Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Sciences

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Tillväxt och hälsa hos smågrisar utfodrade med brokblommig åkerböna i förhållande till tanninninnehållet

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Abstract
There is an increasing need and interest to use domestic protein feed ingredients in Swedish pig diets instead of imported soybean, particularly in organic production. Due to its tannin content, colour flowered faba bean has been disregarded in this sense, yet it yields more and is grown more in Sweden than the white flowered which is void of tannins. From the literature review it appears that tannin slightly reduces the nutritional value of colour flowered faba bean, particularly protein, but is not toxic. With sensible diet optimization using standardized ileal digestibility values and net energy, colour flowered faba bean could be successfully included in pig diets. Thus, to test its use under Swedish circumstances with emphasis on organic conditions, a feeding experiment was conducted to elucidate the effects on pig growth and health. In a randomized block design, 360 newly weaned piglets divided on six batches were fed colour or white flowered faba bean at 10 or 20% inclusion, or a control diet with soybean expeller. The diets were made to simulate organic diets. No differences were found between the treatments, indicating colour flowered faba bean can be used as well as white flowered and soybean. However, during the experiment confounding effects came into play: oedema disease and mouldy feed. Thus, there was a large variation in data, possibly masking treatment effects. Results from a previous part of the same study, testing conventional feeds, found that the faba bean diets had a better feed conversion ratio and higher daily weight gain than the soybean control. In conclusion, colour flowered faba bean has good potential in well balanced diets in Swedish pig production.

Sammanfattning
Det finns ett ökande intresse och behov av inhemska proteinfodermedel i svensk grisproduktion istället för importerad soja, speciellt vad gäller ekologisk produktion. På grund av sitt tanninninnehåll har brokblommig åkerböna förbisetts som ett alternativ, trots att den avkastar mer och odlas i större utsträckning än vitblommig som saknar tanniner. Utifrån en litteraturstudie verkar det som att tanninerna sänker näringsvärdet i brokblommig åkerböna något, framförallt av protein, dock är det inte giftigt. Med väl genomtänkt fodероptimering som utnyttjar standardiserad tunntarmssmältbarhet och nettoenergivärden kan brokblommig åkerböna framgångsrikt inkluderas i grisfoder. För att utforska dess värde under svenska förhållanden med betoning på ekologisk produktion genomfördes ett utfodringsförsök för att se effekterna på tillväxt och hälsa. I en randomiserad blockdesign fördelades 360 nyavvanda smågrisar på sex omgångar och utfodrades med 10 eller 20% brok- eller vitblommig åkerböna, eller sojakaka för kontroll. Fodren var utformade att efterlikna ekologiskt foder. Inga skillnader observerades mellan behandlingarna, varför brokblommig åkerböna kan användas lika väl som vitblommig eller sojakaka. Dock uppkom förväxlingsfaktorer i form av ödemsjuka och mögel i försöksfodret. Därav fanns en stor variation i insamlade data som kan ha maskerat behandlingseffekter. Resultaten från en tidigare del i samma studie, med konventionella foderstater, visade att åkerbönsfodren medförde en bättre foderomvandlingskvot och högre daglig tillväxt än sojafodret. Slutsatsen kan dras att brokblommig åkerböna har god potential i välbalanserade foderstater i svensk grisproduktion.

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1 Introduction

Imported soybean is a common protein source in Swedish pig diets. In 2010, close to 33 000 tons of soybean products were imported to feed pigs in Sweden, most in the form of soybean meal (SJV, 2011). The import is linked to a multitude of problems in both the producing and importing countries, urging the need to explore local protein sources (Heimer, 2010). This is of special interest in organic production, as most of the global soybean in trade is genetically modified and produced at a great distance (Wallenbeck, 2012). Well suited to the Swedish climate, the grain legume faba bean (*Vicia faba* L.) is an alternative, rich in both protein and energy. Already today faba bean dominates European grain legume production but its potential is yet unexploited, particularly in pig diets (Jezierny *et al*., 2010a). In Sweden, as illustrated in Figure 1, production is increasing at the expense of pea (SCB, 2016).

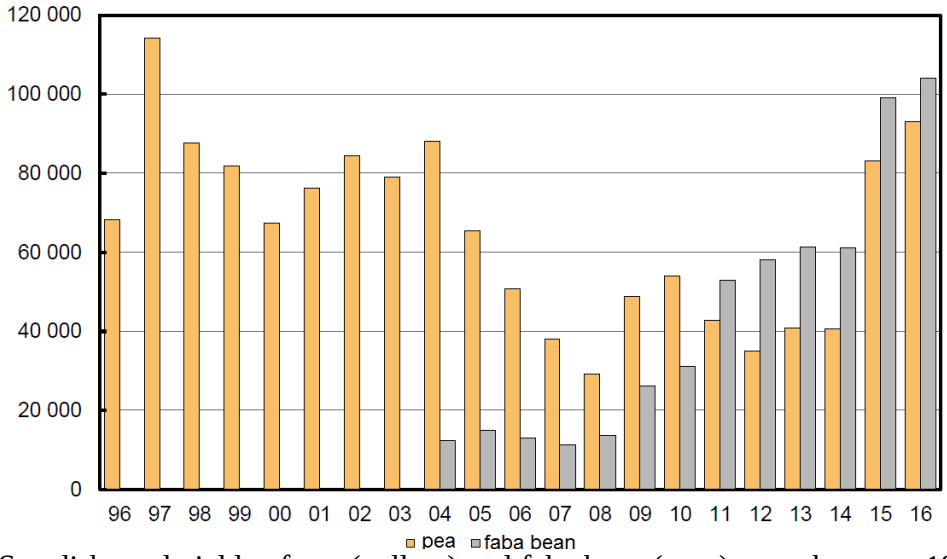


Figure 1. Swedish total yields of pea (yellow) and faba bean (grey) over the years 1996-2015. Expressed in tons with 85 % dry matter. © SCB (2016).

Faba bean is lower in some indispensible amino acids compared to soybean meal, and colour flowered faba bean cultivars contain the antinutritional factor tannin. White flowered faba bean cultivars are void of tannins but yield less, show poorer establishment and lower disease resistance (Martín *et al*., 1991). As a result Swedish faba bean production is currently dominated by colour flowered cultivars (SJV, 2016), as is the case in the rest of Europe (Crépon *et al*., 2010). Fed to pigs, colour flowered cultivars reduce digestibility, mainly of protein by binding to them. Yet there are many examples of studies where colour flowered cultivars are fed successfully to pigs and in a literature review, Crépon *et al*. (2010) conclude that reported detrimental effects may derive from suboptimal diet formulation such as not balancing diets with pure amino acids. According to current organic standards, pig diets may not contain synthetic amino acids, hampering use of home grown protein feed stuffs at organic farms (Wallenbeck, 2012). Feed antibiotics are common in global pig production as an effective preventive means to reduce infection and increase growth performance. In 2006 the EU prohibited habitual use of feed antibiotics and thus there is research into feeds supporting enteric integrity (Maxwell & Carter, 2001). A literature review was undertaken and a feed experiment with faba bean in simulated organic diets performed. The experiment constituted the second half of a study on colour flowered faba bean in weaner (5-9 weeks of age) diets, the first part had focused on conventional diets. The tested hypothesis was that by proper diet optimization faba bean, independent of flower colour, can substitute soybean in

organic weaner diets without compromising pig performance, and that the tannins exert bioactive effects enhancing growth and health.

2 Literature review

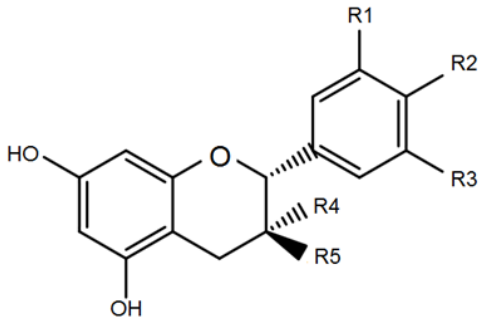
2.1 Tannins

2.1.1 An introduction

Following lignin, tannins compose the second most abundant group of phenolics in vascular plants (Gu *et al*., 2003). They form a heterogeneous group of hydrophilic polyphenols, that distinguish from other plant phenolics in their ability to bind proteins with hydrogen bonds without altering protein structure. The complexes are biologically inactive and indigestible (Mueller-Harvey, 2006), however, dissociation occurs in acid solution (Gu, 2012). Tannins do not participate in primary plant metabolism but defend against phytopathogens and predators by featuring bitter taste, binding to feed nutrients, digestive enzymes and the gut wall. The structure of more than 1000 tannins is established, yet binding activity of most is unknown (Mueller-Harvey, 2006). Vascular plants contain different groups of tannins, those occurring in faba bean are known as condensed tannins (Marquardt *et al*., 1978).

2.1.2 Condensed tannin chemistry

The basic building units of condensed tannins are flavanols (Figure 2). Condensation of flavanols forms polymers: catechins form procyanidins (PC), the most studied condensed tannin type, and gallocatechins form prodelphinidins (PD), the second most studied type (Figure 3). Covalent carbon bonds or ether bonds form between the flavanol monomers, the resulting polymers are linear or branched. Chain length usually ranges between 3 and 20 monomers, the number of monomers is termed the degree of polymerization (DP) (Mueller-Harvey, 2006). In sainfoin cultivars, mean DP varies between 16 and 76.5 (Lorenz *et al*., 2013), 47.9 in blackcurrant, 8.3 in pinto bean and 6.7 in kidney bean (Gu, 2012).



Flavanols	R1	R2	R3	R4	R5
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

Figure 2. Flavanol structures.

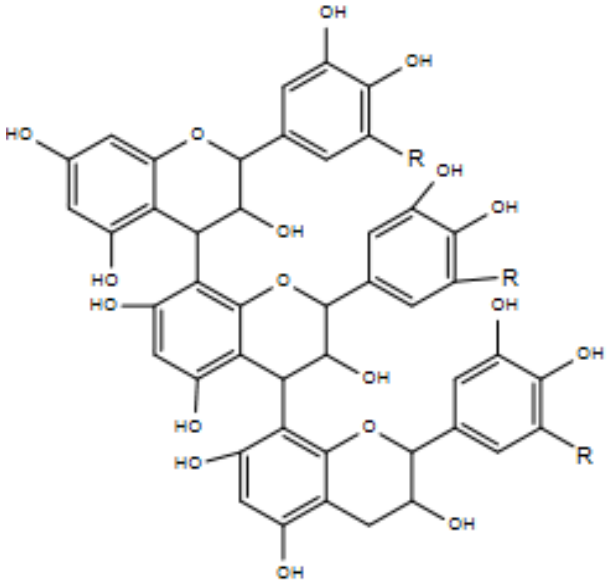


Figure 3. A condensed tannin trimer.
R = H: procyanidins
R = OH: prodelphinidins

2.1.3 Binding activity of condensed tannins

Condensed tannins form hydrogen bonds with macromolecules and metal ions below the acid dissociation constant (pKa) of their phenolic hydroxyls, pH 9-10, as this is when hydroxyls are not ionised and can act as hydrogen donors (Hagerman & Butler, 1981). Protein interactions are preferred, thus in a feeding situation primarily protein digestibility is reduced (Jansman *et al*., 1995). The carbonyl oxygen in the peptide bond serves as the hydrogen acceptor. Hydrophobic interactions also occur between the tannin aromatic ring structure and hydrophobic regions in proteins (Hagerman & Butler, 1981). As it is mainly the hydroxyl groups that are responsible for the protein binding capacity of tannins, prodelphinidins have higher binding capacity than procyanidins (Lorenz *et al*., 2014). Interactions are both tannin and protein specific. Since there is vast variety within both, binding capacity and deriving diverse biological effects are hard to predict. It appears condensed tannins preferentially bind proteins containing proline because of the open structure proline imposes on peptide chains, strengthening the hydrogen bond and making other bonding sites available (Hagerman & Butler, 1981). Tannin factors that affect binding are degree of polymerization, branching, subunit structure and stereochemistry. Protein factors are conformation, size and isoelectric point (pI). Also milieu factors such as pH and temperature are important. Hagerman and Butler (1981) suggest that proteins are most effectively bound at their pI, when their electrostatic repulsions are minimized and they are the least soluble. In the first study to look at the interaction between faba bean tannins and faba bean proteins, Kosinska et al. (2011) found a higher affinity of tannins towards legumin than vicilin and albumin. They examined the effect of pH on binding between faba bean tannins and proteins. Legumin binding peaked at pH 3 and above 6, but dipped at pH 4.5. Vicilin and albumin did not show any peaks or dips but vicilin binding was constantly higher. The pI of the legumin large subunits is 4.6–6.1 and 8.2–8.5 of the small (Matta *et al*., 1981), while that of vicilin is 3.4 (Pearsall & Ewing, 1924). In Figure 4 of the porcine digestive tract is reported the local pH, important digestive enzymes and their pI. The pH values are compiled from Clemens et al. (1975) and enzymes from Yen (2001). Many animal species secrete salivary proline-rich proteins, thought to be a

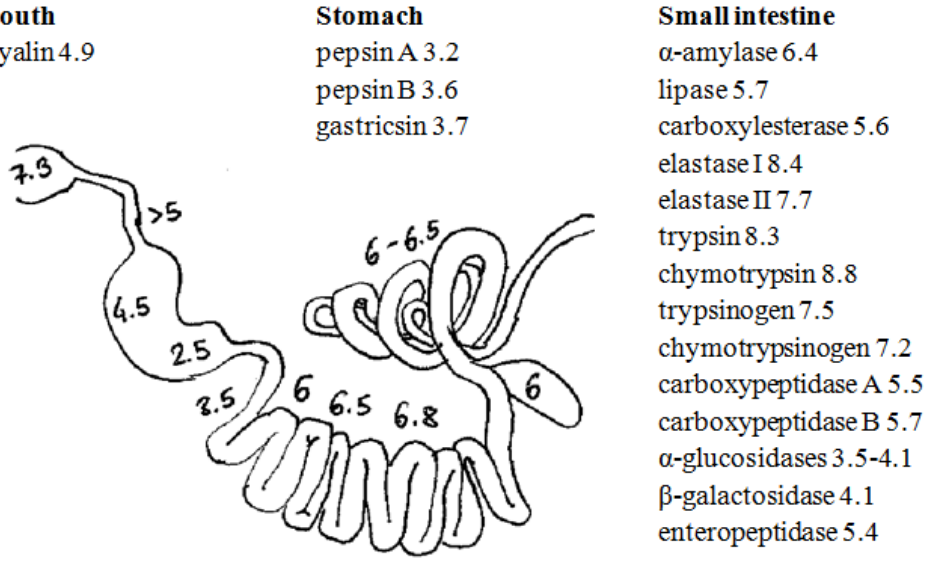


Figure 4. The porcine digestive tract with local pH values. Enzymes found in the different sections are listed, followed by their pI.

defence as condensed tannins will preferentially bind these instead of dietary and digestive proteins, since secretion increases in response to dietary tannin level (Mueller-Harvey, 2006). Porcine saliva contains basal levels of proline-rich proteins, however, in the pig there is no

such marked salivary increase from condensed tannin consumption, only an increase in other endogenous losses as measured at the ileal level, possibly from proline-rich proteins from gastric mucus (Jansman *et al.*, 1995; Cappai *et al.*, 2013).

2.1.4 Bioactive properties of condensed tannins

Due to their size it is unlikely condensed tannins are absorbed by enterocytes, only mono- and dimers are, and poorly so, only 5-10 %. Since they are hydrophilic and recognized as xenobiotics no tissue accumulation occurs, even at high dietary intake. Their hydrophilic properties make them unable to integrate into biological membranes such as tocopherols, absorbed metabolites may only act in blood and cytosol (Gessner *et al*., 2016). Many features are ascribed to flavanols: antioxidant, cytoprotective, antiinflammatory, antibiotic, antiviral, and are applied to condensed tannins. These properties often derive from the same basic mechanisms. Chelating metal ions and macromolecules decreases pathogen growth by rendering substrates required for microbial growth unavailable, as well as reduces oxidative stress since metal ions catalyze oxidation reactions, generating free radicals. Tannins may also bind bacterial and viral components as well as prooxidant enzymes (Koleckar *et al*., 2008; Gessner *et al*., 2016). Direct antioxidative properties derive from the aromatic hydroxyl and its ability to donate an electron to a free radical, producing a more stable and thus less harmful radical. Similar antioxidant properties have been examined in the soybean isoflavones (Yu *et al*., 2016). The more hydroxyls the higher the antioxidant capacity, yet when DP >3 a reduction in antioxidant capacity has been noted (Koleckar *et al*., 2008). Noted prebiotic effects of flavanols could benefit animal growth, though the exact interaction with intestinal microbes is unknown. A beneficial microflora suppresses potentially pathogenic microbes by competitive exclusion, decreasing both intestinal inflammatory gene expression and translocation of bacterial components into the circulation which are inflammatory and initiate oxidative processes (Gessner *et al*., 2016).

2.1.5 Analytical methods

Tannins in feed samples are either free or bound to cell components like fibre and protein, the free being extractable and exhibiting binding potential. Only the extractable fraction is commonly measured (Jansman, 1993). In colorimetric assays a reagent is added to a tannin extract, yielding a coloured product. Its absorbance is measured spectrophotometrically and related to the absorbance of a standard solution. The reagents do not exclusively react with tannins, but often with other phenolic compounds, and even react differently depending on tannin type (Jansman, 1993; Gu, 2012). Quite different results are obtained, an example being three colorimetric assays employed by Jansman (1993) yielding the following tannin percentages in the same faba bean sample: Folin-Denis 1.55, vanillin 0.96, ferric iron 0.78. Liquid chromatography preceded by solid phase extraction can be used to determine tannin quantity; the area under the chromatogram peak is proportional to the quantity and is compared to a standard peak area (Gu, 2012).

For qualitative analysis chromatographic or precipitation assays are employed. Precipitation assays measure how much of a precipitant, usually a protein, is precipitated by a tannin extract, reflecting binding capacity rather than concentration. Depolymerisation followed by liquid chromatography or ionization combined with mass spectrometry, reveals subunit structure and molecular size. Having analysed sainfoin condensed tannins by the former, Lorenz et al. (2014) could not identify properties important for precipitation, and pointed out that the method only provides information on the average tannin size. Today, there is no technique developed for large scale tannin purification and there is a lack of suitable standards (Gu *et al*., 2003).

2.2 Pig feed and protein

2.2.1 Protein utilization

Weaners are in an accelerating growth phase and deposit much muscle relative to their weight. To sustain rapid growth, they require a sufficient intake of amino acids found in protein, particularly the indispensible ones which the pig is unable to synthesize. Some amino acids will not be utilized unless they are supplied at a certain ratio to other amino acids (Lewis, 2001). Current nutrient recommendations have, however, proven to be somewhat high, and requirements are influenced by health status (Høøk Presto *et al*., 2007). In the digestive tract, dietary protein is enzymatically degraded to amino acids, di- and tripeptides which are absorbed in the small intestine. Amino acids reaching the cecum and colon are not absorbed but converted to microbial protein excreted in faeces or fermented to nitrogenous waste products like ammonia, some of which is absorbed. These nitrogenous compounds are, along with excess amino acids, converted to urea in the liver and transported by blood to the kidneys for excretion. The higher the protein quality of a diet, the higher the utilization and the less urea is present in blood, measured as blood plasma urea nitrogen, PUN. PUN changes rapidly in response to dietary changes, pigs with free access to feed exhibit smaller fluctuations than pigs fed twice daily (Pedersen & Boisen, 2010). Porcine PUN literature values vary between 20-220 mg/L (Kohn *et al.*, 2005). Disease and inflammation drains amino acids from growth and raise PUN values. Amino acids are constituents of endogenous antioxidants and thus oxidative stress, common to inflammatory disease, also withholds amino acids from growth (Gessner *et al*., 2016).

2.2.2 Feed stuffs

The 2001 ban on meat and bone meal by the European Commission erased common sources of high value amino acids from pig diets (Jezierny *et al*., 2010a). In general, cereal grain amino acid profiles complement legume amino acids well though the total content is low, the previous being high in the sulphur amino acids and the latter in lysine. In organic production, high quality organic protein sources are scarce and there is difficulty not overfeeding crude protein in meeting the amino acid requirements. EU standards restrict organic production to mechanically extracted expellers of oil seeds like soybean and rapeseed, which are higher in fat compared to the solvent extracted meals. The prohibition of genetically modified organisms render things more difficult as most of the global soybean in trade is genetically modified, and many pure substances added to conventional diets derive from fermentation by genetically modified organisms: amino acids, vitamins and enzymes like phytase. Amino acids may also be synthetically produced, which is not allowed either. This sets up several conflicts of interest in organic production, which is intended to be sustainable yet currently imposes low nutrient utilization and high losses to the environment (Jezierny *et al*., 2010a; Wallenbeck, 2012). In Sweden, common protein sources in organic pig diets are soybean and rapeseed expeller, pea, conventional potato protein (currently there is a 5 % allowance for conventionally produced feed ingredients) and white flowered faba bean. Soybean expeller has a good amino acid profile but is too high in energy and as is the common problem to all grain legumes, contains antinutritional factors, mainly trypsin inhibitors (Wallenbeck, 2012). Legume proteins have a compact structure featuring lower digestibility than for example casein, and the unfamiliar structure may evoke immunological responses, particularly at abruptly introduced and high inclusion levels (Maxwell & Carter, 2001; Salgado *et al*., 2002a; 2002c).

2.3 Faba bean

2.3.1 Production

Being a legume adapted to cool climates, faba bean (*Vicia faba* L.) is cultivated in regions with mild winters and ample rainfall in summer. In Sweden most is grown in the region of Västra Götaland. Harvest occurs in October and November as faba beans mature late, the average yield amounts to 3500 kg per hectare (SCB, 2016). It is beneficial in crop rotation because it fixes nitrogen and has a deep root system, loosening soil structure. White flowered cultivars are bred to be void of tannin (Crépon *et al*., 2010), but are more susceptible to insect attack and disease than the colour flowered, explained by the thinner and tannin free hull, causing weaker microbial protection (Martín *et al*., 1991). This, poor early development and lower environmental stress tolerance result in lower yields. In Sweden today, the acreage seeded with white flowered cultivars is one third that of the colour flowered (SJV, 2016).

2.3.2 Antinutritional factors

Tannins are located in the faba bean grain hull (Marquardt *et al*, 1978). Recently published values in colour flowered cultivars are in the range of 0.2-1 % of dry matter (Jezierny *et al*., 2011), which is similar to older values reported (Marquardt *et al*., 1978). The content peaks 30 days after flowering, thereafter the tannins decrease in concentration but increase in polymerization (Martín *et al*., (1991). As is common to legume grains, faba bean contains antiproteases and lectin, however at concentrations too low to be of practical consequence in animal nutrition. Also present are vicine and convicine, responsible for favism in genetically predisposed humans, these appear to bring no adverse effects in pigs (Crépon *et al*., 2010).

2.3.3 Nutritive content

Faba beans are high in starch and protein, low in fat and sugar. Tables 1 compares the nutritional content of a white (Gloria) and a colour (Fuego) flowered faba bean to that of soybean meal, values are compiled from Jezierny et al. (2011).

Table 1. Nutritional and antinutritional composition of beans, g/kg DM

	Soybean meal	Gloria	Fuego
Crude protein (CP)	541	337	292
Crude fat	28	13	15
Ash	74	43	40
Starch	51	432	425
Sugar	106	30	30
Hemicellulose	40	16	28
Cellulose	71	110	134
Lignin	3	1	3
Lysine	32	21	19
Methionine	7	2	2
Threonine	20	11	10
Tryptophan	7	3	3
Trypsin inhibitor, g/kg CP	6	3	0
Condensed tannins	0	0	7
SID of crude protein, %	87	80	71
SID of lysine, %	90	84	79
SID of methionine, %	91	77	62
SID of threonine, %	85	80	68
SID of tryptophan, %	85	71	53

SID=standardized ileal digestibility

2.3.4 Protein content and digestibility when fed to pigs

The storage proteins in faba beans are albumins, globulins and glutelins: the main portion consists of the globulins vicilin and legumin at a 1:2.5 ratio (Jezierny *et al*., 2010a). Legumin is higher in tryptophan, cysteine and methionine than vicilin (Gunawardena *et al*., 2010). Salgado et al. (2002c) found that vicilin is more immunogenic than legumin to weaners. Faba bean is high in lysine, but the low content of methionine, cysteine and tryptophan makes it unsuitable as the sole protein source in pig diets (Jezierny *et al*, 2010a; Smith *et al*., 2013). The hull constitutes 11 and 16 % of the weight in white and colour flowered cultivars, respectively. The hull fraction contains 5 % crude protein and is high in fibre out of which most is cellulose (Jansman *et al*., 1995). White flowered cultivars are higher in digestible crude protein because they are lower in fibre and tannins, both of which decrease protein digestibility (Jezierny *et al*., 2011). Calculating on the crop yield of *in vitro* digestible crude protein, Neil and Ivarsson (unpublished) found no difference between colour and white flowered cultivars. Many authors have found a linear decrease in standardized ileal digestibility of crude protein with increasing content of colour flowered faba bean in pig diets (Jansman, 1993; Mariscal-Landín *et al*., 2002; Jezierny *et al*., 2011). Jezierny et al. (2010b) found *in vitro* digestibilities to be higher than those predicted *in vivo*, thus they do not provide direct estimates of *in vivo* standardized ileal digestibility of crude protein and amino acids, but there was a close linear relationship between the two. Comparing standardized digestibilities between several colour and white flowered cultivars on growers, Vilariño et al. (2004) and Jezierny et al. (2011) noted that decreases were accompanied with increased tannin levels. When feeding colour flowered cultivars there is a particularly low digestibility of some amino acids compared to white flowered: cysteine, tryptophan, methionine, glycine, proline, threonine (Jansman *et al*., 1995; Jezierny *et al*., 2011), explained by preferential tannin binding, and a tannin induced increase in endogenous secretions which are rich in these amino acids. Jansman et al. (1995) quantified the reduction in apparent ileal digestibility as 55 % from an increase in undigested dietary protein and 45 % from increased secretion or lowered reabsorption of endogenous protein.

2.3.5 Pig growth

Inclusion levels of colour flowered cultivars in feed experiments with growing pigs have varied between 10 and 35 % (Crépon *et al*., 2010). Royer et al. (2010) found 35 % to yield satisfying growth, though marginally lower than that of a soybean control and a white flowered cultivar. Unlikely enough, they found the tannin content to be higher in the diet (2.1 %) than in the beans (1.5 %) though no other dietary compound contained tannins. When Jansman et al. (1993) included 20 % colour flowered faba bean hulls containing 3.3 % tannin in a weaner diet these pigs grew slower and presented a poorer feed conversion than weaners fed white flowered cultivar hulls or pea hulls. Though colour flowered cultivars feature somewhat poor digestibility (Jezierny *et al*., 2011) they can sustain high growth with sensible diet optimization and amino acid supplements (Crépon *et al*., 2010), as has been evidenced by several experiments comparing diets with colour and white flowered faba beans and/or soybean (Flis *et al*., 1999; Royer *et al*., 2010, Smith *et al*., 2013; Møller, 2014; Vils & Vinther, 2016). Smith et al. (2013) speculate that earlier studies with disappointing results of colour flowered cultivars were due to less knowledge on standardized ileal digestibility and net energy, which today make it possible to better balance pig diets. Tannins have been reported to reduce feed intake of pigs, presumably due to a bitter taste (Jansman, 1993). None of the reviewed studies report on a lower feed intake of diets containing colour flowered faba bean compared to control diets.

2.3.6 Enteric effects

Saldago et al. (2002a) found that colour flowered faba bean does not induce changes to intestinal microarchitecture, pancreas weight, pancreatic secretions and enteric enzyme activities that are different from those induced by other legumes. Legume grains in general increase endogenous trypsin and chymotrypsin losses at the terminal ileum (Saldago *et al*., 2002b). However, Jansman et al. (1993) found a particular decrease in trypsin activity from feeding colour flowered faba bean hulls compared to white flowered faba bean hulls. There was no difference in enteric enzyme activity and digestive organ sizes, except weaners on the high tannin diet had smaller ceca, probably due to less fermentation. Intestinal tissue showed no difference in villi shape or mucosal α-glucosidase and aminopeptidase activity. Any reduction in digestive enzyme activity unlikely decreases digestibility as these are secreted in large excess, only their reuptake is decreased (Jansman *et al*., 1995).

Oligosaccharides are common in legume grains, these are indigestible and highly fermentable, especially α-galactosides, and high dietary intake may cause diarrhoea and abdominal pain from gases (Jezierny *et al*., 2010; Smith *et al*., 2013). Faba bean contains α-galactosides, mainly verbascose at 2.2 % of dry matter (Salgado *et al*., 2002a). Contrary to expectations, Smith et al. (2013) observed a higher faecal dry matter when feeding pea and colour flowered faba bean compared to soybean meal, but not between the pea and faba bean. Møller (2014) noted fewer days in treatment for diarrhoea when weaners were fed colour flowered faba bean compared to soybean meal, but not compared to white flowered faba bean. Van der Meulen and Jansman (2010) saw an increased fluid absorption *in situ* of weaners when perfusing pea hulls and colour flowered faba bean hulls through the small intestine, but not when meals or concentrates of these whole legume seeds were perfused. Feeding weaners diets with a colour flowered faba bean or the hulls yielded lower ileal microbial diversity than pea, pea hulls and soybean meal diets (van der Meulen *et al*., 2010). Diversity was lower from the faba bean hull diet than whole seed diet, contrasting to the pea hull diet which yielded a higher diversity than whole pea diet. The authors state that the increased diversity will result in a more stable microflora and that fermentation thus is health supportive.

3 Material and methods

3.1 Faba beans and analysis of tannin content

Based on results from a laboratory evaluation of eleven colour flowered and five white flowered European faba bean cultivars (Ivarsson & Neil, 2016), three cultivars were included at 20 % in weaner feeds. These cultivars are presented in Table 2. The colour flowered cultivars Fuego and Julia were chosen for their high and low content of condensed tannins, respectively. Among the colour flowered cultivars, Julia had the highest digestible crude protein content and lowest tannin content. Fuego is the employed standard in the Swedish national cultivar trials. Gloria was chosen as a tannin free, white flowered cultivar with a high digestible crude protein content for comparison.

Table 2. Analyzed nutritional and antinutritional values of the experiment beans

	Gloria	Julia	Fuego
Flower colour	white	colour	colour
Dry matter (DM), %	87.9	87.2	86.9
Ash, % of DM	3.6	3.3	3.4
Crude protein (CP), % of DM	33.7	34.5	31.5
Condensed tannins, g/kg	0	3.2	4.3
Mean degree of polymerization, number of flavan units	-	4.9	4.2
Prodelphinidin/procyanidin ratio	-	1.7	1.4
Enzymatic *in vitro* digestible CP, % of CP	96.8	95.4	91.5
Standardized ileal digestibility of CP, % of CP[1]	92.8	90.6	85.5

[1]Calculated according to Jezierny et al. (2010)

The cultivars were analysed for dry matter by drying at 103°C for 16 h, ash after ignition at 600°C for 3 h (Jennische & Larsson, 1990), crude protein (nitrogen × 6.25) was determined by the Kjeldahl method (NMKL, 2003) and enzymatic *in vitro* digestibilities were performed as described by Boisen (1991) and Boisen and Eggum (1991). Tannin content was determined at an external laboratory (LUKE, Finland) using depolymerisation followed by liquid chromatography. Depolymerisation is achieved by the presence of a nucleophile, separating tannin extension monomers from the terminal monomer. Liquid chromatography elution separates these units and mean DP is calculated from the ratio of chromatogram peak area of all units to the peak area of terminal units (Gu, 2012). For comparison a precipitation assay, the radial diffusion assay, modified from Hagerman (1987), was also performed. Tannins were extracted from ground beans in test tubes with 70% acetone placed in a sonicator bath for 10 minutes. The tubes were centrifuged to separate the debris, and the tannin-containing supernatant was applied in 5x10 µl aliquots to wells in agar plates containing bovine serum albumin. As the tannins diffuse through the agar it encounters and binds protein, producing a visible ring around the well. The ring diameter is proportional to the amount of tannin with affinity towards the employed protein. Powder tannic acid was employed as a standard. The plates were stored at 30 °C while precipitate rings formed, after 96 h the diameters were recorded using a plastic ruler.

3.2 Experimental diets

Four wheat based pellet (3 mm, 78°C) feeds were formulated by a commercial feed company (Teknosan AB) to be isoenergetic and similar in protein content, simulating organic feeds in that no pure amino acids nor enzymes were added and expeller instead of solvent extracted meal were included. However, raw ingredients were from organic cultivation in the control feed only. Feed compositions are presented in Table 3 and their calculated nutritional values in Table 4. Three feeds contained faba bean and one soybean expeller (46 % crude protein) for control. In the control feed, soybean expeller also substituted rapeseed and some potato protein. The feeds were analyzed for dry matter, ash and crude protein as described previously for the beans. Also, crude fibre was determined according to Jennische and Larsson (1990), ether extract according to The Official Journal of European Communities (1998) and amino acids at a commercial lab (Eurofins) according to US ISO 13903:2005. From these feeds, six experimental diets were prepared: 1) Control (100% Control feed), 2) Gloria (100% Gloria feed), 3) Julia10 (50% Julia feed + 50% Control feed), 4) Julia20 (100% Julia feed), 5) Fuego10 (50% Fuego feed + 50% Control feed) and 6) Fuego20 (100% Fuego feed).

Table 3. Diet formulation of feeds in g/kg as fed

	Control	Gloria	Julia	Fuego
Wheat	450	446	443	437
Oats	250	150	150	150
Maize	20	20	20	20
Soybean expeller	159			
Faba bean, Gloria		200		
Faba bean, Julia			200	
Faba bean, Fuego				200
Rapeseed		50	54	59
Potato protein	26	43	43	44
Salmon concentrate	50	50	50	50
Lucerne meal	1.50	1.50	1.50	1.50
Limestone	12	10	10	10
NaCl	4.6	3.4	3.3	3.3
Monocalcium phosphate	8.8	8.9	7.9	7.8
Vitamin and mineral premix	3.8	3.8	3.8	3.8
Vitamin E	0.5	0.1	0.1	0.1
Yeast (Levucell SB)	0.2	0.2	0.2	0.2

Table 4. Calculated nutritional values of trial feeds as fed, by feed company based on the diet formulation using table values and analyzed values

Parameter	unit	Control	Julia	Fuego	Gloria
ME	MJ	12.29	12.30	12.30	12.30
NE	MJ	9.25	9.49	9.49	9.50
Crude protein	%	17.75	17.72	17.31	17.76
SID crude protein	g/kg	153.47	143.35	139.99	143.85
Lysine	g/kg	9.50	10.67	10.42	10.66
Methionine+Cysteine	g/kg	6.84	6.44	6.41	6.48
Methionine	g/kg	3.23	2.95	2.95	2.95
Fat	%	3.95	4.65	4.85	4.51
Crude fibre	%	6.65	5.65	6.03	5.53
Starch	g/kg	357.75	408.54	404.77	407.77
Phosphorus (P)	g/kg	5.80	5.80	5.80	5.80
Digestible P	g/kg	3.17	2.84	2.82	2.94
Dry matter	%	86.54	85.09	84.83	85.07

SID= standardized ileal digestibility

3.3 Study design

The diets (treatments) were fed in a balanced randomized block design. Six blocks were formed, each with six pens containing ten piglets. The pigs were reorganized from their birth litter at weaning to form even groups regarding birth litter, weight and sex. Thus pen constituted the experimental unit. Within block, the six experimental diets were randomly

distributed, each diet appearing once in each block (batch). Block was repeated six times to obtain more confident results by lowering variance.

3.4 Pigs and health parameters

Approval was obtained from the Ethical committee of the Uppsala region (reference number C119/15). The experiment was performed at the Swedish livestock research centre in Uppsala in the fall of 2016, using a total of 360 pigs (10 pigs per pen • 6 pens • 6 blocks). Yorkshire or Yorkshire-Landrace sow and Hampshire boar crosses were used, because this cross is common at commercial farms and is bred for rapid growth. The pigs entered the experiment at weaning five weeks after farrowing because of their high protein deposition, high relative growth rate and high feed utilization at this age, to reveal differences between the experimental diets. Also, the impact of substances exhibiting antibiotic effects, in terms of improved growth rate and efficiency of feed utilization, is higher in weaners compared to growing and finishing pigs (NRC, 1998). The weaners received the experimental feeds *ad libitum* between 5 and 9 weeks of age, 27 days in total, with free access to water from nipples. They were kept in partly slatted pens, enriched with straw. All pigs were weighed in weeks 5, 7 and 9 of age. Feed intake was judged pen wise on daily basis on a scale of 0-3: 0 implying no intake, 3 implying high intake. When deviating, faecal status was recorded on a scale of 1-5, 1 implying dry and hard, 5 implying watery diarrhoea, 2 implying dry and firm, considered the norm. When deviating, body condition was assessed on a scale of 1-5, 1 implying thin, 3 normal and 5 fat. All health issues and deaths were recorded. Throughout the experiment oedema disease was present in the herd, weaners in the last two batches were vaccinated.

3.5 Outline and complications

Management and housing of pigs was not organic to increase reliability of the results as there would be less variation from factors such as weather, thus more of detected differences can be attributed to the treatments. Unfortunately other confounding effects came into play: oedema disease and mouldy feed. Oedema disease was discovered in the herd prior to the experiment and at least some of the experiment pigs were confirmed affected. Halfway into the last batch mould was discovered in the Fuego experimental feed. Thus in the last week, pigs receiving diets Fuego10 and Fuego20 were fed the control diet instead. All feeds were analyzed for hygienic quality parameters: water activity, colony forming unit count of yeast, aerobic bacteria, mould, coliform bacteria and *Aspergillus fumigatus*. In the first batch, the control pellets was fed crushed not whole due to human mistake.

3.6 Plasma urea nitrogen

Two pigs of different sex from each pen were sampled for blood from the jugular vein in the morning or at midday on the last day of each batch. Plasma was separated from the blood samples and stored at -80°C until analysis. Urea nitrogen in plasma was analyzed using a Technicon AutoAnalyzer 2 (SEAL Analytical). In principle, urea in plasma reacts with diacetyl-monoxime, yielding a colour. The colour intensity is proportional to the urea concentration. Absorbance is measured by a colorimeter and matched to a standard solution, generating the plasma concentrations.

3.7 Statistical analysis

The following mixed model was applied: $yij=\mu+\alpha i+\beta j+eij$, where μ is the general mean value, α effect of treatment, β effect of block, e the residual. Data was computed in the Statistical Analysis System software (SAS, 2009), where the Mixed procedure was employed to estimate variance components and p-values calculated. For growth responses pig was the experimental unit, and the fixed effects of treatment, sex and weight at weaning, and random effects of

block and litter within block were included. For feed intake and feed conversion ratio pen (mean of all pigs in one pen) was the experimental unit, and the fixed effect of treatment and random effect of block included. For plasma urea nitrogen, the fixed effects of treatment, sex and weight at 9 weeks, and random effect of block were included. Classification variables were treatment, sex, batch and birth litter. Pair wise differences of treatment least square means were tested with the Tukey method and correlations estimated.

4 Results

4.1 Radial diffusion assay for tannins

The white flowered faba bean, Gloria, did not yield any visible precipitate ring around the agar wells. The colour flowered cultivars, Fuego and Julia, evoked a visible ring of about 1.1 cm in diameter, however no distinction could be made between them, therefore values are not presented here. Using tannic acid as a standard to condensed tannins clearly was not suitable as the difference in binding capacity in this assay was very different (Figure 5).

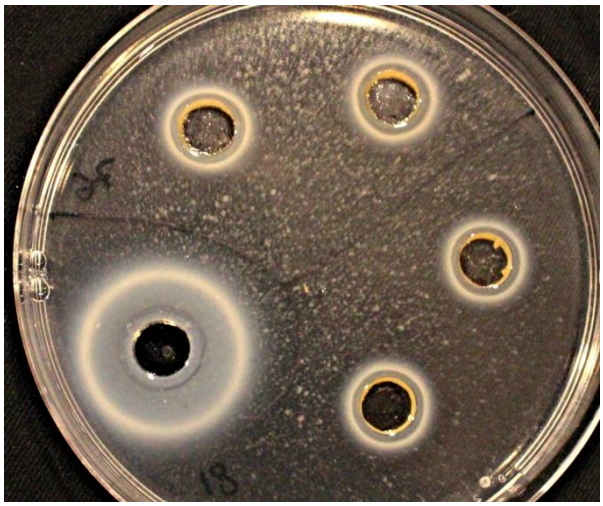


Figure 5. The white precipitate ring formed by faba bean tannins in albumin agar, the large tannic acid standard to the left, two replicates of an extract form Julia above and two replicates of Fuego at the bottom right.

4.2 Feed analysis

The hygienic quality analysis revealed no *Aspergillus fumigatus*. Water activity (a_w) was above the recommendation (<0.7 a_w) in most feeds, as well as aerobic bacteria count. The analyzed nutritional values are presented in Table 5.

Table 5. Analyzed nutritional values of trial feeds in g/kg, as fed

	Control crushed	Control	Gloria	Julia	Fuego
Dry matter	883	873	872	875	866
Ash	60	62	48	45	49
Crude protein	184	204	199	203	189
Crude fibre	45	50	44	52	50
Ether extract	45	34	54	50	45
Lysine	9.7	10.5	11.0	10.7	10.1
Methionine+Cysteine	6.6	6.5	6.3	6.4	6.1
Threonine	7.0	7.9	7.9	7.9	7.3

4.3 Pig health and weight

Table 6. Number of days barn staff noted health issues and average weights over the entire study period (162 days in total)

	Control	Gloria	Julia10	Julia20	Fuego10	Fuego20
Health remarks, days						
Feces[1]	22	26	29	15	27	14
Feed intake[2]	20	13	13	14	17	14
Body condition[3]	-	3	-	-	1	2
Deaths	-	1	-	2	3	-
Average weight, kg						
5 weeks of age	12.2	12.2	12.2	12.2	12.2	12.3
7 weeks of age	16.3	16.4	16.2	16.5	15.9	16.1
9 weeks of age	26.4	25.7	26.5	25.6	25.1	25.3

[1]Sum of days when faecal status deviated from 2 (scale 1-5).
[2]Sum of days when feed intake was below 2 (scale 0-3).
[3]Sum of days when body condition deviated from 3 (scale 1-5).

4.4 Growth parameters and plasma urea nitrogen

Weight at weaning imposed a significant effect on average daily gain. P-values presented in Table 7 are computed without batch 9, since it overall featured poor average weights, feed intake and feed conversion ratio. After finishing the experiment, a high mortality due to oedema disease was noted in this batch. Setting the limit for tendency at $p < 0.10$ and significance at $p < 0.05$, excluding batch 9 lowered p-values but did not change significances. The only treatment effect detected was for average daily gain between weaning and 7 week of age. Adjusted p-values based on Tukey's test showed Julia20 tended to have a higher average daily gain than Fuego10 ($p=0.025$). Correlation estimates detected a significant ($p=0.0036$) inverse correlation ($r= -0.35$) between weight at 9 weeks and PUN.

Table 7. Effect of experimental diet on pig average daily gain (ADG), feed intake (ADFI), feed conversion ratio (FCR) and plasma urea nitrogen (PUN). Least square means ± pooled standard error of mean (SEM)

	Control	Gloria	Julia10	Julia20	Fuego10	Fuego20	pooled SEM	p-value
ADG, g								
weaning – 7 weeks of age	316[ab]	311[ab]	305[ab]	327[a]	282[b]	293[ab]	22	0.030
7 weeks – 9 weeks of age	721	663	690	705	664	662	48	0.185
weaning – 9 weeks of age	526	493	505	523	480	485	33	0.092
ADFI, g								
weaning – 9 weeks of age	835	855	828	883	792	792	58	0.411
FCR, kg feed/kg growth								
weaning – 9 weeks of age	1.60	1.75	1.65	1.70	1.57	1.55	0.14	0.685
PUN, mg/L	123	106	115	107	112	107	6.5	0.382

[ab] Means with different superscript differ ($P < 0.05$).

5 Discussion

5.1 Current experiment

5.1.1 Pig growth and health

The lack of difference in pig performance between the experimental diets encourages replacing imported soybean with domestic faba bean as there is no advantage to pig health and growth of using soybean. Regarding health, there was no differences between cultivars, indicating that whatever the effect of tannins it does not impair health at inclusion levels up to 20 % to weaners. The large variation in data might have contributed to the high p-values obtained. The results differ somewhat from the previous part of this study, testing the same cultivars in conventional feeds, where the control soybean meal diet had the poorest feed conversion ratio and Julia20 the best (Neil & Ivarsson, unpublished), further supporting the use of faba bean. No reductions in feed intake related to tannin content were noted in the current study, any unpleasant aroma might have been covered by pelleting or, as supported by the reviewed studies, pigs are not sensitive to the tannin taste (Flis *et al*., 1999; Royer *et al*., 2010, Smith *et al*., 2013; Møller, 2014; Vils & Vinther, 2016). Analyzed nutritional values of feeds agree well with those calculated, only crude protein values were higher. Also, the crude protein value differed between the crushed and whole pellets. Feeding the control diet crushed in the first batch probably did not affect the results as there was little difference in intake between the treatments. Also due to the outline of the feeding machine, the whole pellets was crumbled upon reaching the feed tray, thus the difference in consistency was not that remarkable when consumed by the animals.

5.1.2 Beans and their properties

The *in vitro* analyzed digestible crude protein values are impossibly high, probably consequent to an inherent method flaw, and should not be used as such but surely for comparison between cultivars. This is similar to the findings by Jezierny et al. (2010b). Calculated standardized ileal digestibilities are lower, yet not as low as those measured *in vivo* (Table 1) by Jezierny et al. (2011), indicating that the use of *in vitro* methods to determine standardized ileal digestibilities in feed batches requires further development. As can be seen in Table 2, organic matter and crude protein digestibility decreased as the tannin content increased, in line with the linear relationships calculated by other workers (Jansman, 1993; Mariscal-Landín *et al*., 2002; Jezierny *et al*., 2011). The tannin mean degree of polymerization of the experimental cultivars is somewhat lower than values of other beans species found in literature, but well in range compared to berries and sainfoin. Julia had the highest prodelphinidin/procyanidin ratio and highest mean degree of polymerization. Thus, the condensed tannins in Julia might be more potent than those in Fuego. The superior farming qualities of colour flowered cultivars appear to be an effect of their tannin content, in that case breeding for low tannin cultivars is inefficient. Since no performance reduction was found in the current study there appears to be even less reason to do so concerning pig feeding. The mould probably established due to the high water activity.

5.1.3 Protein utilization

Differences in PUN values between cultivars could have resulted from tannins decreasing protein absorption and absorption of indispensible amino acids which in turn alters the protein value of the diet and the amount that can be deposited. In the present study, no differences were found. Compared to literature values the experiment pigs featured PUN concentrations within a common range. Since the pigs were fed *ad libitum*, sampling time of day was allowed to vary in the range of a few hours without values being affected (Pedersen & Boisen, 2010). Compared to the previous part of this study, PUN values were generally higher. This is

likely an effect of the restrained organic feed optimization and resulting high crude protein content and suboptimal amino acid balance of the diets. This will reduce nitrogen retention, increasing nitrogen leakage per unit growth, and increases the amount of amino acids passing to the cecum and colon for fermentation, contributing to a milieu beneficial to pathogens. This disagrees with both animal welfare and environmental considerations. The inverse correlation between weight at the end of this feeding experiment and PUN, indicates that heavy pigs efficiently utilized feed protein for growth and not immune defence. Inflammation causes a metabolic shift towards catabolism which inhibits growth (Gessner *et al*., 2016). Body reserves are degraded to produce immune proteins and provide immune cells with glucose from glucogenic amino acids and glycerol from lipolysis. The amino group from amino acid deamination ends up as ammonia which is converted to urea prior to urinary excretion. Possibly this is the physiological background to the negative correlation between weight and PUN.

5.2 Previous studies

5.2.1 Analytical methods

It is hard to compare physiological responses between studies where only the tannin quantity is reported: as evidenced in the current study there are differences in condensed tannin structure between cultivars (Table 2) though not striking. The colorimetric methods developed decades ago are still used in animal nutrition studies today, but need to be abandoned for the research area of the role of tannin in nutrition to proceed. A range of colorimetric assays for tannin analysis are employed in the reviewed studies, none has used liquid chromatography or mass spectrometry. Their unspecificity and insensitivity add to the difficulty of comparing studies. This is illustrated by the higher tannin content analyzed in the feed than beans by Royer et al. (2010) - likely some other feed ingredient contained similar phenolic compounds or the tannins were modified by feed processing to a shape generating a different absorbance. Precipitation assays are considered to better reflect the binding properties of tannins, however, precipitation is only tested towards the one chose protein. Considering the large variation in possible binding activity of tannins towards different proteins, there is a limit to the conclusions to be drawn from such assays. Furthermore, all mentioned methods measure only extractable tannins. Depending on binding properties to the debris, these tannins may dissolve in response to milieu changes as they pass through the gastrointestinal tract, particularly in acid regions. This fraction could contribute to binding enzymes, other dietary compounds, microbes or intestinal cell components.

5.2.2 Growth and enteric effects

Since the tannin content is largely the same in colour flowered faba beans today compared to older studies, and the inclusion levels have been in a similar range, differences in production results between older and more recent studies cannot be contributed to varying tannin levels in beans and diets. In the reviewed feeding experiments different standards for diet optimization were used. As pointed out by Smith et al. (2013), divergence in pig performance between studies can be explained by improved feed optimization in recent years. Tryptophan analysis is neglected in many experiments, although it is indispensible and its concentration is low in faba bean (Table 1). Especially regarding organic diets, this underlines the importance of feed analysis prior to optimization. Any slight reduction in feed conversion should be contrasted to the higher prize of soybean compared to home grown faba bean. The antinutritional substances in raw soybean are sensitive to heat and pressure, thus it is somewhat unfair to compare raw faba bean to soybean expeller or meal. Also the protein content of soybean expeller is higher than that of the raw bean.

5.2.3 Binding activity

Plants contain tannin mixtures, not a pure tannin, adding to the complexity of elucidating effects. Since satisfying methods for their purification is as of yet not developed there is not much progress in the research area at the moment. Going the other way about it by measuring metabolic responses is cumbersome. In Figure 4, enzymes with pI close to the pH at their operational site are pepsins and pancreatic amylase. Duodenally, a range of enzymes with acidic pI might be complexed but should soon dissociate. None of these include chymotrypsin, trypsin and their zymogens: their pI are far more alkaline than small intestinal pH. This is in contrast to the finding by Jansman et al. (1993). As reviewed, other workers have questioned the importance of pI. The observation that salivary proteins are effectively precipitated by tannins is also contradictory in this sense: saliva being neutral and the pI of salivary proteins alkaline, for example 10.5 for PRB4 and 10 for histatin III (SIB, 2016). These values are also close the pKa of phenols, at which point there is no binding activity anyway. Protein pI might be one of multiple factors affecting binding, but is not decisive. If there is preferential binding of condensed tannins to certain proteins or amino acids, whatever the underlying mechanism, established standardized ileal digestibility values of amino acids may deviate greatly from the true since feed-specific amino acid composition in endogenous losses will be very different from that assumed, hampering diet formulation. As the limiting amino acids in faba bean, the sulphur amino acids and tryptophan, exhibit a particularly low digestibility in colour flowered cultivars compared to white, these should receive special consideration in diet formulation. The pH at which legumin subunits are bound by faba bean tannins (3, 6<) do not match their pI (4.6-6.1, 8.2-8.5). Also, the particularly reduced amino acid digestibility noted in colour flowered faba bean applies to methionine, cysteine and tryptophan, yet these are not abundant in legumin, the most common faba bean protein, indicating the primary interest of tannins lies elsewhere.

5.2.4 Bioactive effects

The reviewed studies (Van der Meulen and Jansman, 2010; Smith *et al*., 2013; Møller, 2014) give the impression it is both the fibre and tannin content affecting intestinal function and faecal consistency, both of which are concentrated in the hull. Since the hull fraction is larger and thus fibre concentration is higher in colour flowered cultivars, part of the reductions in digestibility attributed to tannins could derive from fibre. In the present study, there was no decrease in the occurrence of oedema disease or faecal remarks ascribed to feeding the tannin containing cultivars. The lower ileal microbial diversity of weaners fed colour flowered faba bean hulls observed by Van der Meulen et al. (2010) and smaller ceca of weaners fed colour flowered faba bean observed by Jansman (1993) could be *in vivo* proof of antibacterial properties of tannins. This can derive either from tannins directly inhibiting microbes or making their substrates unavailable to them. The mechanism likely also applies to enteric cell proteins, enzymes and nutrients. For example, dietary protein is a substrate to pathogens causing post weaning colibacillosis: tannins reduce protein availability to the pathogen but simultaneously also to the weaner, who is in need of protein to develop an immune response to the pathogen. Whatever the benefits of condensed tannins in these terms, they hardly overcome the accompanying depressing effects which appear to derive from the same modes of action. Advantage could be drawn from that the effects are both tannin, substrate and circumstance dependant. Elucidating the ultimate tannin, inhibiting pathogen growth yet not that of beneficial microbes and intestinal protein absorption in the enteric milieu is a demanding task. Most of the bioactive effects ascribed to condensed tannins deduce from studies of other tannin types like hydrolysable tannins or the mono- and dimeric building units of condensed tannins such as catechin. Catechin is a known antioxidant and if there is some enteric degradation of condensed tannins it is possible catechins are absorbed, however it is

unlikely that the doses are high enough to be relevant in production. The non-existent enterocyte absorption of condensed tannins and extremely low absorption of possible metabolites render *in vitro* findings of antioxidant properties dull. Enteric effects are more likely of relevance. However, experimental doses are higher than those naturally occurring in faba bean (Gessner et al., 2016) and in order to obtain physiologically significant quantities for the antioxidant and antimicrobial effects, extraction and purification methods must develop. This will also ease *in vitro* assays such as the radial diffusion assay performed in the current study, as it was clear tannic acid, a hydrolysable tannin, is not a suitable standard to the condensed tannins in faba bean. Finding enhancers of enteric integrity is especially important to weaners since their enteric function is sensitive and undeveloped. For now, other preventive measures should not be overlooked, such a clean environment and sectional production which both effectively reduce infectious pressure.

5.3 Considerations for the future

The pigs were only examined over a short period, future experiments should examine feeding colour flowered cultivars over the entire rearing period, as well as to sows and breeding hogs since condensed tannins have no known detrimental effects on fertility. White flowered cultivars have been successfully fed at 10 % to gestating and lactating sows (Neil & Sigfridson, 2012). If such experiments proved successful, it would prompt utilization of colour flowered faba bean in Swedish pig production. Processing methods such as soaking, dehulling, germination, roasting and air-classification have not been considered in this work, they offer potential to increase digestibility but increase production cost. Although vicilin is present at one third the amount of legumin, both belong to the most abundant group of proteins in faba bean. Thus feeding a faba bean protein concentrate could increase the risk of evoking an immune response from vicilin. Whether this response could be overcome or outbalanced by antiinflammatory properties of faba bean tannins makes solid work for future studies. Increasing the inclusion of faba bean to weaners, independent of cultivar, might be limited by the content of antinutritional fibres and immunogenic proteins as well as the lack of sulphur containing amino acids. Inclusion levels to growing and finishing pigs could be higher as they are less prone to develop an immune response to plant storage proteins.

6 Conclusion

The results from this experiment did not prove faba bean cultivars, colour or white flowered, to be any better nor worse than soybean expeller in terms of growth and health. Thus, colour flowered faba bean has good potential in well balanced diets in Swedish pig production. Possible, but rather improbable, bioactive properties of faba bean tannins did not show any effect.

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