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Novel protein crops as pig feed in organic farming

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Summary

From 25 August 2005 to the 1st of January 2012 organic farmers are obliged to gradually increase the use of exclusively feeds that are produced by organic farming methods from 85 to 100%. In this desk study the characteristics and potential of organically-produced proteinaceous crops will be described.
Legume seeds, which fit in the European setting, have a high protein content as compared to other seed crops but lower than soy bean (*Glycine max* Merr.). The most important of these grain legume crops are: pea - *Pisum sativum* L., field bean - *Vicia faba* L., lupin species- *Lupinus albus* L., *Lupinus luteus* L. and *Lupinus angustifolius* L. Legumes in general have a high quality of amino acids, although the levels of tryptophane and sulphur-containing amino acids, cystein and methionin, are low. The high levels of lysin and threonin make legumes complementary to cereal grains. Legumes also contain so-called antinutritional factors (ANFs), which owe their name to a reduction of the nutritional value, especially when used as feeds for farm animals. The major ANFs are: tannins, phytate, protease inhibitors, (flatulence inducing) oligosaccharides, alkaloids, lectins and saponins.
In addition to legumes, quinoa may be an interesting proteinaceous crop due to its relatively high fat and protein content, low levels of ANFs and the high quality of protein-bound amino acids as compared to cereal grains.
Investigations with pigs and poultry show that EU-grown legumes, obtained by breeding for low ANF levels, may replace soy beans in their diets.
For the organic farmer, field beans probably are a more attractive crop than peas due the lower sensitivity for fungal and other diseases. On the long term lupins and quinoa may also prove to be attractive crops for organic farming, when cultivars would become available that are adapted to the climatic and other agronomic conditions in the EU. In this study, for some new cultivars selected for lower ANF-content of field bean, lupins and quinoa, occurrence of ANFs was studied under organic farming conditions. It was shown that ANF-contents were reduced in cultivars selected for low content of specific ANFs. ANF-contents under organic farming for other ANFs were in similar to contents found for conventional farming. Also, occurrence of lectins/estrogens, heavy metals, dioxins, pesticides and dioxins was studied of organically grown crops of these crops. Studied samples from organic farming trials showed that none of these harmful components had concentrations that were above normal, safe levels. Only some lectin activity was found in field beans and some pea samples. One sunflower meal sample contained too high Aflatoxin B levels.

1. Introduction

Since the ban on animal-derived protein sources in animal diets the use of plant-derived protein in concentrates has strongly increased. The major source of such protein has been extracted soybean meal. From 25 August 2005 onwards to the 1st of January 2012, the use of raw materials and feeds for organic farming has to be gradually increased from 85 to 100% from organically-grown sources. Since the major part of soybean meal is produced from genetically transformed cultivars, soybean meal will not be allowed as feed component in organic farming. Due to the limited availability of protein from organically-grown potatoes, this source will also not be feasible for feed production. Therefore, alternative crops, which can be grown by organic farming, have to be explored as sources of protein for concentrates. This study describes the characteristics and potential of such alternative proteinaceous crops. Most of these crops are legumes, but also quinoa has been taken into consideration since it has a relatively high fat and protein content, a high quality of protein-bound amino acids and contains low levels of antinutritional factors (ANFs).
In the past 15 years a series of conferences has been held in the EU community devoted to ANFs in legumes produced by conventional farming practices (Huisman *et al.*, 1989; van der Poel *et al.*, 1993; Jansman et al, 1998; Muzquiz *et al.*, 2004), which were attended by scientists of the “old” and “new” member states. The conference proceedings of these symposia review the entire scope of research including animal nutrition, chemical analysis, breeding and processing of legumes (and some other crops) with regard to the presence of ANFs. Due to the relevance of organically-grown legumes as feeds in the context of this survey, the most relevant ANFs were measured in a few *faba* bean and lupin cultivars and in quinoa, which were all grown under organic farming conditons. occurrence of some other unwanted components was studied (lectins/estrogens, heavy metals, dioxins, pesticides and dioxins) of organically grown crops of these alternative proteinaceous crops.

1.1 Agronomy of pulses (legumes)

The EU area (EU25) for pulse growing has been estimated 1.9 million ha of which the proportion of certified organic farming was about 4 % in 2003 and is still increasing. Legumes are particularly interesting since they provide in their nitrogen requirements by converting atmospheric N2 through symbiosis with *Rhizobium* bacteria. Since this nitrate and its derivatives can be accumulated in the soil by plowing the non-edible parts, legumes can also fulfil an important role as green manure in organic farming.

The low relevance of pulses as source of feeds in ‘common’ agronomy has an economic background. For organic farming there are additional reasons for the low interest:

1. extended crop rotation with non-legume crops is required due to foot rot and other soil-born diseases;
2. when legumes are incorporated in the agronomic schedule for human consumption peas are prefered due to their higher economic profitability;
3. the contribution of nitrogen fixation is limited since for this purpose lucerne and grasses/clover are more effective and easier to incorporate;
4. the amino acid composition is not optimal for concentrates;
5. the price is too high for use in concentrates.

The major research and practical interest in pulses from the side of organic farming was devoted to their potential in nitrogen fixation and much less to their function as source of proteinaceous component of feeds. Legumes are known to fix atmospheric nitrogen by symbiosis with bacteria of the genus *Rhizobium*. In doing so, they may provide subsequent crops with sufficient nitrogen to avoid the need for exogenous nitrogen supply by chemically prepared fertilizers or animal-derived manure. This will reduce the amount of leaching of nitrate to surface waters (Hansen *et al.*, 2001; Jensen & Hangaard, 2003, Ridley *et al.*, 2004).

In addition, such use of legumes as “green manure” may improve soil texture and soil biological activity, e.g. presence of earthworms etcetera.

The major limiting factor for growing pulses is the requirement for a wide crop rotation schedule. Crop rotation is especially relevant to avoid a build up of pressure by pathogens, causing foot rot and other soil-born diseases. Recently, an extensive long-term research program has been started in Denmark to determine the most important pathogens and their hosts (Jensen, 2003). Seed-related and soil-borne pathogens are also here considered as the greatest barrier for the desired extension of legume agronomy. In areas with a history of pea growing, 10-20% of all plots cannot be used for pea culitvation for probably the next 20 years due to a high level of pathogens in the soil. For this purpose a 4-year research program has been initiated to explore pathogens, with regard to specific or common virulence to lupin, pea and field bean. The research program includes:

- Resistance of pea towards *Aphanomyces eutiches* and *Fusarium* spp;
- Resistance of field bean towards *Fusarium* spp;
- Resistance of lupin towards *Fusarium* spp and *Colletotrichum* spp;
- Characterization of naturally occurring soil pathogens on pea, field bean and lupin;
- Characterization of hosts for *F. avenaceum* and *F. oxysporum* on pea, clover, field bean and lupin;
- Determination of yield loss by soil pathogens on ten other legume crops.

The results of this study in Denmark will be very valuable for other EU countries.

1.2 Nutritive value

Seeds of the grain legumes grown in the EU: pea (*Pisum sativum*), French bean (Phaseolus vulgaris), field bean (*Vicia* faba), lupin (*Lupinus* albus, L. luteus, L. angustifolius) have a high protein content as compared to other seed crops, but the protein content and (ileal)protein digestibility are lower than in extracted soybean meal. Amino acid composition is also of high quality, although tryptophane and sulphur-containing amino acids are suboptimal. Lysine and threonine levels are very high which makes legumes a feed source of high quality when used in combination with cereals. In addition, some legumes have also a high fat and/or fibre content (Table 1). For organic feeds the inclusion rates for field bean, lupin and pea are 30%, 15% and 30%, respectively (Arp *et al.*, 2001). More detailed information for field bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and Lupin spp is given in the CVB veevoedertabel (CVB, 2005).

1.3 Antinutritional Factors (ANFs)

All grain legumes, and to a lesser extent also quinoa, contain so called antinutritional factors (ANFs): compounds which negatively affect the nutritive value. The most important ANFs are: tannins, protease inhibitors, phytate, lectins, flatulence-inducing oligosaccharides, alkaloids, pyrimidin glucosides (vicine/convicine) and saponins (Table 2).

1.3.1. Tannins

Tannins belong to a chemical family of compounds known as polyphenols. Tannins can be divided in two chemical subclasses: condensed tannins and hydrolysable tannins (Haslam, 1981). Hydrolysable tannins (Fig. 1, right panel) may be hydrolysed by acids and bases into its major components: sugars, mostly glucose, or related polyols, and a phenolic acid. Condensed tannins, also called proanthocyanidins, are polymers of flavan-3-ols in which the interflavan bonds are commonly C-4 to C-8 (Fig. 1, left panel), but also C-4 to C-6 bonds are observed.

Condensed tannins are considered the most active as antinutritional factors and are commonly observed in the seed coat of coloured-flowering *faba* beans (Helsper *et al.*, 1993) and peas (Buraczewska *et al.*, 1989). White-flowering cultivars are free of condensed tannins but may contain low levels of hydrolysable tannins. Tannins effectuate their antinutritional acitivity by aspecifically binding proteins and polysaccharides (e.g. starch), which reduces the digestibility of these feed components.

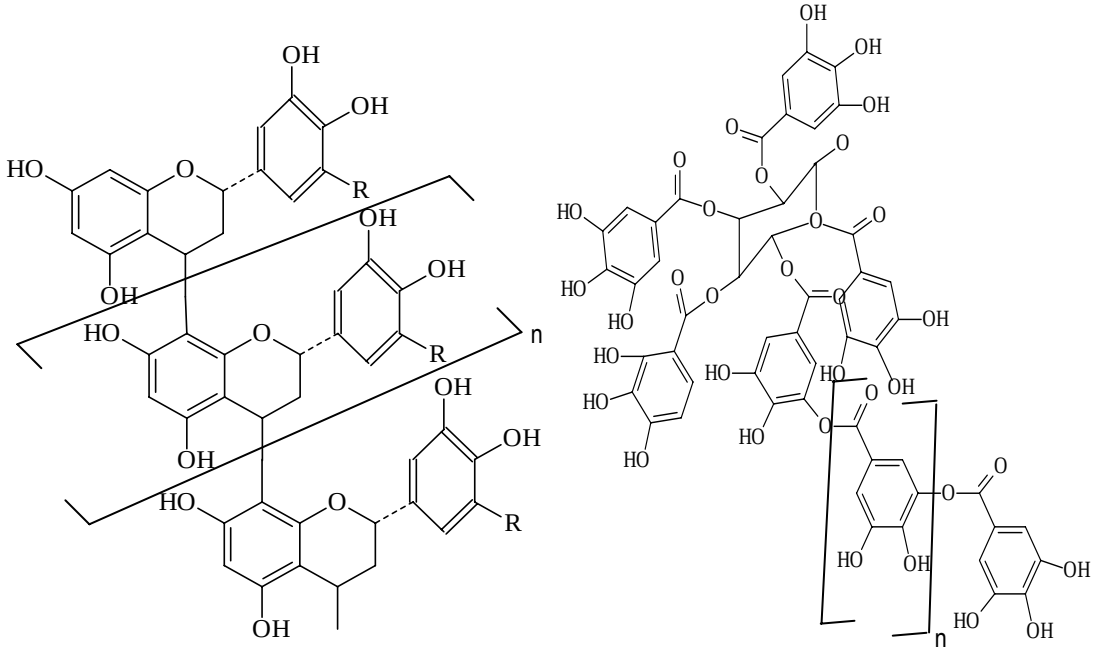

Figure 1. Structure of condensed tannins (left panel, R= H, OH) and gallotannin (a hydrolysable tannin, right panel). Source: Haslam, 1981. n = 1,2,3,..

1.3.2. Protease inhibitors

Legumes may contain various types of protease inhibitors and the activity of these varies widely between and within crop species (Valdebouze *et al.*, 1980; Birk, 1989). The most well described protease inhbitors are the so-called Bowman-Birk protease inhibitors and Kunitz trypsin inhibitors. In addition to these proteinaceous protease inhibitors, condensed tannins have been shown to exhibit protease inhibitor acitivity, probably by aspecific binding to proteins (Helsper *et al.*, 1993a, 1993b).

The highest levels are observed in soybean but also winter peas and *faba* beans may show high trypsin inhibitor activity (Boisen, 1989). When feasible, trypsin is mostly used as protease for the assay of their activity. In addition to a direct effect on digestive enzymes, long-term exposure to protease inhibitors may lead to growth inhibition and increased pancreas size (pancreas hypertrophy) of animals. Epidemiological studies have shown that protease inhibitors can also have a benificial activity as protective agents against breast, colon, and prostatic cancer (Birk, 1989). The activity of protease inhibitors is sensitive to heat treatment and can also be inactivated by fermentation (Frokiaer *et al.*, 2001).

1.3.3. Phytate

Phytate (myo-inostol hexakisphosphate, fig. 2) can bind metal ions and thus affect the uptake of iron and zinc. In addition, phytate from peas has been shown to inhibit protein availablity (Frederikson *et al.*, 2001). Formulation of feeds with the enzyme phytase of microbial origin will greatly reduce these effects but is not permitted in organic pig farming. However, in some cereals like wheat, barley and rye intrinsic phytase is present which can hydrolyse phytate.

Another aspect of phytate, especially relevant in organic farming, is that phytate-bound phosphorus is not available for digestion and will therefore be excreted with the faeces. Thus, in intensive poultry and pig farming this may lead to higher emissions of phosphate to the environment as compared with conventional farming. Large proportions of phosphate in plant-derived food sources are phytate-bound: in peas ±50%, lupin ±60% and field beans ±60%. In cereal crops this can amount to ±85%. The presence of (intrinsic) phytase activity makes the phosphorus available for uptake by the animal (Jongbloed *et al.*, 2000). Concentrations of total phosphorus should be collected from various sources of information, e.g. Selle *et al.* (2003) reports ±3.5-4.5 g/kg for legumes and 2.5-3.5 g/kg for cereals, while the CVB veevoedertabel (CVB, 2005) lists ±1.2 g/kg for lupin, 2.5g/kg for *faba* beans and 2.2 g/kg for peas.

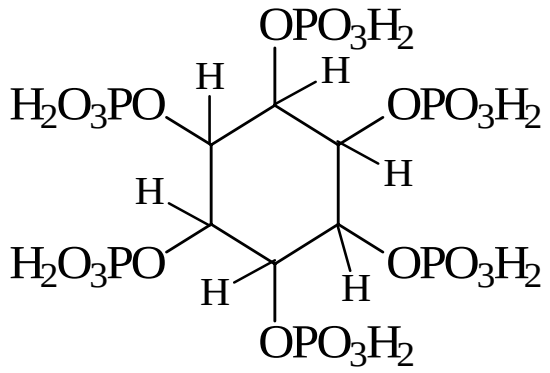

Figure 2. Chemical structure of phytic acid

1.3.4. Lectins

Lectins are proteins that can bind to specific carbohydrates. Some grain legume seeds contain high concentrations of lectins. Their physiological effect is strongly dependent on the sugar to which a particular lectin shows binding affinity. Phaseolus haemagglutinin exhibits accute toxic effects such as diarrhoea and vomiting, while pea lectin does not result in accute symptoms. Long-term effects are unkown, but physiologically effective lectins are likely to lead to growth inhibition. Lectins are readily inactivated by heat processing.

1.3.5. Flatulence-inducing oligosaccharides

Legumes contain substantial amounts of non-digestible raffinose-type oligosaccharides (raffinose, stachyose, verbascose and others, see fig 3). After fermentation by the intestinal microflora the lactose moieties may lead to flatulence which causes considerable incovenience for the animal. Oligosaccharides may be removed from the meal by treatment with galactosidases.

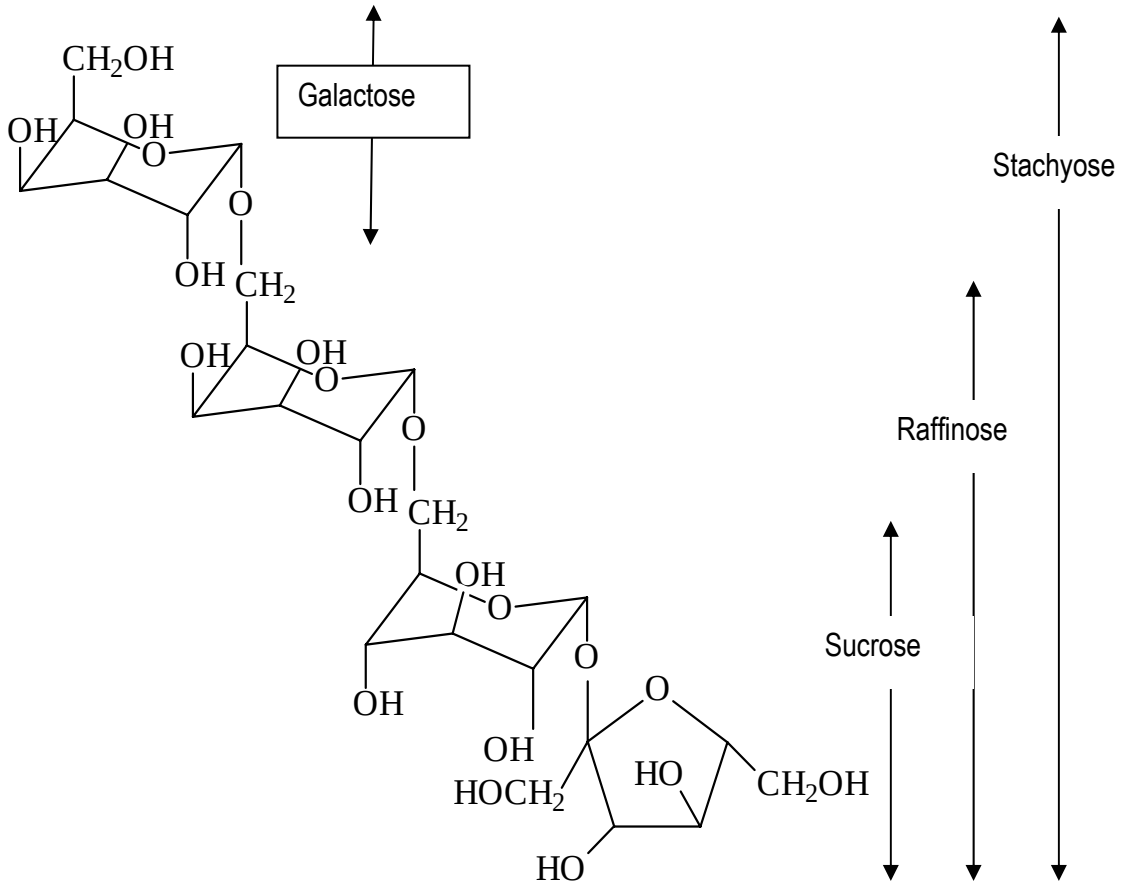

Figure 3. Flatulence-inducing oligosaccharides of the raffinose series. Verbascose has an additional galactose residue as compared to stachyose

1.3.6. Alkaloids, pyrimidin glucosides (vicin/convicin) and saponins

Lupins contain various types of alkaloids from which the quinolizidin alkaloids are the most relevant with regard to antinutritional activty in feeds. In particular pigs appear to be very sensitive for this type of alkaloids (Cheeke and Kelly, 1983). The exact mode of action of these alkaloids is unknown, but their bitter taste may result in inhibition of feed intake, but also neurophysiological effects, e.g. tremors, convulsions and pulmonary arrest have been described (Kingsbury, 1964). Low-alkaloid genotypes, also known as sweet lupins, are available.

Pyrimidin glucosides, from which two compounds are described, vicine and convicine, are specific for field beans. They disturb fat metabolism in laying hens and also affect fertility. In France, *faba* bean genotypes which are virtually free of pyrimidin glucosides, have been developed since about two decades (Duc *et al.*, 1989).

Saponins are steroid glycosides to which both positive and negative physiological effects have been ascribed. As for alkaloids the bitter, astringent taste may inhibit feed intake, but also haemolytic activity on red blood cells and foaming (detergent) activity may impair the nutrititive quality of feeds in which they are included.

Table 1. Survey of the most important characteristics of field bean, lupin, pea and quinoa (various sources)

Crop species	Field bean	Lupins	Pea	Quinoa
DM yield (kg/ha)	3000-5000	1500-2500	3000-3500	3000-3500
Protein content (%)	24 - 26	35 - 48	20 - 25	12 - 18
Lysine (mg/g protein)	47	30 - 60	43 - 72	61
Methionine (mg/g protein)	6.0	3 - 10	6.0	3-11
Cysteine (mg/g protein)	9.3	7 - 48	8 - 9	7-18
Faecal protein digestibiliity (%)	82-92	About 83.3	71 - 90	?
Fat (%)	1- 3	4 - 15	1.1	7-9
Starch (%)	40-57	29 – 46	41.6	67
Crude fibre (g/kg)	77 - 88	168	53 - 67	68
ME (MJ/kg; pigs)	14.4	14.7-15.5	15.5	13.8
DE (MJ/kg); (poultry)	11.6 – 11.9	13.4-16.0	14.0 - 14.2	?
NEv (MJ/kg)	8.4 – 8.9	8.4 – 8.6	9,3	?

Table 2. Characteristics of anti-nutritional factors in field bean, lupin pea and quinoa (various sources)

Crop species	Field bean	Lupin	Pea	Quinoa
Alkaloids	no	no, in alkaloid-free cultivars	no	no
Tannins	no, in tannin-free cultivars	no	no	yes
Convicine/vicine (only relevant for laying hens)	yes in most cultivars; no, in few cultivars	no		no
Phytate	yes	yes	yes	yes
Protease-inhibitorrs	yes	no	yes in most cultivars; no, in few cultivars	yes
Lectins	yes	yes	yes	yes
Flatulence inducing oligosacharides	yes	yes	yes	no
Saponins	yes	no	no	no, in saponin-free cultivars

2. Field Bean (*Vicia faba* L.)

2.1 Agronomy

White-flowering varieties have a low content of total tannins and this trait is linked to a virtual absence of condensed tannins (Helsper *et al.*, 1993b). This allows a larger inclusion rate in feeds than for coloured-flowering cultivars. Cultivars are available which are low in pyrimidin glycosides as well as in condensed tannins (Grosjean *et al.*, 2001). In various EU countries. e.g. France and UK, field bean culitvars have been developed which, under the appropriate climatic conditions, can be sown in the fall. In general, these have a higher average yield than spring-sown field beans. The English winter field beans are all tannin-containing. Some of the French winter culitvars are free of pyrimidin glycosides. In Northern Europe the climate is not feasible for growth of winter *faba* beans.

Faba beans are susceptible for soil-borne pathogens, like Fusarium spp. and Pythium spp. The white-flowering varieties are in general more susceptible than the coloured-flowering ones. Studies with various near-isogenic lines have shown a causal relationship between the absence of condensed tannins and Fusarium susceptibility (Helsper *et al.*, 1994). In the same and other studies it was shown that there is no relationship between the absence of condensed tannins and susceptibilty for leaf and stem pathogens, like Uromyces *Vicia*e-fabae, Botrytis fabae (see also Kantar at el., 1996), and Ascochyta fabae.

"Top yelllows" is caused by the same virus that induces this disease in peas and may cause severe damage. Alfalfa may be a source of infection. A plant infested with "top yelllows" is more susceptible for chocolate spot disease (Botrytis fabae L.). In organic farming a wide crop rotation schedule is very important to reduce pressure by soil-born pathogens. In addition, cultivar choice is very relevant. Data of yield reduction due to diseases were not available for *Vicia faba* L.

2.2 Nutritive value

Makkar *et al.* (1997) have compared the nutritive value of six coloured-flowering cultivars with six white-flowering ones. The seed samples originated from different breeding companies and are therefore not completely comparable. Despite this, the comparison between white- and coloured-flowering cultivars showed clear differences. Protein, fat, crude fibre, starch and ash content were not significantly different between the two groups. The calculated organic matter digestibility, metabolisable energy and in vitro N-digestibility were significantly higher in white-flowering as compared to coloured-flowering cultivars. As expected, condensed tannins were absent in the white-fowering cultivars while total polyphenols were low in all genotypes. The levels of other ANFs, e.g. trypsin inhibitor activity, phytate and lectins were low. In vitro studies using liquor and particulate matter from rumen of dairy cows showed a strong negative correlation between tannin level on the one hand and in vitro rumen protein digestibility ($r=-0.92$, $p<0.001$), metabolisable energy ($r=-0.89$; $P<0.001$) and organic matter digestibility ($r=-0.89$; $P<0.001$) on the other, while a strong positive correlation was observed between the content of condensed tannins and saponins ($r=0.96$; $P<0.001$). Tables 3 and 4 show the amino acid composition of the proteins and the average ANF levels. Similar studies were performed with six pairs of near-isogenic lines, selected for the same contrast (Van der Poel *et al.*, 1992; Helsper *et al.* 1996) show the same correlations between presence of condensed tannins and digestibity values.

The same six near-isogenic lines were used to study the effect of absence versus presence of condensed tannins in seed coats on other ANFs. The tannin-free line showed a higher content of pyrimidine glucosides (vicine plus convicine), but no differences in protein-bound trypsin inhibitor activity and lectins. Near-isogenic lines with the same contrast (absence versus presence of condensed tannins) and also near-isogenic lines with a contrast in presence versus (virtual) absence of pyrimidine glucosides were also used by Grosjean et al (2001) also used near-isogenic lines to study the effects of tannins and pyrimidin glucosides on fecal digestibility in castrated pigs of 30-40 kg (Table 5). The diet contained 50% of 3mm-flattened beans. Results were comparable to that described above for the condensed tannins, while no

effect was shown for the contrast in content of pyrimidin glucosides. There are two genes regulating content of condensed tannins in field beans: zt-1 and zt-2. Most tannin-free cultivars, e.g Caspar which is widely used, posess zt-1. The gene zt-2 appears to result in a higher protein digestibility caused by higher protein and energy levels and a lower fibre content (Crofton *et al.*, 2001).

Tabel 3. Amino acid composition in proteins of six coloured- (Scirrocco-Herz-Freya) and six white-flowering (Caspar-Cresta) cultivars of field beans (source: Makkar et al., 1997).

Amino acids	*Scirocco*	*Alfred*	*Carola*	*Condor*	*Tina*	*Herz Freya*	*Caspar*	*Albatros*	*Gloria*	*Tyrol*	*Vasco*	*Cresta*	*FAO Protein*[a]
Lysine	6·28	6·89	7·93	7·72	8·56	7·55	6·55	6·24	7·22	6·68	8·16	7·23	5·80
Leucine	7·57	7·71	7·57	8·11	7·35	8·27	7·37	7·16	7·52	7·77	7·40	7·50	6·60
Isoleucine	4·19	4·09	3·97	4·64	3·29	4·26	3·81	3·81	4·24	4·12	3·76	3·79	2·80
Methionine	0·90	0·95	0·92	1·0	0·91	1·01	0·90	0·88	0·81	0·95	1·1	0·79	2·50
Cystine	1·19	1·42	1·28	1·39	1·38	1·27	1·41	1·29	1·18	1·31	1·10	1·18	
Phenylalanine	5·25	4·35	4·13	4·77	4·45	4·68	4·11	3·58	4·86	4·45	4·31	4·34	6·30
Tyrosine	3·88	3·88	3·93	4·60	3·67	3·97	3·86	3·46	4·31	4·27	3·76	3·86	
Valine	3·75	4·44	4·89	5·64	4·76	4·98	4·20	4·49	4·87	5·14	4·99	4·58	3·50
Histidine	3·29	2·89	3·29	3·34	3·63	3·46	2·70	2·86	2·91	3·28	3·47	4·15	1·90
Threonine	4·39	4·09	4·33	4·34	4·15	3·88	4·07	3·77	3·76	3·90	3·76	3·79	3·40
Serine	5·39	5·51	4·89	5·21	5·23	5·53	5·35	5·18	4·68	5·00	5·03	5·09	—
Glutamic acid	17·29	15·77	16·54	16·36	16·60	15·86	15·25	14·20	14·82	15·47	15·89	14·81	—
Aspartic acid	10·28	10·47	10·54	10·98	10·72	10·12	10·24	9·67	9·77	10·40	10·27	10·15	—
Proline	4·42	5·99	4·69	5·08	6·18	6·29	5·74	4·68	4·94	5·69	5·75	5·45	—
Glycine	4·48	4·87	5·00	4·82	4·93	4·47	4·71	4·15	4·31	4·52	4·52	4·22	—
Alanine	4·10	4·57	3·53	4·04	3·63	4·60	4·41	4·30	3·43	3·90	4·27	3·99	—
Arginine	9·76	12·1	10·18	9·76	11·80	11·18	10·80	8·80	10·47	9·92	11·75	10·94	—

[a] For 2–5-year-old child.

Table 4. Antinutritional factors in field beans (source: Makkar et al., 1997).

Contents of total phenols, tannins, condensed tannins and trypsin inhibitor activity of white- and coloured-flowering *Vicia faba* beans (mean ± SD, $n = 6$)

Beans	*Total phenols*[a] ($g\ kg^{-1}$)	*Tannins*[a] ($g\ kg^{-1}$)	*Condensed tannins*[b] ($g\ kg^{-1}$)	*Trypsin inhibitor activity*[c]	*Lectin activity*[d] ($mg\ ml^{-1}$)	*Saponin*[e] ($g\ kg^{-1}$)	*Phytate* ($g\ kg^{-1}$)
White-flowering	4·5 ± 0·4	0·14 ± 0·06	nd	3·05 ± 0·34	27·2 ± 9·4	18·3 ± 1·2	15·0 ± 2·7
Colour-flowering	20·1 ± 5·7 (***)	14·1 ± 4·4 (***)	26·2 ± 7·1 (***)	1·85 ± 0·09 (***)	27·1 ± 5·1 (NS)	31·7 ± 5·4 (***)	16·6 ± 2·3 (NS)

[a] As tannic acid equivalent.
[b] As leucocyanidin equivalent.
[c] As mg trypsin inhibited g^{-1} dry matter.
[d] Minimum amount per ml assay medium which produced haemagglutination.
[e] As diosgenin equivalent.
***, $P < 0·001$; NS, not significant; nd, not detected.

Table 5. Effect of the presence (+) versus absence (-) of trypsin inhibitor activities (T) and pyrimidin glucosides (P = vicine + convicine) on digestibility values of types of field beans in pigs (source: Grosjean et al., 2001)

Tannins/Pyrimidin glucosides	Digestibility (%)		
	Organic matter	Energy	Protein
T - ; P +	90.5	94.3	90.7
T - ; P -	89.3	88.2	89.1
T + ; P +	80.9	76.7	82.9
T + ; P -	82.2	81.0	79.5

2.3 ANFs in some organically grown *faba* bean cultivars

From the values in Table 6 it becomes obvious that there is a large variability in trypsin inhibitor activities (TIA) in these *faba* bean cultivars, which were all grown under conditions of organic farming. There is a fourfold difference between the highest and lowest TIA observed. Surprisingly, the extremes were observed for a single cultivar (Gloria) from two different locations, Belgium and Switzerland. The variability between cultivars shows that breeding might lead to significant improvement of nutritive value. The TIA values in general are similar to those observed for field beans, grown under conditions of conventional farming, as is true for the other ANFs. Concentrations of phytate and flatulence-inducing oligosaccharides vary very little between these cultivars with the extremes being only 50% different. Also the proportional distribution of individual FIO component sugars shows very little variation with raffinose, stachyose and verbascose accounting for 8%, 27% and 65%, respectively.

In organic poultry farming the cultivar Divine is the most popular, since this cultivar is almost free of convicine/vicine. Convicine/vicine reduces productivity of laying hens, but not affect pig production. For organic pig production, cultivars low in tannins are needed, but these cultivars do not need to be low in convicine/vicine.

Table 6. Trypsin inhibitor activities (TIA), phytate and flatulence-inducing oligosaccharides (FIO) concentrations in some organically-grown faba bean cultivars. Levels in raffinose (Raff), stachyose (Stach) and verbascose (Verb) are given as averages, the other values are expressed as average ± standard deviation (n=4)

Cultivar	TIA* (TIU/mg DM)	Phytate* (mg/g DM)	FIO* (mg/g DM)			
			Raff	Stach	Verb	Total FAS
Faba bean						
Divine	5.5 ± 0.8	9.9 ± 1.4	1.7	7.7	19.9	29.3 ± 2.5
Dixie	5.5 ± 0.3	10.8 ± 1.6	2.1	5.9	12.0	19.9 ± 1.9
Melody	5.7 ± 0.7	11.2 ± 1.3	1.3	5.8	15.6	22.7 ± 2.9
Gloria (B)	6.7 ± 0.1	8.2 ± 1.1	1.8	6.9	17.0	25.7 ± 1.9
Gloria (CH)	1.9 ± 0.3	7.2 ± 0.8	2.7	6.2	12.0	20.9 ± 1.7
Victoria	4.8 ± 0.3	7.7 ± 0.9	2.1	7.5	17.5	27.1 ± 2.7
Aurelia	2.4 ± 0.1	8.9 ± 0.9	1.4	5.7	17.7	24.9 ± 2.7
Scirocco	2.0 ± 0.2	8.3 ± 0.6	2.7	6.2	12.0	20.8 ± 1.7
Soy bean meal						
Range in literature	1.8-5.0	7-18	11	46	1	58

* TIA has been determined according to Kakade *et al.* (1974) with modifications as suggested by Liu & Markakis (1989) and Valdebouze *et al.* (1980). Phytate was measured according to Vaintraub *et al.* (1988). FIO have been analysed using Dionex anion exchange chromatography of ethanolic extracts with pulsed amperometric detection, where 100 mM sodium hydroxide, including a 0-37.5 mM sodium actetate gradient was used as the mobile phase and a Carbopac PA1 column as the stationary phase..

Data on soy bean meal, e.g. Mielke, C. D., and D. J. Schingoethe. 1981: toasted soy bean meal vs. raw beans: 2.7 vs 24.0 TIU/mg, respectively.

2.4 Lupin (*Lupinus* spp.)

The genus *Lupinus* includes about 300 species, originating from two centres of diversity: the Mediterranean and the west coast of Middle-America. Variation between and within the species is very large and offers great opportunities for plant breeding.
Four annual species are grown as a seed crop:
Lupinus albus (white lupin) in the Mediterranean
Lupinus mutabilis (Andes lupin) in the Andes
Lupinus luteus (yellow lupin) in the Mediterranean
Lupinus angustifolius (blue or small-leaved lupin) in the Mediterranean.
The use of lupin as a crop has been extensively described by Hondelmann (1996) and Cowling et al (1998). Much information has been retained from these publications. In addition information is available at the Website of the University of Giessen: http:/bibd.unigiessen.de/2000/uni/p000003/g_lupin.htm. Yellow and blue lupin are grown since low-alkaloid culitivars became available in about 1920. Seeds of the four species contain 35-42% protein. In addition, seeds of the Andes and white lupin contain fat at 13-23% and 10-16%, respectively. In contrast to field beans and peas, lupin seeds contain hardly any trypsin inhibitor activity and only low levels of saponins which increases their nutritive value as compared to the former crops.

2.5 Agronomy

Lupins are very active in nitrogen fixation; a part of the fixed nitrogen will become available for the next crop. Lupins have a tap root and a strongly branched root system which contributes to the improvement of soil structure. The crop is robust and the pulses remain on the stem which facilitates mechanical harvesting. A soil pH higher than 6 damages the crop. A crop rotation schedule of one to four (maximally one lupin crop per four consecutive years) is required. Maize and cereals are good catch crops for lupin, other pulses will contribute to a build up of the pressure by soil pathogen populations from which many are common for more than one genus.
In Australia blue lupins are grown at a large scale, from which a part is exported as a soybean substitute. In the former USSR, other East European countries and in Germany much attention has been paid to breeding of yellow, white and blue lupin. In the Netherlands, the focus was on breeding of yellow lupin (Lambeerts and Tolner, 1952). Low bitterness and seed retention were important breeding traits.
Yields have increased considerably by adaptation of the crop to climatic and soil conditions. In France and the UK short, determinate winter types of white lupin have been developed which mature early and give higher seed yields than spring-sown genotypes. In Poland thermoneutral cultivars of yellow lupin have been developed which show early flowering and maturation. Especially for organic farming, lupin has recently received much attention as a subsititute of imported, mostly genetically transformed soybeans as a component of feeds.
In Switzerland cultivars of white and blue lupin have been compared (Frick *et al.*, 2002); seed yield of white lupin amounted to 4000 kg/ha with a protein content of 34-39% and blue lupin showed an average seed yield of 2800 kg/ha with a protein content of 32-43%.
The most severe problem in white lupin is susceptibility to anthracnosis during flowering which may result in a yield loss of up to 50%. Anthracnosis is caused by Colletotrichum gloeosporioides, which is transferred by seeds. Healthy plants can be infested by splashing. Humid and warm weather enhances the disease spread. Use of healthy seeds is the only way to control the disease, but also seed treatment at high temperatures has given positive results (Römer, 2001). A new race of the fungal pathogen developed in the 1990s which makes lupin growth by organic farming almost impossible. Anthracnosis resistance is present in blue lupin. Due to anthracnosis susceptibility, organic farming of white and yellow lupin is

considered not feasible in Denmark, leaving blue lupin as the only remaining option. New, less branching crop types are very promising with stable seed yields of 5000-6000 kg/ha.
A disease affecting growth of blue lupin as a crop is due to susceptibility to Botrytis and Fusarium (F. oxysporum and F. avenaceum; Joernsgaard *et al.*, 2002). Fusarium resistance is observed in some blue lupin and in all investigated yellow lupin genotypes. White lupin cultivars are all very susceptible (Kupstou *et al.*, 2002). The inheritance of fusarium resistance has been investigated in blue lupin; two dominant, non-allelic genes result in complete resistance. The presence of one of the two genes is not effective. No information is available on yield losses. Table 7 shows some characteristics of the three lupin species.

Table 7. The most important characteristics of three lupin species

	L. albus	*L. angustifolius*	*L. luteus*
Raw protein (%)	34 - 45	28 - 38	36 - 48
Fat (%)	10 - 15	5 - 7	4 - 7
Carbohydrates (%)	35 - 46	37 - 46	29 - 39
Crude fibre (%)	3 - 10	13 - 17	15 - 18
Seed yield	2500 – 4000 kg/ha	2000 – 3000 kg/ha	1000 – 2000 kg/ha
Soil pH	5 - 7.5	5 - 7	< 7
Vegetation period	140 – 175 days	120 – 130 days	130 – 150 days
Diseases	anthracnosis (via seed), Fusarium (soil)	Fusarium (soil)	anthracnosis (via seed)
Catch crop	Minimal 4 years no legumes; cereal and maize are a good catch crop		
N-fixation	If no lupin has been grown before, grafting with an appropiate Rhizobium race is required		

2.6 Nutritive value

The nutritive value of lupins as poultry and pig feed has recently received much attention, especially with regard to which antinutritional factors (ANFs) are limiting for the inclusion rate and what treatment may reduce the effect of these ANFs. The most important ANFs in all three lupin species are alkaloids, especially of the quinolizidin family (Liener, 1989). Lupins contain very low protease inhibitor activity (see also Table 9) or saponins. From in vitro assays it was concluded that the effect of lectins on the digestibility of blue lupin may be reduced by adding egg powder to the diet (Van Nevel *et al.*, 1998). However, Van Nevel *et al.* (2000) were not able to show a positive effect of this treatment on the digestibility of L. albus in pigs of about 24 kg. Therefore, lectins do not seem to play a significant antinutritive role. Ferguson *et al.* (2003)

Table 8. Nutrient composition of three lupin species (source: Cowling et al., 1998)

	Lupinus albus	*Lupinus luteus*	*Lupinus angustifolius*
Nutrient composition (% as received):			
Moisture	8.6	8.5	8.9
Protein (N x 6.25)	35.8	38.3	32.0
Ash	3.3	3.5	2.7
Fat	9.4	5.6	5.9
Crude fiber	10.6	16.3	15.4
ADF	14.6	24.9	19.7
NDF	17.6	34.3	23.5
Oligosaccharides	6.6	8.9	4.1
Lignin	0.7	0.7	0.9
Amino acids (% in seed):			
Lysine	1.58	2.07	1.46
Available lysine (pigs)	1.02	-	1.04
Available lysine (poultry)	1.37	-	1.35
Methionine	0.24	0.27	0.20
Cystine	0.49	0.88	0.42
Cys + Meth	0.74	1.15	0.62
Tyr + Phe	2.76	2.68	2.33
Energy values (MJ/kg):			
GE	18.7	-	18.4
DE (pigs)	16.0	-	14.6
AME (poultry)	13.2	-	10.4
ME (cattle)	11.9	-	12.0
Testa (hull) and pod (%):			
Testa (% of seed)	18	25	24
Pod wall (% of fruit)	33	46	34
Seed weight (mg)	342	157	144

ADF = acid digestible fibre; NDF = neutral detergent fibre; GE = gross energy;
DE = digestible energy; AME = apparent metabolizable energy;
ME = metabolizable energy

concluded from studies with pigs that the active ANFs in lupin are present in the grain tissues and not in the testa. Pigs did not like the diet consisting of lupin without the testa present and had actually a preference for diets containing the testa as the only lupin-derived component. The higher concentration of flatulence-inducing sugars like raffinose and stachyose in the grain may be the reason for this. A study by Gdala *et al.* (1997) with yellow and blue lupin shows that addition of α-galactosidase improves the digestibility, which is strong evidence for the antinutritive effect of the raffinose-type sugars. Table 8 shows the nutrient composition of lupins.

2.7 ANFs in some organically grown lupin species and cultivars

Variability and absolute values of ANFs in the organically grown cultivars tested (Table 9) are comparable to those observed for conventionally grown lupins. As expected, trypsin inhibitor activities in lupin are lower than in Faba beans (Table 6), while phytate concentrations are similar and flatulence-inducing oligosaccharides are much higher. The variability between cultivars shows that breeding for TIA is not very useful since both the absolute values and variability between species are low. Concentrations of phytate show more than fourfold variability between species but less between cultivars. Hence, it may be concluded that only interspecific breeding techniques can lead to lowering of the levels in this ANF. Also flatulence inducing oligosaccharides vary very little between the cultivars tested with the extremes being 100% different. Between lupin species the distribution over individual flatulence-inducing sugars shows much more variability than for Faba beans. Raffinose is slightly more abundant (14%), stachyose much more abundant (62%) and verbascose (24%) less abundant than in Faba beans.

Table 9. Trypsin inhibitor activities (TIA), phytate and flatulence-inducing oligosaccharides (FIO) concentrations in some organically grown lupin cultivars. Species names are given in brackets: ang= L. angustifolius, lut = L. luteus and alb = L. albus. Levels in raffinose (Raff), stachyose (Stach) and verbascose (Verb) are given as averages, the other values are expressed as average ± standard deviation (n= 4). Dieta has been grown in two countries.

Species or cultivar	TIA* TIU/mg DM	Phytate mg/g DM	Alkaloids mg/kg DM	FIO in mg/g DM			Total FIO
				Raff	Stach	Verb	
Bora (ang)	1.55 ± 0.09	4.5 ± 0.6	680 ± 170	8	32	16	56 ± 5
Wodjil (lut)	0.82 ± 0.11	18.9 ± 1.6	80 ± 8	16	32	38	96 ± 16
Amber (lut)	1.10 ± 0.10	10.7 ± 1.7	n.d.	10	41	28	78 ± 17
Dieta (alb, UK)	1.56 ± 0.06	5.2 ± 0.5	850 ± 270	8	58	6	71 ± 11
Dieta (alb, NL)	1.38 ± 0.22	8.8 ± 1.1	510 ± 130	7	34	3	44 ± 3
Soy	1.8-5.0	7-18	-	11	46	1	58

***** TIA has been determined according to Kakade et al. (1974) with modifications as suggested by Liu & Markakis (1989) and Valdebouze et al. (1980). Phytate was measured according to Vaintraub et al. (1988). FIO have been analysed using Dionex anion exchange chromatography of ethanolic extracts with pulsed amperometric detection, where 100 mM sodium hydroxide, including a 0-37.5 mM sodium actetate gradient was used as the mobile phase and a Carbopac PA1 column as the stationary phase. Total lupin alkaloids have been measured according to Cortez Sanchez et al (2005) and are the sum of angustifoline, (iso) d-lupanine, lupanine, 11,12-dehydrolupanine and 13-OH-Lupanine.

Since these flatulence-inducing sugars might differ in specific activities (flatulence-inducing activity per gram sugar) the distribution over the individual sugars should be taken into account when different crops are compared.

The sweet-tasting *L. luteus* did only contain very low levels of alkaloids or did not contain any detectable amounts of alkaloids at all. The species *L. angustifolius* and *L. alba* can contain high concentrations of lupin-specific quinolizidin alkaloids. The cultivars tested here are low alkaloid cultivars, but still contain alkaloids at levels that make it necessary to limit the proportion of these low alkaloid cultivars in rations for pigs. Above a concentration in the feed of more than 200 mg/kg of alkaloids feed intake by pigs is sharply reduced (Dunshea *et al.*, 2001). With alkaloids levels lower than 200 mg/kg pig intake is not affected. With concentrations in these low alkaloid lupin cultivars of L. ranging from 510 to 850 mg/kg, maximum inclusion rates in pig diets should be less than 20 to 40 %. It should be noted that also within the same cultivar (see results of Dieta), alkaloid level varies between production location. Therefore either an inclusion rate in pig diets of less than 20 % should be used or else alkaloid analysis is recommended to establish the maximum inclusion rate.

The most abundant alkaloid measured is lupanine (>75% of the total), followed by 13-hydroxylupanin and angustifoline in *L. angustifolius* (about 10%) and 11,12 sec dehydromultiflorin, 11,12 dehydrolupanine and angustifoline (each about 5 %) in *L. albus* cv Dieta.

3. Peas (*Pisum sativum* L.)

After World War II, the EU has stimulated the cultivation of peas as a proteinaceous feed source. After 1960, the acreage decreased for various reasons, the most important being:

- Limited possibilities for mechanical harvest;
- Labour intensive;
- Chemical weed control is difficult;
- Low economic profitability as compared to cereals;
- Low yield stability due to a dense plant structure and disease susceptibility.

Subsidies by the EU have stimulated pea cultivation after 1978, but gradually lowering of these subsidies resulted in a decrease in acreage in the late 1980s. The present acreage is ….ha, with a negligible proportion amounting for organic farming.

3.1 Agronomy

Peas are susceptible for a wide scope of pests and diseases; fungal and viral pathogens, insects and nematodes are the most important in this framework. Among these causes there is a great variation in resulting yield loss over the years. Fungal diseases cause the highest damage. The dense crop structure and specifically the associated sensitivity for lodging create good growing conditions for fungi. However, the introduction of semi-leafless varieties has limited the susceptibility for leaf pathogens.

Foot rot

A broad spectrum of fungal species from various genera can cause foot rot in pea: Ascochyta, Fusarium, Pythium, Aphanomyces and Thielaviopsis. A single parcel may be contaminated with spores of more than one of these pathogens. Once in the soil, they may initiate disease development for many years thereafter. The damage may be considerable and the entire crop may be lost for that season. Seed treatment gives some protection and may prevent seed rot and loss of seedlings. In general, this is not sufficient to prevent infection throughout the growing season. Agronomic measures, directed towards the build up of the disease form the basis for preventing foot rot. This includes a wide crop rotation schedule of one to six (maximally one pea or other legume crop per six consecutive years). A number of pathogens can be transferred via seeds. Therefore, the use of clean starting material is a prerequisite for a succesful crop.

Sclerotinia sclerotiorum

Sclerotinia can affect many crops: peas, beans, potatoes, coleseed, chicory, carrots, etcetera. Sclerotidia can survive in the soil during the winter. Under humid conditions and at temperatures between 10°C and 25°C mushrooms may develop on which new spores will grow which may infect the growing crop. Both in- and outside the stems a white fungal fluff develops in which the black sclerotidia are formed. Rotting phenomena evolve which will also affect the pulses. The disease occurs spotwise, especially in heavily growing crop types. Yield losses can be considerable.

Botrytis cinerea
In peas Botrytis cinerea is one of the most frequent causes of damage. The fungus attacks diseased crops and requires dead, organic material to establish. Upon infection a grey fungal fluff develops on the stems, leaves and pulses. Infested pulses can rot partially or completely, leading to serious yield losses.

Mycosphaerella and Ascochyta pinodes
Micosphaerella pinodes is the sexual form of Ascochyta pinodes. Ascochyta spores can survive in seed and crop remainders, which subsequently affect the emerging crop. Mycosphaerella spores can be distributed by the wind over large distances.

Yield losses
Information on yield losses as a consequence of above mentioned pathogens is very scarce. At the "Proefstation voor de Akkerbouw en Groententeelt in de Vollegrond" the effect of spraying fungicides on yield in the Netherlands has been studied and was found to be approximately 10% (Table 10). In Canada, the acreage of dry peas has been considerably enlarged during the past decade to 1.2x106 ha (3x106 acres) in 2000. Here, Mycosphaerella is one of the most important diseases and yield losses are also estimated at 10%. Resistance towards this pathogen has not been described (Xue *et al.*, 2003).

Tabel 10. Effect of spraying (2 x 1 kg/ha Ronilan) on seed yield (14% moist content) of dry peas. Average of two cultivars: Finale and Solara at 55 plants/m2. Lelystad 1984 – 1986 (Source: Teelt van droge erwten, PAGV, 1989).

Year	Fungal disease	Yield (kg/ha)		Increase by treatment (%)
		Untreated	Treated	
1984	*Sclerotinia, Botrytis*	5510	6450	14.6
1985	*Mycosphaerella, Botrytis*	3790	4220	8.0
1986	none	5590	5710	2.1
Average		4960	5420	8.5

3.2 Nutritive value

White-flowering, round peas are used at a large scale for feeds. These pea types contain a little amount of tannins and are often low in trypsin inhibitor activity (TIA). Other pea types such as grey peas are less appropriate due to their high TIA. White-flowering round winter peas show fourfold higher TIA than spring types (Mariscal *et al.*, 2002). Cultivar choice is therefore very important. An example of variability is nutrient composition is given in Table 11.
Phytate is another important ANF in pea. The concentration is highly variable with the cultivar, differs between locations and is dependent on the maturity stage of the seed. Soaking of pea meal at 45 °C is very effective to decrease phytate levels (Fredrikson *et al.*, 2001).
In a study on the effect of TIA on amino acid digestibility in poultry it was shown that pea cultivars with a low TIA show a high amino acid digestibility and vice versa. Breeding for this trait is possible and very effective (Wiseman *et al.*, 2003). Results of this study are summarized in table 12. Comparable effects have been found for TIA and digestibility in pigs (Grosjean *et al.*, 2000).

Table 11. Nutrient composition of two pea cultivars g/kg (Source: Mariscal et al., 2002)

	Finale (spring)	Frilène (winter)	Frilène (extruded)
Nutrient composition			
Dry matter	868.2	876.7	888.8
Protein	250.6	266.9	268.1
Ash	29.9	31.5	33.3
NDF	180.5	172.5	139.5
ADF	90.0	127.3	87.3
ADL	2.4	15.5	1.8
Crude fibre	85.5	103.2	94.2
Tannins	2.3	2.3	2.1
Trypsin inhibitor activity (TIU/mg)	<2.0	7.6	<2.0
Essential amino acids			
Arginine	8.57	10.20	8.68
Histidine	2.66	2.44	2.36
Lysine	7.57	6.66	6.70
Phenylalanine	4.58	4.32	4.20
Leucine	7.26	7.12	6.72
Isoleucine	4.70	4.47	3.89
Valine	4.98	4.78	4.64
Methionine	1.06	0.89	0.82
Threonine	3.75	3.72	3.56
Tryptophane	0.88	0.86	0.75

Table 12. Apparent ileal digestibility of a few amino acids in near-isogenic lines of pea (Pisum sativumI L.), showing different trypsin inhibitor activities (TIA) in young broiler chicks (Wiseman et al., 2003)

	Pea A5		Pea B5	
	High TIA	Low TIA	High TIA	Low TIA
TIA (TIU/mg DM)	8.73	1.45	7.40	1.78
CAID cystine	0.738	0.812	0.721	0.804
CAID methionine	0.887	0.930	0.885	0.929

TIU: trypsin inhibitor units; CAID: coefficient of apparent ileal amino acid digestibility.

4. Quinoa (*Chenopodium quinoa* Willd.)

Quinoa (*Chenopodium quinoa* Willd.) has its origin in the Andes. It is genetically related to orache, spinach and sugar beet. Plants grow to 150 cm height and make a plume as inflorescence. The seeds have a high protein content and a high quality amino acid profile as compared to cereals. Breeding activities at Plant Research International (Wageningen, The Netherlands) have made saponin-free cultivars available.

4.1 Agronomy

Quinoa grows on all soil types, provided that they are reasonably permeable. Heavy and easily compactible soils are less appropriate. A pH between 6 and 8 is optimal, but under favourable growth conditions a pH of 5 is also tolerable.
Seed production requires sowing until ultimately half May (in the Netherlands). Quinoa seed is relatively small and requires a fine and medium moist sowing bed for good germination. The seed can be sown with regular sowing machines at a depth of 1 to 2 cm at 10 kg/ha. At a row distance of 50 cm weeding can be done mechanically, a row distance of 25 cm will result in early coverage which makes mechanical weeding not feasible.
A good quinoa crop will withdraw 100 kg N, 30 kg P and 400 kg K per ha. This is sufficient for seed production. To control weed the use of a false sowing run before the final sowing is advisable. Soil with heavy white goosefoot infestation is not feasible for seed production.
In a crop rotation scheme quinoa should be sown as a follow-up crop of after potato. Quinoa is related to beet and should, therefore, not be preceded by beet as a crop. Quinoa itself is resistant towards nematodes and rhizomania.
The seeds can be harvested with a combine, about seven weeks after flowering when 80% of the plumes have turned brown. Sowing at early May in The Netherlands will allow seed harvest at mid September. Earlier sowing will make an earlier harvest possible. The tuning of the combine is comparable to that for coleseed. Harvest under dry circumstances is advisable since the plume easily attracts moisture. At harvest the seed has a moisture content of 15 to 20% and should be dried to maximally 14% immediately after harvest to prevent fungal growth (H. Mastebroek, personal communication).

4.2 Nutritive value

Considering the chemical composition of quinoa seed, the nutritive value should be better than that of wheat and maize. Especially the amino acid composition of the protein and a higher fat content are remarkable (Table 13). However, quinoa also contains a number of ANFs: saponins (9-21 g/kg), phytate (10 g/kg), tannins (5 g/kg) and trypsin inhibitor activity (1.4 to 5 trypsin inhibitor units/mg) (Ahamed *et al.*, 1998). The bitter tasting saponins accumulate mainly in the seed coat and may be removed by soaking. In experiments with poultry, fed with 10 to 40% quinoa in the feed, the effect of removal of saponins was not evident. It was concluded that other factors cause the antinutritive effect (Jacobsen *et al.*, 1997). In a second experiment in which the inclusion rate was 15%, there was no difference in weight increase as compared to the control diet. Similar results have been obtained by Improta and Kellems (2001). Plant breeding has provided new cultivars, which are low in saponins. No feeding experiments have been decribed for these cultivars. Table 13 and 14 shows the most relevant data of quinoa as a feed crop.

Table 13. Some characteristics of quinoa

Protein (%)		12-19
Fat (%)		5-10
Carbohydrate (%)		61-74
Crude fibre (%)	2-3	
Seed yield (average)		3000-3500 kg/ha
Soil pH		6-8, (5-6 is tolerable under good soil conditions)
Vegetation period		140 days (The Netherlands)
Diseases		Possibly false mildew. After grass crops: larvae of *Agriotes lineatus* and larvae of *Tipula paludosa* (are called leatherjackets).
Possible crops before cultivation of quinoa		Grass, potato, not beet
Nitrogen fixation	No	

Table 14. Chemical analysis of quinoa, wheat and soy meal (Source: Jacobsen et al., 1997).

Chemical analysis of quinoa products and other dietary ingredients used for broiler feed

	Quinoa			Wheat	Soybean meal dehulled
	Seed	Dehulled	Germ		
Moisture (g kg^{-1})	141	141	141	150	140
Crude protein (g kg^{-1})	120	112	281	123	479
Crude fat (g kg^{-1})	59	45	146	19	12
Dig. carbohydrates (g kg^{-1})	586	626	332	585	140
Crude fibre (g kg^{-1})	21	17	21	23	34
Ash (g kg^{-1})	25	18	57	16	60
Estimated ME [a] (MJ kg^{-1})	13.8	13.7	14.9	12.3	10.1
Essential amino acids (g kg^{-1} of crude protein)					
Lysine	53	53	52	27	61
Methionine	19	19	20	17	14
Cystine	16	16	17	20	15
Threonine	35	36	33	29	39
Saponins (g aescin kg^{-1})	18	3	18		

[a] ME = 0.155 × Crude protein + 0.343 × Crude fat + 0.167 × Dig. carbohydrates.

5. Antinutritional factors in an organically grown quinoa cultivar (Chenopodium quinoa, cv Atlas)

Measurement of trypsin inhibitor activity (1.06 ± 0.13 TIU/g DM) and flatulence-inducing oligosaccharides (total 0.45 ± 0.13 mg/g DM) shows that these antinutrional factors are much lower, while phytate levels (11.7 ± 1.6 mg/g DM) are similar to those observed in legume crops. To our knowledge intrinsic phytase activity has hitherto not been reported for quinoa. Obviously, from the point of view of antinutritional factors quinoa has some significant advantages to grain legumes.

6. Analysis of potentially harmful or toxic components

Organically grown field bean, lupin, quinoa and pea samples analysed for ANF-content were also analysed for several potentially harmful and toxic components. The samples have been analyzed for isoflavones,

lectins (fasin activity), heavy metals, mycotoxins, pesticides and dioxins. Only field beans and peas showed lectin activity ranging from 1:500-1: 5000. No other levels were above normal safe levels. Next to these organically grown alternative proteinaceous crops, also a few organically produced oil seed meals were analysed (soy bean, rape seed, crambe, sesame and sunflower). Of these, only the sunflower meal sample contained too much Aflatoxin B, although no general conclusions can be drawn on the occurrence of Aflatoxin B in sunflower meal, because of the limited scale of testing of sunflower meal. Soy bean meal (flakes) showed some estrogenic activity. All other oil seed samples showed no other components with levels above normal safe levels. The detailed laboratory results are summarized in Appendix 1.

7. Discussion and conclusions

The perspective for growing and use of homegrown proteinaceous feeds is dependent on various factors. A positive contribution has been given by the development of cultivars of pulses and quinoa which lack or are low in ANFs. This allows a higher inclusion rate in the mixed feeds. In addition, there are many efforts throughout the EU to develop cultivars with higher yield and disease resistance aiming to improve crop performace and, thus, make it more profitable. These factors are especially relevant with regard to the introduction of pulses and quinoa in organic farming practices, not only for human consumption but also for the production of feeds in the organic farming of pigs, poultry, etcetera. Table 15 shows some of the bottlenecks for each crop.

Table 15. Opportunities and bottlenecks for the growth and use of new proteinaceous crops in organic pig husbandry.

Crop	Most important ANF	Agronomy	Included in diets of pigs up to X % without large negative effects on feed conversion
Tested experimentally in this study			
Faba bean	Trypsin inhibitors, tannins no problem with right genotypes	Soil pathogens, N-fixation positive	Up to 10 % (Van der Peet-Schwering et al. (2006)
Lupin	Alkaloids no problem with right genotypes; oligosaccharides	Soil pathogens, not optimally adapted for all European regions, N-fixation positive	Up to 10 % (Van der Peet-Schwering et al. (2006)
Pea	Protease inhibitors	Soil pathogens, crop structure, N-fixation positive	Up to 20 % (feed industry data)
Quinoa	Protease inhibitors, although much lower than many legumes	No soil pathogen problems, still not fully adapted to European climate as the crop was only recently introduced in Europe, no N-fixation	Up to 20 % (Van der Peet-Schwering et al. (2006)
Proteinaceous meals from oil crops on the basis of literature study			
Sunflower	Phytate (75 % of phosphate)	Only suited for Southern Europe, no N-fixation	Leibholz (1992): substantial reduction in piglet growth (< 20 kg) with sunflower compared to soy bean meal
Rapeseed	Phytate, glucosinolates	No N-fixation	Up to 30 % with pigs > 20 kg; up to 5 % for piglets (< 20 kg).
Crambe	Glucosinolates	No N-fixation	Up to 20 % for growing pigs (>20 kg) (glucosinolates < 3 mumol/g). No experience with piglets < 20 kg.
Soy bean	Phytate, oligosaccharides	European climate not well suited	Weaned piglets (0-14 d) with up to 25 % inclusion in diets.

As a consequence of the required wide crop rotation schedule and the higher added value of grass-clover mixture to the agronomic planning possibilities, the acreage for pulses will be insufficient to comply with the needs of organically grown proteinaceous feeds. Import from the Eastern EU countries may provide a solution. For reasons of national supply quinoa growing may be an attractive option. Problems related to dry and cool storage, required to prevent losses, have to be solved for quinoa. For feed production the seeds must be flattened, milled and converted to a high value formula by the feed producer. From a financial and technical point of view it will be necessary for some EU countries to sell the primary product to a feed producer.

Table 16 shows the financial picture for prevailing, organically-produced protein (Gotink, 2003). At the current cost price level, estimated seed yield and protein content, field bean appears to be cheapest and pea the most expensive source for protein. White lupin and quinoa also have an attractive fat content. (Note: income as a consequence of rest components, with the exception of straw and McSharry subsidies, are not accounted for). However, for the grower pea seems to be most attractive crop. No market price for quinoa is available yet. Therefore, and also for reasons of its better protein composition and fat content, a comparison with the other three crops is difficult to make.

Table 16. Costs and profits per kg seed and per kg protein of three pulse crops and quinoa grown either under conventional or organic farming conditions in Europe, cost prices are given for EU-grown crops (base year 2002) (FAO statistics 2003; Gotink, 2003; Mastebroek, 2004); market prices for conventionally produced oil seed meals from FAO-statistics production year 2003. Market prices for organically produced meals based on information from organic producers of pig feeds and world market prices for organically produced oil crop meals.

	Yield (kg/ha)	Total costs from own production €/ha	Cost price (€ per kg seed or meal)	Market price	% Protein	Cost price (€/kg protein)	Market price
Conventional							
Field bean	5000	624	0.12	0.19	25	0.48	0.76
Lupin	3000	735	0.25	0.20	35	0.72	0.57
Pea	4500	604	0.13	0.15	20	0.65	0.75
Quinoa	3000	360	0.12	0.17	17	0.71	1.75
Soy bean meal			-	0.21	40	-	0.53
Sunflower meal			-	0.12	35	-	0.34
Rapeseed meal			-	0.16	35	-	0.46
Crambe meal			-	0.15	35	-	0.43
Organic3							
Field bean	4000	624	0.16	0.25	25	0.64	1.00
Lupin	2400	735	0.31	0.25	35	0.88	0.71
Pea	3600	604	0.17	0.25	20	0.85	1.25
Quinoa	2500	360	0.14	0.20	17	0.82	1.17
Soy bean meal			-	0.40	40	-	1.00
Sunflower meal			-	0.23	35	-	0.66
Rapeseed meal			-	0.30	35	-	0.86
Crambe meal			-	0.28	35	-	0.80

***** Income from straw yield and McSharry subsidies have been subtracted from total costs to arrive at the total costs from own production of feed; cost price per kg of seed is calculated as total costs divided by yield. Cost price can vary markedly in the EU with land prices, labour costs and soil fertility (and therefore yield). See Gotink, 2003 & Mastebroek, 2004 for more details for pulses and quinoa respectively.

2 Market price of quinoa imported from outside EU for human consumption is 1.00 €/kg; market price for contract production in EU for conventional feed use is between 0.30-0.50 €/kg seed at the current small scale of production. When larger scale quinoa production would occur, market price will be much lower than current levels; assumption is that the market value will decline to the feeding value shadow price calculated as 0.17 €/kg seed, based on composition of quinoa with 17 % protein, 9 % oil and 74 % 'wheat-quality' starch plus cell wall with market values of 0.375 €/kg protein, 0.375 €/kg oil and 0.10 €/kg 'wheat').

3 Market prices for field bean, lupin, pea and quinoa have been calculated on the basis of equivalent market price per kg protein. Prices for organic oil seed meals (cake derived after pressing) are 2005 cost prices paid by Dutch organic feed producers.

The market prices for raw materials, produced by organic farming, are considerably higher than for conventionally produced crops. For pulses these are approximately € 0.25/kg of seed. The cultivation costs are about equal, the costs for herbicides and crop protection chemicals are comparable to the extra labour costs for weed control. Seed yield is approximately 20% lower for organic farming. Under these conditions organic farming is financially more attractive for the farmer than conventional farming, only for field bean and pea, not for lupin. On a short term lupin appears not to be an attractive crop, if the product is sold directly to feed producers. This crop has, as the only one of the three pulses, a negative financial balance. On the long term lupin will also form an attractive crop when disease resistance and high-yielding cultivars will become available. White lupin has, in addition to a high protein content, also 10% fat in the seed which increases its nutritive value. New cultivars which are currently being developed will have to be tested under the various climatic conditions throughout the EU. The development of quinoa as a protein-

rich feed crop is just starting and requires extra research. Current market prices for EU-grown quinoa are between 0.30 and 0.50 €/kg of seed, but with increased scale of production, market prices for quinoa based feed production can go down to 0.20 €/kg of seed for quinoa. This holds both for conventional and organic production of quinoa. Cost price is lower than the market value, so own production of quinoa could be viable option.
Organic feed production of the studied crops by farmers for their own use, is cheaper than buying these same feeds on the market only for field beans, peas and quinoa, not for lupin. However, when enough organically produced oil seed crop meals would be available on the market, these oil seed crop meals would be cheaper than own production of pulses or quinoa. However, most oil seed crop meals are obtained after hexane extraction and oil seed crop meals from hexane extraction are not allowed in organic farming. Oil pressing is the only allowed technology to produce organic oil seed crop meals. The cheapest oil seed crop meal would be sunflower meal and then crambe meal. Sunflower however was not successfully used with just weaned piglets, but can be used for growing pigs (> 20 kg). Quantities of crambe meal available are still very low as Crambe production in Europe is still starting up. Crambe meal has not extensively been tested on just weaned piglets, but with growing pigs inclusion of up to 20 % of crambe meal was possible without adverse effects (Liu *et al.*, 1993).

Given the fact that cost prices per kg protein from organic homegrown crops are lower than market prices of organically produced imported soy bean meal, there is scope for EU grown proteinaceaous feed crops. A local production of feed protein for organic pig husbandry would then be possible, which is to be preferred over imports of organic soy bean from other continents. Also organically produced imports of feed stuff into Europe create a mineral nutrient surplus (N, P, K) in Europe. A local production would help to reduce the imbalance in nutrients flows.

This study shows that with the genetic improvement in some alternative protein crops, the occurrence of antinutritional factors in new cultivars has been reduced, also under organic farming conditions. With organic farming conditions, yields are lower than with conventional farming, but still high enough to achieve a cost price of homegrown protein feeds that can compete with imports of for example oil seed meals, albeit at relative low margins per ha. Continued plant breeding will be able to create further improved cultivars with higher yields, less susceptibility to plant diseases, especially soil borne plant pathogens that will be easier to combine with other crops in crop rotations. It remains a question whether organic pig farmers will organize the crop production themselves or whether this will be carried out in co-operation with specialized organic arable farmers.

Lastly, this study has shown the potential of some recent advances in plant breeding in leguminous crops and in quinoa. However, the proof is in the eating. Only feeding trials with pigs will show the real value of these new cultivars of alternative protein crops. Such feeding trials have been carried out in a second study using field beans, lupin and quinoa which will be reported later (Jongbloed *et al.* 2006, in press).

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Appendix 1

Appendix 1. Analysis of potentially harmful or toxic components

RIKILT Analysis 2004

Level of heavy metals mg/kg

RIKILT Sample ID and description	Cd	Pb	As	Hg	Rikilt ID
136984 lupin blue, alkaloid low, Bora	<0.02	<0.1	<0.1	<0.01	136984
136985 lupin yellow, alkaloid low, Wodjil	0.081	<0.1	<0.1	<0.01	136985
136986 lupin yellow, alkaloid low, Amber	0.43	<0.1	<0.1	<0.01	136986
136987 lupin white, alkaloid low, Dieta UK	<0.02	<0.1	<0.1	<0.01	136987
136988 lupin white, alkaloid low, Dieta NL	0.027	0.92	<0.1	<0.01	136988
136989 quinoa, saponin low, Atlas	0.12	0.17	<0.1	<0.01	136989
136990, field bean, Divine	0.025	<0.1	<0.1	<0.01	136990
136991, field bean, Dixie	<0.02	<0.1	<0.1	<0.01	136991
136992, field bean, Melody	<0.02	<0.1	<0.1	<0.01	136992
136993, field bean, Gloria, BE	0.094	<0.1	<0.1	<0.01	136993
136994, field bean, Victoria	0.057	<0.1	<0.1	<0.01	136994
136995, field bean, Aurella	<0.02	<0.1	<0.1	<0.01	136995
136996, field bean, Gloria, ZW	<0.02	<0.1	<0.1	<0.01	136996
136997, field bean, Sirocco	<0.02	<0.1	<0.1	<0.01	136997
138006, Organic soy bean meal	Not analysed in 2004	Not analysed in 2004	Not analysed in 2004	Not analysed in 2004	138006

Levels of mycotoxins in mg/kg in product

Mycotoxins RIKILT Sample ID	AB1	DON	FB1	FB2	HT2	OTA	T2	ZON
136984	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136985	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136986	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136987	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136988	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136989	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136990	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136991	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136992	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136993	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136994	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136995	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136996	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
136997	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	<0.05
138006	<0.005	<1.0	<0.10	<0.10	<0.10	<0.05	<0.50	0.09

Fasin activity and phytoestrogen levels	**13698 4**	**13698 5**	**13698 6**	**13698 7**	**13698 8**	**13698 9**	**13699 0**	**1369 91**	**13699 2**	**13699 3**	**13699 4**	**13699 5**	**1369 96**	**13699 7**
Fasine activity (lectins)	neg.	neg.	neg.	neg.	1:20	neg.	1:200 0	1:500 0	1:100 0	1:200 0	1:200 0	1:100 0	1:500 0	1:500
Daidzin (HPLC)	<0.05	<0.1	<0.05	<0.05	<0.05	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Malonyl-daidzin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-daidzin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total daidzein from glucons	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Daidzein (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Glycitin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Malonyl-glycitin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-glycitin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total glycitein from glucons	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Glycetein (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Genistin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Malonyl-genistin (HPLC)	<0.05	<0.05	<0.05	<0.1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-genistin (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total genistein from glucons	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Genistein (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total isoflavones aglucons	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total isoflavons	<0.1	0.11	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

For fasine activity:
neg. = negative lectin activity at 1:10 dilution
1:20, 1:500, etcetera = positive at that dilution
Units for all other components: mg/g

Results of analysis of dioxin, non-ortho-, mono-ortho and indicator-PCB in feed sampels
Levels in ng/kg product, total levels in ng TEQ/kg product

Dioxines	136984	136985	136986	136987	136988	136989	136990	136991	136992	136993	136994	136995	136996	136997	138006
2,3,7,8-TCDF	<0.05	<0.05	<0.05	<0.05	<0.05	0.06	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1,2,3,7,8-PeCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,3,4,7,8-PeCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,6,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,3,4,6,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,7,8,9-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,6,7,8-HpCDF	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
1,2,3,4,7,8,9-HpCDF	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
OCDF	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
2,3,7,8-TCDD	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1,2,3,7,8-PeCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,7,8-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,6,7,8-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,7,8,9-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,6,7,8-HpCDD	<0.25	<0.25	<0.25	<0.25	0.26	0.30	<0.25	<0.25	<0.25	0.32	0.35	0.37	<0.25	<0.25	<0.25
OCDD	2.26	1.00	1.29	3.19	1.64	1.53	0.59	0.66	1.48	2.47	3.69	3.05	1.17	1.41	0.68
Total amount TEQ[lb]	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Total amount TEQ [ub]	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**

lb = with lower bound detection limits
ub = with upper bound detection limits

Dioxins and PCBs (continued).
Levels in ng/kg product, total levels in ng TEQ/kg product

non-ortho-PCB's	136984	136985	136986	136987	136988	136989	136990	136991	136992	136993	136994	136995	136996	136997	138006
PCB 81	0.05	<0.05	<0.05	<0.05	0.06	0.19	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.12
PCB 77	0.29	0.23	0.21	0.19	0.61	4.23	0.32	0.35	0.52	0.34	0.36	0.26	0.33	0.37	1.6
PCB 126	<0.05	<0.05	<0.05	<0.05	0.07	0.49	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.099
PCB 169	<0.05	<0.05	<0.05	<0.05	<0.05	0.06	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total level TEQ[lb]	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**
Total level TEQ [ub]	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.05**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
mono-ortho-PCB's															
PCB 123	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 118	<10	<10	<10	<10	<10	37.90	<10	<10	<10	<10	<10	<10	<10	<10	21
PCB 114	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 105	<10	<10	<10	<10	<10	13.70	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 167	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 156	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 157	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 189	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Total level TEQ[lb]	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Total level TEQ [ub]	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
indicator-PCB's															
PCB 028	<100	<100	<100	<100	<100	116.00	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 052	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 101	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 118	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 153	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 138	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
PCB 180	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
Som TEQ [lb]	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.06**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**
Som TEQ [ub]	**0.31**	**0.31**	**0.31**	**0.31**	**0.31**	**0.36**	**0.31**	**0.31**	**0.31**	**0.31**	**0.31**	**0.31**	**0.31**	**0.31**	**0.32**

Levels of pesticides and indicator pcb's in µg/kg product	136984	136985	136986	136987	136988	136989	136990	136991	136992	136993	136994	136995	136996	136997	138006
DICHLORVOS	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
MEVINPHOS	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
ETHOPROPHOS	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PHORATE	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
DIAZINON	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CHLORPYRIFOS	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
FENTHION	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PARATHION-METHYL	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
HCB	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
a-HCH	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
g-HCH	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
b-HCH	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
DELTA-HCH	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
g-CHLORDANE	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
a-CHLORDANE	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Endosulfan I	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Endosulfan II	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Endosulfan-sulfaat	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
o,p'-DDT	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
p,p'-DDT	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
p,p'-TDE	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
p,p'-DDE	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
METHOXYCHLOR	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 28	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 52	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 101	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 118	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 138	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 153	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
PCB 180	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

RIKILT Analysis 2005: Oil seed crop flakes, crambe seed and peas

RIKILT Sample ID and description
138006 organic soy flakes
147460 sunflower flakes
147461 sesam flakes
147462 peas
147463 rape seed flakes
147491 crambe

Fasin activity and phytoestrogen levels	**138006**	**147460**	**147461**	**147462**	**147463**	**147491**
Fasine activity (lectins)	neg.	neg.	neg.	‘1:5000	neg.	neg.
Daidzin (HPLC)	0.14	<0.05	<0.05	<0.05	<0.05	<0.05
Malonyl-daidzin (HPLC)	0.09	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-daidzin (HPLC)	<0.05	<0.05	<0.05	<0.05	0.12	<0.05
Total daidzein from glucons	0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Daidzein (HPLC)	0.2	<0.05	<0.05	<0.05	0.06	<0.05
Glycitin (HPLC)	0.1	<0.05	<0.05	<0.05	<0.05	<0.05
Malonyl-glycitin (HPLC)	0.06	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-glycitin (HPLC)	<0.05	0.36	0.31	<0.05	<0.05	<0.05
Glycetein (HPLC)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total glycitein	0.12	0.20	0.18	<0.05	<0.05	<0.05
Genistin (HPLC)	0.32	<0.05	<0.05	<0.05	0.07	<0.05
Malonyl-genistin (HPLC)	0.41	<0.05	<0.05	<0.05	<0.05	<0.05
Acetyl-genistin (HPLC)	0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Genistein (HPLC)	0.14	<0.05	<0.05	<0.05	<0.05	<0.05
Total genistein	0.58	<0.05	<0.05	<0.05	0.07	<0.05
Total isoflavones aglucons	0.89	0.21	0.2	<0.05	0.16	0.06
Total isoflavons	1.41	0.36	0.34	<0.05	0.27	0.08

For fasine activity:
neg. = negative lectin activity at 1:10 dilution
1:20, 1:500, etcetera = positive at that dilution
Units for all other components: mg/g

Level of heavy metals mg/kg	**138006**	**147460**	**147461**	**147462**	**147463**	**147491**
Cd	0.064	0.21	0.021	<0.02	0.073	0.081
Pb	<0.1	0.12	<0.1	<0.1	0.13	<0.1
As	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hg	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Levels of mycotoxins in mg/kg in product	**138006**	**147460**	**147461**	**147462**	**147463**	**147491**
AB1	<0.005	13	<0.005	<0.005	<0.005	<0.005
DON	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
FB1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
FB2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
FB3	ND	<0.10	<0.10	<0.10	<0.10	<0.10
HT2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
OTA	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
T2	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
ZON	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Results of analysis of dioxin, non-ortho-, mono-ortho and indicator-PCB in feed sampels
Levels in ng/kg product, total levels in ng TEQ/kg product

Dioxins	138006	147460	147461	147462	147463	147491
2,3,7,8-TCDF	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1,2,3,7,8-PeCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,3,4,7,8-PeCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,6,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,3,4,6,7,8-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,7,8,9-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,6,7,8-HpCDF	<0.25	0.33	0.31	<0.25	<0.25	<0.25
1,2,3,4,7,8,9-HpCDF	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
OCDF	<0.50	1.61	2.12	<0.50	<0.55	<0.50
2,3,7,8-TCDD	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1,2,3,7,8-PeCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,7,8-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,6,7,8-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,7,8,9-HxCDD	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3,4,6,7,8-HpCDD	<0.25	0.85	0.63	<0.25	<0.25	<0.25
OCDD	0.68	4.20	4.22	0.63	0.80	0.68
Total amount TEQ[lb]	**0.00**	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**
Total amount TEQ [ub]	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**	**0.29**

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Results of analysis of dioxin, non-ortho-, mono-ortho and indicator-PCB in feed sampels
Levels in ng/kg product, total levels in ng TEQ/kg product

non-ortho-PCB's	**138006**	**147460**	**147461**	**147462**	**147463**	**147491**
PCB 81	0.12	0.25	0.45	*	<0.05	0.12
PCB 77	1.6	5.43	11.40	1.1	0.65	1.6
PCB 126	0.099	*	*	<0.05	<0.05	0.099
PCB 169	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total level TEQ[lb]	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**
Total level TEQ [ub]	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
mono-ortho-PCB's						
PCB 123	<10	<10	<10	<10	<10	<10
PCB 118	21	12	14	<10	12	21
PCB 114	<10	<10	<10	<10	<10	<10
PCB 105	<10	<10	<10	<10	<10	<10
PCB 167	<10	<10	<10	<10	<10	<10
PCB 156	<10	<10	<10	<10	<10	<10
PCB 157	<10	<10	<10	<10	<10	<10
PCB 189	<10	<10	<10	<10	<10	<10
Total level TEQ[lb]	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Total level TEQ [ub]	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
indicator-PCB's						
PCB 028	<100	<100	<100	<100	<100	<100
PCB 052	<100	<100	<100	<100	<100	<100
PCB 101	<100	<100	<100	<100	<100	<100
PCB 118	<100	<100	<100	<100	<100	<100
PCB 153	<100	<100	<100	<100	<100	<100
PCB 138	<100	<100	<100	<100	<100	<100
PCB 180	<100	<100	<100	<100	<100	<100
Som TEQ [lb]	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
Som TEQ [ub]	**0.32**	**0.32**	**0.32**	**0.31**	**0.31**	**0.32**

Levels of pesticides and indicator pcb's in µg/kg product

Rikilt ID -->	**138006**	**147460**	**147461**	**147462**	**147463**	**147491**
DICHLORVOS	<10	NA	NA	NA	NA	NA
MEVINPHOS	<10	<10	<10	<10	<10	<10
ETHOPROPHOS	<10	<10	<10	<10	<10	<10
PHORATE	<10	<10	<10	<10	<10	<10
DIAZINON	<10	<10	<10	<10	<10	<10
CHLORPYRIFOS	<10	<10	<10	<10	<10	<10
FENTHION	<10	<10	<10	<10	<10	<10
PARATHION-METHYL	<10	<10	<10	<10	<10	<10
HCB	<10	<10	<10	<10	<10	<10
a-HCH	<10	<10	<10	<10	<10	<10
g-HCH	<10	<10	<10	<10	<10	<10
b-HCH	<10	<10	<10	<10	<10	<10
DELTA-HCH	<10	NA	NA	NA	NA	NA
g-CHLORDANE	<10	<10	<10	<10	<10	<10
a-CHLORDANE	<10	<10	<10	<10	<10	<10
Endosulfan I	<10	<20	<20	<20	<20	<20
Endosulfan II	<10	<20	<20	<20	<20	<20
Endosulfan-sulfaat	<10	NA	NA	NA	NA	NA
o,p'-DDT	<10	<10	<10	<10	<10	<10
p,p'-DDT	<10	<10	<10	<10	<10	<10
p,p'-TDE	<10	<10	<10	<10	<10	<10
p,p'-DDE	<10	<10	<10	<10	<10	<10
METHOXYCHLOR	<10	NA	NA	NA	NA	NA
PCB 28	<10	<10	<10	<10	<10	<10
PCB 52	<10	<10	<10	<10	<10	<10
PCB 101	<10	<10	<10	<10	<10	<10
PCB 118	<10	<10	<10	<10	<10	<10
PCB 138	<10	<10	<10	<10	<10	<10
PCB 153	<10	<10	<10	<10	<10	<10
PCB 180	<10	<10	<10	<10	<10	<10

Other components found with the library search "RIKILT pesticides MS Library"

In sample 147460	Fluorantheen	present
PAH were found	Anthraceen	present

Using HR-GCMS an estimation has been made of the levels, the levels are only indications.

ng BAPeq/gram product	15.0	0.21	0.03	0.16	0.18