]	2.4[a]	1.7[c]	0.1	0.001
Glutamic acid	32.0[b]	33.8[b]	40.2[a]	33.2[b]	1.0	< 0.001
Glycine	8.3[b]	8.3[b]	9.7[a]	7.7[b]	0.3	0.001
Serine	7.6[c]	8.2[b]	9.2[a]	7.7[bc]	0.1	< 0.001
Tyrosine	6.1[c]	6.5[b]	7.7[a]	6.2[c]	0.1	< 0.001

Total amino acids	182.2[c]	196.2[b]	228.4[a]	185.1[c]	3.9	< 0.001

[a-d]Means within a row without a common superscript differ ($P < 0.05$).

[1]Least square means based on 8 pig observations per diet.

[2]SEM, standard error of the mean.

Chapter 4: General discussion

4.1 Main findings and conclusions

The objective of this MSc thesis was to evaluate effects of feeding modern faba bean cultivars on dietary energy and nutrient digestibility and growth performance in pigs. In Chapter 2, increasing dietary inclusion of faba bean grain to 30% in Phase 2 likely contributed to the ability to detect differences in diet ATTD of dry matter and gross energy and growth performance, probably due to increased dietary content of tannins, vicine and covicine, fibre and resistant starch. Similar ATTD of gross energy in zero-tannin Snowbird and Fabelle with greater tannin content indicated that level of tannins in modern colour-flowered faba bean cultivars may not reduce digestibility of carbohydrates, particularly starch (Jansman, 1993) and thus did not reduce diet energy digestibility. Furthermore, lowest ATTD of crude protein in Malik matching its greater resistant starch and total dietary fibre indicated greater microbial fermentation of carbohydrates in the hindgut of pigs that did reduce ATTD of protein possibly due to increased microbial protein formation. Greater feed disappearance and weight gain for Fabelle than Malik indicated that effects of vicine and covicine, resistant starch and dietary fibre on growth performance should be of greater consideration than solely tannin content at formulating swine diets including modern faba bean cultivars. Finally, greater weight gain of pigs fed Fabelle diets despite lower ATTD of gross energy and crude protein than Snowdrop and Tabasco implied that growth performance in pigs was more related to feed intake than energy and nutrient digestibility.

In Chapter 3, the study was conducted to determine ileal digestibility of nutrients (starch, crude protein, and amino acids) and energy in growing pigs. Hindgut fermentation in pigs modifies digesta protein and amino acid profile and reduces its starch content (Stein et al 2005; Stein and Bohlke, 2007). Results from the present study indicated that tannin content in mid-tannins cultivars reduced AID of starch digestibility, thereby increasing starch available for hindgut fermentation. Moreover, greatest resistant starch in Florent and greater total dietary fibre in Fabelle than Snowdrop might also explain the lowest AID of starch in mid-tannin faba beans cultivars. Consequently, greater AID of energy in zero-tannin cultivars matched their greatest AID of starch and lower content of tannin. However, lower CATTD of energy in Florent than Snowbird, Snowdrop and Fabelle indicated that greater variation in hindgut fermentation associated with variations in resistant starch and total dietary fibre content among faba bean cultivars likely affected digestible energy value. Approximately 15% of total starch in pulse grains is fermented in the hindgut (Fouhse and Zijlstra, 2017) thereby yielding volatile fatty acids. However, fermentation of starch into volatile fatty acids is at least 14% less efficient in yielding net energy than enzymatic digestion of starch to glucose (Jørgensen et al., 1997). Thus, the equation (Noblet et al., 1994) used in the present study to predict net energy value of ingredients using total starch may have overestimated net energy value. Lower SID of crude protein and most essential amino acids in Florent than other faba bean cultivars might be caused partly by greater combined content condensed tannin, vicine and covicine content and resistant starch. Thus, lower protein deposition in pigs fed Florent than pigs fed Snowbird, Snowdrop or Fabelle would be expected.

In conclusion, vicine and covicine instead of tannins appeared to be more relevant anti-nutritional factors in reducing the growth performance in weaned pigs fed balanced diets including up to 30% modern faba bean cultivars. Combined tannins, vicine and covicine contributed to both lower energy and nutrient digestibility in pigs fed Malik (Chapter 2) and Florent (Chapter 3) than zero-tannin cultivars. Cultivar variation in content of macronutrients including total and resistant starch, crude protein and dietary fibre contributed to difference in energy, protein and amino acid digestibility and should be considered in diet formulation.

4.2 Practical implications

Increased domestic use of faba bean by the swine industry would provide an alternative market to a highly competitive human food export market. Because of combined effects of mid-tannins and vicine and covicine in reducing nutrient digestibility and growth performance in pigs, faba bean cultivars with lower content of these two antinutritional factors should be prioritized for feeding pigs. Furthermore, because of variations in harvest yield among cultivars, greatest net energy value and SID lysine yield per hectare would be expected with Fabelle due to its greatest grain yield per unit of land (Table 4.1).

Cost per kg gain did not differ ($P < 0.05$; Table 4.2) among pigs fed five faba bean cultivars in Chapter 2. Therefore, growth performance rather than solely feed cost should be considered feeding modern faba bean cultivars to weaned pigs. However, based on energy and amino acid digestibility results in Chapter 3, lower cost per calorie and lysine would be expected feeding Snowbird and Fabelle respectively as compared with other cultivars.

Despite the greatest growth performance observed in pigs fed the Fabelle diet, the lowest vicine and covicine in Fabelle cultivar would indicate that this cultivar is best suited for human consumption due to no risk associated with favism and therefore, might only be included in swine diets if it does not meet food grade quality for human consumption. However, the greatest harvest yield in Fabelle (Table 4.2) likely due to greater tolerance to frost damage would imply that excess grain production can be used in swine feed. Furthermore, frost damaged faba bean grain can be included in swine feed as frost mostly darkens the hull sparing the cotyledons. Frost also affect grain more in pods located on the upper part of the plant as compared to pods in than lower part of the plant spared by heat convection from soil.

Expanded inclusion of faba bean in rotational cropping system would reduce N-fertilizer use due to increased rhizobia N-fixation reducing N_2O emission and eutrophication in water bodies. Additionally, increased use of locally grown commodities like faba bean inclusion in swine diets would reduce the regional environmental carbon footprint of pork production. Finally, inclusion of faba bean to reduced importation of both corn grain and soybean meal would reduce its transportation carbon footprint, crop inputs and amount of pressure put on land for both corn grain and soybean production to safeguard the sustainable use of environmental resources such as forests.

4.3 Limitations

The two experiments were well designed and executed thoroughly to test the hypotheses. However, some limitations did exist in the conduct of these thesis projects:

For Chapter 2, the start of the animal experiment was delayed by approximately 6 months due to restrictions associated with the Coronavirus (COVID-19) outbreak. These restrictions meant no access to research facilities and congregation for activities such as diet mixing, animal selection and weighing that required teamwork but could not happen due to social distancing rules. Furthermore, there was a limited number of pigs available to select from because of increased demand from other campus research and teaching activities following the lifting of some of COVID-19 restrictions. The study hypothesis was tested by grouping 4 pigs in each pen as the experimental unit and feeding five diets randomly assigned to each area block. Thus, more than 240 pigs were needed to yield sufficient replication. Generally, increasing the number of replications results in more accurate estimation of experimental error and increases the ability to detect treatment effects (Aaron et al., 2004). Thus, due to these limitations, the study was amended to extend the trial timeline and the number of weaning groups increased from 3 to 6 over time to select sufficient pigs of adequate initial body weight.

The net energy values of the test faba bean cultivars used in formulation were predicted based on crude protein, starch, fat, and acid detergent fibre values acquired from Cho et al. (2019) and Kopmels et al. (2020) using the equation by Noblet et al. (1994) adopted by NRC (2012). Despite using similar faba bean samples, the anticipated (Cho et al, 2019) and measured macronutrient values differed. This difference could be attributed to variation in sampling procedures (Gonçalves et al., 2016) and laboratory assays despite similar analytical methods. As a result, predicted NE values of faba bean cultivars were lower in the present study than anticipated when nutrient values were substituted for measured values obtained from lab analysis after the study was conducted. Thus, for proper energy prediction, these variations can be addressed by performing lab analyses of test ingredients prior to diet formulation. Lack of labs in Canada to perform condensed tannin analysis in faba bean cultivars was also a limitation to this project. The samples were therefore sent to different commercial labs used different assays to estimate the tannin content. In Chapter 2 assay estimated condensed tannins with failure to detect in some cultivars while in chapter 3, the assay estimated total tannins.

For both Chapters 2 and 3, transitioning pigs from the adaptation diet to experimental diets proved to be a challenge. Study 1 in Chapter 2 used weaned pigs. For 5 to 7 days prior to weaning, pigs received a common creep feed then a commercial starter diet for 5 days after weaning. In commercial swine production, the goal is to transition pigs to a low-cost, grain-based diet as quickly as possible after weaning without reducing growth performance. However, feed intake after weaning is critical for the developing digestive system in young pigs. Therefore, the study was split into 2 Phases and diets for each phase were formulated differently to meet changing nutrient requirements (NRC, 2012). Consequently, difficulties in getting pigs to start consuming faba bean diets during the first 2 to 3 days of Phase 1 were observed in most pens. This issue was evidenced by lower feed intake and loose faeces consistency at the start that kept improving as pigs grew older and accustomed to the taste. Study 2 in Chapter 3 used ileal-cannulated growing pigs that had been used previously on another study feeding complete diets, not just mostly the sole ingredient. Consequently, getting pigs to consume diets containing 950 g faba bean/kg diet was a challenge. In the future, this challenge might be mitigated by introducing the test article progressively and placing additional pigs on test. Most pigs had difficulties consuming the diet containing Florent faba bean likely due to greater content of both tannins and vicine and covicine. Furthermore, restricted feed allowance based on metabolic BW reduced digesta output in some pigs due to low intake of dry matter. Therefore, the study was amended to extend the adaptation and(or) specimen collection phase of some study periods to allow for more time for pigs to consume the experimental diets and collect sufficient digesta.

Additionally for Chapter 3, ileal-cannulated pigs were growing too big for the surgically installed T-cannula. Consequently, the cannula in two pigs dropped out, forcing the termination of these pigs before the planned study end date. Consequently, the study was somewhat limited in the number of analyses and duplicates that could be performed on some samples particularly for the nitrogen-free diet.

Moisture content in freeze-dried digesta samples also proved an important factor to address in Chapter 3. The presence of mucin in some samples made it difficult to completely evaporate moisture during initial freeze drying. Excess moisture content was mitigated by extending time for freeze drying to ensure that moisture trapped in the mucin was evaporated of samples prior to grinding for lab analyses. Finally, the reported digestible and net energy values from all treatments were standardized to 12% moisture prior to statistical analysis. Analysed nutrient values were also standardized to 12% moisture for even comparisons among faba bean cultivars.

4.4 Future research

Based on these findings, future research should address the following identified knowledge gaps: First, vicine and covicine are crucial antinutritional factors in faba bean grain and were associated with lower feed disappearance and weight gain of pigs as observed in Chapter 2. However, this conclusion is only verified in previous studies conducted using other monogastric animal species, e.g., rats and chickens (Losowsky and Walker, 1969; Hegazy and Marquardt, 1984). Thus, further research is needed to confirm this hypothesis by conducting a study to define tolerable level of dietary vicine and covicine and effect on health and growth performance in weaned and growing pigs. Furthermore, no study has evaluated feeding faba bean to gestating or lactating sows, therefore effects on sow reproductive performance over several parities need to be tested and reported.

Second, a study is needed to evaluate the effect of varying content of dietary fibre and resistant starch in faba bean cultivars on gut microbiota and energy and amino acid digestibility in weaned pigs. In addition, conducting a study to determine beneficial or detrimental effects of tannins on gut microbiota would increase our understanding about data from Chapter 2, indicating that pigs fed Fabelle diets had greater weight gain than pigs fed other cultivars despite its greatest tannin content.

Lastly, tannins and dietary fibre are mostly concentrated in the seed coat of faba bean seed. Therefore, a study should investigate effects of processing, e.g., dehulling, on nutrient digestibility and growth performance in weaned and growing pigs although feeding dehulled faba bean cultivars to broiler chickens did not support this approach (Cho, et al. 2019).

4.5 References

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Table 4.1. Digestible energy and nutrient content of faba bean cultivars per yield (kg/hectare)[1]

Item	Snowbird	Snowdrop	Fabelle	Florent
AID starch kg/ha[2]	1,856	1,540	1,941	1,747
NE, MJ/kg per ha[3]	60,829	47,481	65,359	53,895
SID CP, kg/ha[4]	1,298	1,231	2,050	1,288
SID Lys, kg/ha[5]	88	78	125	88

[1] Yield of faba bean cultivars (kg/hectare): Snowbird = 5821, Snowdrop = 5122, Fabelle = 7043, Florent = 5995 (Pulse Crop News, 2018).

[2] AID starch, kg/hectare = 0.001 x (starch x CAID starch) x yield of faba bean. Starch content taken from Table 3.3; CAID starch taken from Table 3.4.

[3] NE, MJ/kg per hectare = NE MJ/kg from Table 3.4) x yield of faba bean cultivar.

[4] SID CP, kg/hectare = 0.001 x (CP x CSID CP) x yield of faba bean. CP content taken from Table 3.3; CSID CP taken from Table 3.6.

[5] SID lysine, kg/hectare = 0.001 x (lysine x CSID lysine) x yield of faba bean. Lysine content taken from Table 3.3; CSID lysine taken from Table 3.6

Table 4.2. Effect of feeding faba bean cultivars to weaned pigs on feed cost per kg gain[1]

Period[2]	Snowbird	Snowdrop	Tabasco	Fabelle	Malik	SEM	*P*-value
Diet cost ($/kg)[3]							
d 0 to 7	2.53	2.45	2.58	2.60	2.45	0.066	0.743
d 7 to 14	4.20[b]	4.21[b]	4.38[ab]	4.66[a]	4.19[b]	0.066	0.004
d 14 to 21	3.69	3.75	3.79	3.99	3.72	0.066	0.230
d 21 to 28	5.00[ab]	5.01[ab]	5.08[ab]	5.27[a]	4.80[b]	0.066	0.027
d 0 to 28	3.85[b]	3.86[b]	3.96[ab]	4.13[a]	3.79[b]	0.070	<0.001
Total feed cost[4]	15.42	15.42	15.83	16.52	15.16		
Feed cost/kg gain[5]							
d 0 to 7	1.02	1.02	1.00	1.00	1.05	0.012	0.638
d 7 to 14	1.12	1.15	1.18	1.15	1.15	0.012	0.658
d 14 to 21	0.85	0.82	0.82	0.84	0.86	0.012	0.812
d 21 to 28	0.89	0.91	0.93	0.90	0.92	0.012	0.879
d 0 to 28	0.97	0.98	0.98	0.97	0.99	0.014	0.761
Total cost/kg gain[6]	3.88	3.90	3.93	3.89	3.98		

[a-c] Means within a row without common superscript letters differ ($P < 0.05$).

[1] Price of ingredients ($/1000kg): faba bean cultivars and wheat = 367, soybean meal = 500, lactose = 2,000, soy protein concentrate = 1,800, fish meal =3,000, canola oil =1,000, limestone = 121, mono-/di-calcium phosphate = 920, salt = 170, vitamin premix = 5000, trace mineral premix = 5,000, L-lysine HCL = 1,850, L-threonine = 2,290, DL-methionine = 3,300, L-tryptophan = 18000, L-valine = 35,000, celite = 2800, Ronozyme HiPhos 2500 = 10,000, choline chloride 60% = 2,120.

[2] Week effect ($P < 0.05$).

[3] Feed cost = diet cost x (ADFD x period length, 7d).

[4] Total feed cost = sum of feed cost for 4 periods.

[5] Feed cost per kilogram of gain = feed cost/ (weight gain × period length, 7d).

[6] Total Feed cost per kilogram of gain = sum of feed cost per kilogram of gain for 4 period.

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