Developments in Plant Breeding For Improved Nutritional Quality of Soya Beans II. Anti-nutritional factors

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Short title: Reduction in anti-nutritive factor content of soya beans

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SUMMARY

Nutritional value of most plant materials is limited by the presence of numerous naturally occurring compounds which interfere with nutrient digestion and absorption. Although processing is employed widely in removal of these factors, selection of cultivars of soya beans with inherently low levels would have a considerable impact on efficiency of non-ruminant livestock production. The review considers the role of plant breeding in achieving this objective.

The most abundant trypsin inhibitors are the Kunitz and the Bowman Birk inhibitors, containing 181 and 71 amino acids respectively. The Kunitz inhibitor is present at a concentration of 1.4g/kg of total seed contents and the Bowman Birk inhibitor 1.6g/kg. A large number of isoforms of the Bowman Birk inhibitor have been described in soya bean cultivars and it has been shown that the general properties of the inhibitor are, in fact, attributable to different isoforms.

Nulls for both Bowman-Birk and Kunitz trypsin inhibitors have been identified, allowing new low trypsin inhibitor cultivars to be produced. However, research into breeding for low trypsin inhibitor cultivars currently has limited application as trypsin inhibitors contribute a major proportion of the methionine content of soya beans. Trypsin inhibitors are thought to be involved in the regulation of and protection against unwanted proteolysis in plant tissues and also act as a defense mechanism against attack from diseases, insects and animals. Hence, in breeding programes for low trypsin inhibitor cultivars, alternative protection for growing plants must be considered.

Use of soya beans in non-ruminant animal feeds is limited by the flatulence associated with their consumption. The principal causes appear to be the low molecular weight oligosaccharides containing ∀-galactosidic and ∃-fructosidic linkages; raffinose and stachyose.Non-ruminants do not have the ∀-galactosidase enzyme necessary for hydrolysing the ∀-galactosidic linkages of raffinose and stachyose to yield readily absorbable sugars.

Soya beans contain between 6.8 and 17.5g/kg of phytic acid; a ring form of phosphorus (P) which chelates with proteins and minerals to form phytates not readily digested within the gut of non-ruminants. One approach for over-coming the effects of phytic acid is through synthesis of phytase in the seeds of transgenic plants. Currently, recombinant phytase produced in soya beans is not able to withstand the processing temperatures necessary to inactivate proteinaceous anti-nutritional factors present.

Soya bean lectins have the ability to bind with certain carbohydrate molecules (N-acetyl-D-galactosamine and galactose) without altering the covalent structure. Lectins are present in raw soya bean at a concentration of between 10 and 20 g/kg. Purified soya bean agglutinin is easily inactivated by hydrothermal treatment but in complex diets binding with haptenic carbohydrates may confer protection against denaturation. The majority of research into soya bean lectins in carried out using laboratory animals so very limited information is available on their *in vivo* effects in farm animals. This review is concerned specifically with breeding but there are other means of improving nutritive value, for example processing which may alter protein structure and therefore functionality of proteinaceous antinutrtional factors present.

INTRODUCTION

Soya beans are of major importance worldwide as a plant protein component of diets for non-ruminant livestock. It is accepted that limitations to their use are associated with comparatively modest concentrations of protein and nutritionally essential amino acids (although levels are still higher than most other plant sources) and there is considerable interest in selecting cultivars with improved nutritional quality (reviewed by Clarke & Wiseman 1999). However a further area of fundamental importance is the presence of a number of naturally occurring factors which are anti-nutritional insofar as they interfere with nutrient digestion, absorption and assimilation in animals. Some of these factors are heat labile and are reduced below levels likely to cause problems, although necessary processing is associated with increased cost. These factors, however, are heat stable and effective means of their removal remain to be identified.

An alternative approach is to reduce concentrations of these factors through plant breeding and the review will address this subject.

PROTEASE INHIBITORS

Inhibitors of digestive enzymes active in the gastro-intestinal tract of non-ruminants are peptides widely distributed in plants; they differ in both specificity and potency of inhibition depending on the origin of the target enzyme (Birk 1989). Some, which have a broad specificity, possess a single reactive site whereas others are capable of inhibiting two enzymes (trypsin and chymotrypsin) simultaneously and are termed polyvalent or double headed. Several mechanistic classes of inhibitor are known; serine-, sulphydryl-, acid- and metalloproteases (Xaviera-Filho & Campos 1989). It has long been known that soya beans contain serine protease inhibitors capable of acting as antinutritional factors.

The affinity of protease inhibitors for trypsin means that their presence is usually detected by their bonding with trypsin (Kakade *et al.* 1969). Consequently, the correct term for protease inhibitors evaluated in this manner is trypsin inhibitors (TIs). However, it has been shown that inhibitors that strongly inhibit bovine trypsin do not inhibit human trypsin (Mallory and Travis 1975). Since the inhibitory capacities of protease inhibitors are usually measured with bovine pancreatic proteases, their relevance and significance should be questioned (Birk 1989). Trypsin and chymotrypsin isolated from the target animal should, therefore, be used and differences in the origin of the trypsin may account for the variation in levels of TIs reported in the literature. Although detailed studies into the binding of porcine trypsin

with soya bean trypsin inhibitor have been conducted (Song & Suh 1998), no data are reported on the use of porcine or avian trypsin in a trypsin inhibitor assay. Whilst trypsin from poultry is not readily available, porcine trypsin is easily obtained and considerably cheaper than bovine trypsin.

The wide range of means of expression used in measurement of trypsin inhibitor activity (TIA) may also lead to confusion: TIA may be expressed as trypsin units per gram material, trypsin units per gram protein, parts per million of Kunitz units, mg pure trypsin inhibited per gram and, most commonly, as mg pure trypsin inhibited per gram sample. One trypsin unit is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction mixture under the strict conditions of the Kakade test (Kakade *et al.* 1974).

Chemical Structure

The most abundant trypsin inhibitors in the soya bean are the Kunitz inhibitor (KSTI) and the Bowman Birk inhibitor (BBI), containing 181 and 71 amino acids respectively. They form well characterized stable enzyme-inhibitor complexes with pancreatic proteolytic enzymes on a molar 1:1 ratio. KSTI is present at 14g/kg of the total seed contents and BBI 1.6g/kg (Orthoefer1978).

KSTI was the first plant proteinase inhibitor to be isolated and characterized (Kunitz 1947*a*, *b*). It has a molecular weight of about 21,000 and includes two disulphide bridges. It is primarily a single-headed inhibitor of trypsin but was also shown to be weakly reactive against chymotrypsin at two reactive sites, one of them overlapping with the trypsin reactive site (Kassell 1970). The reactive site in soya bean has been localized at the ARG (63)-ILE (64) bond and the three dimensional structure of this inhibitor has been determined (Sweet *et al.* 1974).

BBI has a molecular weight of approximately 8,000 with a high content of cysteine, forming seven disulphide bridges. The increased number of disulphide bridges in BBI endow it with greater structural stability than KSTI, making it more resistant to denaturation by heat. It forms a 1:1 complex with either trypsin or chymotrypsin and a ternary complex with both enzymes. The soya bean BBI consists of two domains, each containing a genetically distinct reactive site. The sequence alignment of these domains shows a large homology, and it is generally assumed that these double headed inhibitors have evolved by gene duplication (Birk 1985). Odani & Ikenaka (1977) separated the two internal homologous regions and have shown that both reactive sites are each individually reactive with proteases. The three dimensional structure for the BBI of the soya bean has been established and confirmed (Werner & Wemmer 1991). The protein exists as two domain structures

and, in addition, the proteinase inhibiting sites are located in exposed external loops. Each inhibitory domain is held in place by the disulphide bridges within a domain, and by cross links of a domain to an intervening sequence. This may explain their fairly rigid structure. In the soya bean BBI the reactive sites have been identified at LYS (16)-SER (17) and at LEU (42)-SER (43) (Sweet *et al.* 1974).

Isoforms

The variation in nutritional quality of soya bean cultivars stems partly from their different levels of trypsin inhibitor and from varying proportions of trypsin inhibitors of the two classes. A large number of Bowman-Birk trypsin inhibitors have been described in soya bean cultivars since the first one isolated independently by Bowman & Birk (Birk 1961). Isoforms have been classified by Tan-Wilson *et al.* (1987) into four sub-groups by virtue of their distinctive amino acid compositions, molecular weights, spectrum of enzyme inhibitor activity and immunochemical cross-reactivity.

Tan-Wilson *et al.* (1987) introduced the 4th subgroup shown in Table 1 on discovering two molecules that were structurally different from the other three BBI subgroups. The fact that these molecules only have one disulphide group would disqualify them as members of the Bowman-Birk class of inhibitors (Laskowski & Kato 1980), in spite of the relatively strong immunological reaction with the classical BBI molecule antibody. However, the strong trypsin inhibition of subgroup 4 isoinhibitors demonstrates they are functionally closer to the classical BBI molecule than the isoinhibitors in subgroups II and III. Table 1 shows that the general properties of Bowman Birk inhibitors are, in fact, attributable to different isoforms.

(Table 1 about here)

Role of Trypsin Inhibitors in Plants

The roles of trypsin inhibitors in plants are diverse: they are thought to be involved in the regulation of and protection against unwanted proteolysis in plant tissues and also act as a defence mechanism against attack from diseases, insects and animals (Xavier-Filho & Campos 1989). Injury to plants due to phytophagus insects or mechanical damage induces accumulation of proteinase inhibitor proteins (Ryan 1990). Accumulation of proteinase inhibitors occurs both locally, at the site of injury, and systemically in other organs of the plant distal to the primary wound site (Botella *et al*. 1996). Being proteins, with high concentrations of cysteine in BBI, they are able

to fulfill a secondary role; this involves recycling their constituent amino acids for use as building blocks in *de novo* protein synthesis. Proteases are influential in the mobilisation of proteins in plants during germination and it seems to be achieved by an interplay of many proteases. They have also been detected as a 'cloud' that has leaked into the soil to surround the germinating seed and guard it against attack from micro-organisms (Wilson 1980). Characterisation of 11 wild perennial species of soya bean revealed that seeds of all species studied contained both trypsin and chymotrypsin inhibitors (Kollipara & Hymowitz 1992).

Technologies to Denature Trypsin Inhibitors

KSTI is usually referred to as the 'heat-labile' inhibitor, while BBI is often referred to as the 'heat-stable' inhibitor. In most cases, descriptions of BBI as 'heat stable' are derived from the early work of Birk (1961), in which purified BBI retained its antiproteolysis activity after being heated in aqueous solution at 100°C for 10 min. The original experimental evidence that led to the labeling of KSTI was that of Rackis (1966) where the inactivation of soya bean protease inhibitor (SBPI), which had been purified by ion-exchange chromatography, was examined. Since the purified SBPI was rapidly inactivated by heat, it was assumed to be KSTI rather than BBI. In later years, this led to the belief in general that KSTI is more heat labile than BBI. However, work by Di Pietro and Liener (1989) produced results conflicting with this view: by using immunochemical and enzymatic techniques to distinguish between the two inhibitors, inactivation of KSTI and BBI during various types of heat treatment was investigated. It was found that when the inhibitors were heated (75-95°C) within a soy flour matrix, purified BBI was inactivated more quickly than purified KSTI, suggesting heating conditions may influence whether BBI is considered a heat-stable protease. More recently, Armour *et al.* (1998) found that trypsin inhibitory activity in soya bean was less readily abolished by aqueous heat treatment than chymotrypsin inhibitory activity and suggested that KTI and BBI *in situ* may have quite different heat-stabilities than they have after isolation and purification.

Antinutritional effects of Trypsin Inhibitors

Osborne & Mendel (1917) observed that only soya beans that had been cooked could support growth in rats. Following this observation, research was extended to many other animal species (Liener 1958). Initially it was assumed that this growth reduction was due to limited proteolysis in the gut due to trypsin inhibition. However, it was reported that there was still a growth reduction in rats when predigested

proteins or free amino acids were fed together with a high antitryptic fraction prepared from soya beans (Liener & Kakade 1980). This result indicated that the antinutritional effect of TI cannot only be explained by the inhibition of trypsin activity in the gut. In other studies it was shown that TI also influenced the secretion of other pancreatic enzymes (Schneeman *et al.* 1977). When trypsin is inhibited by TI, cholecystokinin (CCK) production is enhanced resulting in an increased production of pancreatic digestive enzymes. Hence the growth depression observed is a combined effect of endogenous loss of essential amino acids and decreased intestinal proteolysis.

Due to the enhanced enzyme production, hypertrophy and hyperplasia of the pancreas occurs; Chernick *et al.* (1948) discovered pancreatic enlargement in chicks caused by feeding raw soya beans. This finding was confirmed in several other studies, not only in chicks but also in rats, mice and young guinea-pigs (Hasdai *et al.* 1989; Gallaher & Shneeman 1986; as reviewed by Liener & Kakade 1980). Subsequent work by Khalifa *et al.* (1994) suggests that control of the composition of pancreatic secretions may not only be attributable to CCK but also to other intestinal hormones together with metabolites resulting from the transformation of other nutrients. Long term (700 day) feeding trials with rats induced an extensive increase in relative and absolute weights of pancreas and caused an increase in the occurrence of macroscopic pancreatic nodules and possible pancreatic neoplasia (Grant *et al.*, 1995). The negative feedback mechanism regulating the secretion of pancreatic enzymes found in rats also exists in pigs and calves, but without causing pancreatic hypertrophy (Gallaher & Schneeman 1986).

Breeding for Low Trypsin Inhibitor Content

Elimination of inhibitors would improve nutritional quality of soya beans. Orf & Hymowitz (1979) identified a variant, being a null in Kunitz inhibitor in PI 157.440 and PI 196.168; genetic studies showed that four alleles in a multiple allelic system control the various forms of Kunitz inhibitor and the absence of all forms is controlled by a single recessive allele, *ti*. The recessive allele has been backcrossed into the elite cultivars Williams 82, Clark 63 and Amsoy 71, all of which have been released as germplasm (Bernard & Hymowitz 1986). These varieties have been used for research but have not been grown commercially.

Variants have also been reported for the Bowman-Birk proteinase inhibitor (Stahlhut & Hymowitz 1983). However, little progress has been made, probably because this protein contains a relatively high level of cysteine and its elimination would reduce the overall level of this amino acid. Another possible reason for the

slow progress in reducing level of trypsin inhibitors overall is that these proteins are readily denatured upon heat treatment. More recently research has focused on producing cultivars with lowered Kunitz trypsin inhibitor content.

Low trypsin inhibitor cultivars in animal feed

A soya bean variant low in Kunitz inhibitor activity was first identified by Singh *et al.* (1969). Recently, a new varient has been developed that is isogenic to the conventionally grown Williams 82 cultivar except that it lacks the Kunitz trypsin-inhibitor allele. Cook *et al.* (1988) reported that this varient was nutritionally superior to conventional raw soya beans for growing-finishing pigs. Han *et al.* (1991) found weight gain and feed efficiency of chicks fed diets where 250 and 500g/kg of the protein was the raw, low Kunitz cultivar were not significantly different from those of chicks fed a diet where all of the soya bean protein was from heat-treated conventional soya beans (Table 2).

(Table 2 about here)

It has also been suggested that even higher levels of the low trypsin inhibitor variant could be used in diets of older birds without adversely affecting performance, as the adverse response to raw dietary soya beans is, to some degree, age-dependent (Crenshaw & Danielson 1985). However, similar experiments using laying hens (Table 3) have subsequently shown that the relative nutritive value of KSTI free soya beans for chickens does not differ greatly with age of birds (Zhang *et al.* 1991).

(Table 3 about here)

Attempts to replace the soya bean content (250g/kg diet) of broiler chick diets entirely with raw low trypsin inhibitor soya beans lead to growth and feed conversion ratios similar to raw, conventional cultivars (Chohan *et al.* 1993; Table 4). Friedman *et al.* (1991) found the low Kunitz inhibitor varient contained less than 0.002 of the Williams 82 cultivar Kunitz inhibitor content; it was also found that raw soya flour prepared from the isoline was nutritionally superior to raw flour prepared from the conventional soya bean, as measured by PER and pancreatic weights, leading to the suggestion that further work could lead to the discovery of soya beans which require minimal heating.

(Tables 4 and 5 about here)

A considerable reduction in processing costs can be achieved by using low trypsin inhibitor cultivars, in addition to lessening risk of overcooking (Friedman *et al.* 1991). Ironically, the Bowman-Birk and Kunitz trypsin inhibitors contribute to the nutritional quality of soya beans by virtue of their relatively high cystine content. This supplements the low or negligible amounts of sulphur-containing amino acids in the storage proteins that comprise the bulk of the protein reserve in the seed. Hence the effect on the amino acid profile of breeding Kunitz or BBI free soya beans must be carefully considered.

CARBOHYDRATE CONTENT

Soya beans contain approximately 350g total carbohydrates/kg DM, making this fraction proportionately the second largest component. However, only trace amounts of the soluble carbohydrates in soya beans are monosaccharides such as glucose and arabinose. More measurable amounts of soluble carbohydrates in soya beans are present as di- and oligosaccharides (see Table 6).

(Table 6 about here)

One of the important factors limiting the use of soya beans in non-ruminant animal feeds is the flatulence associated with their consumption. The principal causes appear to be the low molecular weight oligosaccharides containing ∀-galactosidic and ∃-fructosidic linkages, namely raffinose and stachyose

Role in Plants.

The biosynthesis of raffinose saccharides in soya beans is believed to start with the initial reaction catalysed by galactinol synthase (GS) to produce galatinol from UDP (uridine diphosphate)-galactose and myo-inositol. Subsequently, galactinol is used to add galactosyl residues to sucrose and raffinose to form the corresponding higher homologue. Each of the steps is catalysed by specific synthases (Dey 1985).

The onset of desiccation tolerance in immature soya bean seeds and storability of mature seeds has been associated with the accumulation of non-reducing soluble carbohydrates, specifically stachyose, in addition to sucrose (Lin & Huang 1994). The flatulence producing potential of stachyose has prompted the search for soya bean genotypes with low stachyose content (Kinney 1996). If

stachyose is required for desiccation tolerance and long-term storability of conventional seeds, alternatives to stachyose must be found to maintain quality seed stocks in low stachyose genotypes. During water deficiency stress, soya bean leaf and young stem tissues accumulate other non-reducible soluble carbohydrates including certain galactosyl cyclitols (Bohnert *et al*. 1995). Obendorf *et al.* (1998) reported that galactosyl cyclitols accumulate in the axis and cotyledon tissues of developing soya bean seeds in association with onset of desiccation tolerance. In future work it would be of interest to determine whether galactosyl cyclitols may substitute for stachyose in providing protection for desiccation tolerance and storability in soya bean seeds.

Anti-nutritional effects of soluble carbohydrates in soya beans

Flatulence is generally attributed to the fact that non-ruminant animals are not endowed with the enzyme (∀-galactosidase) necessary for hydrolysing the ∀-galactosidic linkages of raffinose and stachyose to yield readily absorbable sugars (Gitzelmann & Auricchio 1965). Consequently, the intact oligosaccharides enter the lower intestine where they are metabolised by microflora producing such gases as carbon dioxide, hydrogen and, to a lesser extent, methane. It is the production of these gases which is responsible for nausea, cramps, wet droppings and diarrhoea. In poultry the ∀-galactoside family of oligosaccharides has been implicated in reducing soya bean meal true metabolisable energy (TME), fibre digestion and intestinal transit time (Coon *et al.* 1990). ∀-galactosides can also increase the osmotic pressure of the lumenal contents.

In pigs the large intestine has a sufficient population of microbes to degrade non-starch polysaccharides and provide a potential source of energy (lactic acid and volatile fatty acids) for the animal to absorb. However Veldman *et al.* (1993) reported that the presence of ∀-galactosides in the diet caused fluid retention and increased microbial activity which may result in systemic and local effects such as stimulated gut motility, gut wall damage and decreased hydrolysis of dietary constituents resulting in a diminished overall digestion.

The nutritional significance of soya bean meal oligosaccharides, however, remains controversial, with some studies indicating a significant antinutritional effect and others failing to show any negative effect. The apparent contradictory results may be related to experimental technique and, in particular, to the method of reducing the concentration of ∀-galactosides. Removal of ∀-galactosides using ethanol extraction results in improvement in TME of soya bean meal (Coon *et al*. 1990) (Table 7) but interpretation of these data is confounded by the simultaneous

probable extraction of other meal components.

(Table 7 about here)

In contrast, Angel *et al*. (1988) reported that removal using endogenous soya bean ∀-galactosidase failed to produce any beneficial effect on the nutritional value of soya flakes and concluded poor energy utilisation from soya bean meal (toasted-defatted soya flakes) by poultry is not related exclusively to the presence of the oligosaccharides raffinose and stachyose (Table 8).

(Table 8 about here)

Veldman *et al*. (1993) found that the addition of velasse, the residue after evaporation of a 0.8 ethanol extract of soya bean meal generated during the production of soya protein concentrate, had a significant adverse effect on the ileal digestibility of nutrients and resulted in fluid retention and enhanced microbial fermentation in the gut when fed to piglets. However, the addition of an ∀-galactosidase to the velasse diet did not overcome these problems.

Irish *et al.* (1995) evaluated the effects of removing the ∀-galactosides of soya bean using either ethanol extraction or exogenous ∀-galactosidase enzyme (∀-D-galactoside galactohydrolase) with and without invertase (∃-fructofuranoside fructohydrolase) on the nutritional value of soya bean meal. It was shown that the performance of broilers and the TME value obtained with adult birds was not improved by removing stachyose and raffinose from soya bean meal using either ethanol extraction or ∀-galactosidase (Table 9). From these results Irish *et al.* (1995) concluded soya bean meal oligosaccharides have little or no anti-nutritional effect.

(Table 9 about here)

Experiments with soya bean mutants of low raffinose saccharides have helped researchers to define their targets for genetic engineering approaches (Liu & Clemmer 1997). One molecular strategy to decrease oligosaccharides may involve blocking the expression of GS gene for the production of galactinol in the seed by antisense techniques because GS is considered to be the key enzyme in the biosynthesis of oligosaccharides (De Lumen 1992). Mutant phenotypes have been generated in plants specified by antisense RNA techniques (Mol *et al*. 1990).

Breeding for low oligosaccharide content

An ultimate solution to the flatus problem would be the genetic removal of oligosaccharides by plant breeding. It is known that there is considerable variation in the raffinose and stachyose content among varieties of soya beans (Hymowitz *et al*. 1972). More recently the use of mutation breeding or genetic engineering has created lines with low oligosaccharides which are available for mass production (Kinney 1996). The meal from these lines has shown an improved ME content when fed to animals. In the registration of a low oligosaccharide content germplasm (D90-7256), Hartwig (1996) reported the combined raffinose and stachyose content of D90-7256 as 97.3g/kg in comparison with 119.6g/kg in its parent cultivar, Forrest.

Hartwig *et al*. (1997) studied seed protein and its relationship to soluble sugars in soya bean and found the correlation between protein and stachyose + raffinose to be negative but non-significant. However, the results of this study demonstrate the feasibility of developing soya bean germplasm that has higher seed protein and lower levels of stachyose and raffinose per unit of protein.

Two separate methods of conventional breeding (germplasm screening and chemical mutagenesis) have been devised to modify the soluble carbohydrate biosynthetic pathway and soya bean strains with low raffinose oligosaccharide contents or with high sucrose and low raffinose oligosaccharide contents have been developed (see table 10). By using these varieties, a novel soya flour with low raffinose oligosaccharides can also be made available for human consumption (Kinney, 1996; Kerr, 1996).

(Table 10 about here)

PHYTIC ACID

Plant phosphorus (P) is often found in the form of phytic acid (or its salt, phytate) which has a ring structure containing six $P(OH)_3$ groups (*myo*-inositol 1,2,3,4,5,6-hexakis; dihydrogen phosphate). Earlier work by Maga (1982) found soya phytate levels generally vary between 10 and 15 g/kg. However, more recent analysis by Raboy *et al.* (1984) indicates phytic acid levels in soya beans may be higher; 13.9-18.2g/kg. Phytic acid is the primary phosphorus and *myo*-inositol reserve in the seed (Reddy *et al.*1989). It is also thought to store other cations and is an energy-yielding component (Cosgrove 1980; Greenwood 1990). It is believed to protect plants against oxidative damage during storage and from moulds (Graf *et al.* 1987; Gupta & Vankitasubramanian 1975).

Anti-nutritional Effects

Phytates play an important role in mineral availability to the animal (Philippy & Johnstone 1985). The anti-nutritional properties of phytate result from its ability to form chelates with iron, manganese, copper, molybdenum, calcium and particularly zinc (Beleia *et al.* 1993). These complexes are extremely stable even at low pH (3 or 4) and are not readily digested within the gut. Consequently the utilisation of phosphorus in the form of phytate is poor in non-ruminants as they do not possess endogenous phytases. Phytic acid will also bind to proteins where they react strongly with positively charged ions and functional groups. The solubility of these complexes is governed by pH: the stability decreases with increasing pH (Cheryan 1980). When bound to protein, phytate induces a decrease in solubility and functionality of the protein (de Rham & Jost 1979). Consequently, in order to meet dietary requirements, inorganic phosphorus is routinely added to pig and poultry feed. Furthermore, non-utilised phytate is excreted by animals and applied to the soil as manure, which contributes to environmental pollution in areas of intensive animal production.

Increasing regulatory scrutiny of animal waste disposal has also fostered interest in finding solutions for decreasing phosphorus output. One immediate solution has been supplementation with industrial exogenous phytase.

Plant breeding for lowered phytate levels.

The ultimate solution is to introduce phytase genes directly into transgenic soya beans, which could reduce phytate content substantially. Recently, Denbow *et al*. (1998) reported ongoing research of phytase gene engineering. Two different gene sources are in use; one from *A. niger* and one from soya beans.

Insertion of phytase producing genes has been successfully achieved and the enzyme produced has pH and temperature optima that were indistinguishable from commercially available fungal phytase (Li *et al.* 1997). Currently, recombinant phytase produced in soya bean is not able to withstand the processing temperature necessary to inactivate proteinaceous anti-nutritional factors such as lectins and trypsin inhibitors. This could be overcome in the future by using recombinant phytase to lower phytate content during seed maturation. However, this would require the addition of specific targeting sequences to facilitate the localisation of phytase to the protein bodies which are the site of phytate accumulation. Current constructs are not suitable for obtaining co-localisation of enzyme and substrate (Li *et al.* 1997).

LECTINS

Lectins are proteinaceous compounds found in most plants, usually in the form of glycoproteins (Jaffé 1980). They have the ability to bind to certain carbohydrate molecules without altering the covalent structure (Pusztai *et al*. 1990). This affinity is usually highly specific; soya bean lectin binds with terminal N-acetyl-D-galactosamine and to a lesser extent with D-galactose. The majority of dietary lectins are able to resist gut proteolysis to varying degrees and bind to glycoproteins in the gut wall causing serious damage (King *et al*. 1983).

Seed lectins are primarily localized in the protein bodies of the cotyledon cells. Soya bean lectin sediments with the 7S fraction during ultracentrifugation. First purified and studied by Liener & Hill (1953), the lectin found in soya bean seed is a tetrameric protein with a molecular weight of 120 kDa, consisting of equal amounts of two identical subunits, each of which has a MW of 30 kDa (Goldstein & Poretz 1986). Lectin content of soya bean meal is reported to range from 2.2 to 4.0 g/kg DM (Pusztai 1991) and a lectin content of between 10 and 20 g/kg is normally present in native raw soya bean (Huisman & Tolman 1992).

For some time lectins have attracted the attention of food scientists and nutritionists because some of these proteins, such as ricin from the castor bean, are toxic to animals. The ability of soya bean lectin to inhibit the growth of rats and chicks was first demonstrated by Liener (1953) who showed that it accounted for about 0.25 of the growth inhibition produced by raw soya beans. Such growth inhibition cannot be explained by trypsin inhibitor activity alone and was confirmed by several later investigators including Donatucci (1983). In addition to growth inhibition, the soya bean lectin is linked to an enlargement of the pancreas, a lowering of blood insulin levels, an inhibition of the disaccharides and proteases in the intestines, degenerative changes in the liver and an interference with absorption of non-haem iron and lipid from the diet.

The function of lectins in plants are numerous. As is the case with many ANFs, lectins confer some chemical resistance against pests (Janzen *et al*. 1976). There is also evidence to suggest that lectins are involved in recognition of Rhizobium (Pusztai 1989) even though soya bean lines that do not contain lectins still nodulate readily (Pueppke 1983). Lectins sharing 0.63 of the N-terminal amino acid sequence have also been detected in vegetative tissues of soya bean (Spilatro *et al.* 1996). Research has indicated that some lectins are responsible for the induction of *de novo* synthesis of proteins necessary for successful nodulation (Hirsch *et al*. 1995). The interaction between lectins and a number of seed components led Bond *et al.* (1985) to suggest lectins may have an important function in the maturation and or

germination of seeds.

The main lectin found in soya bean is soya bean agglutinin. In addition to this, Campillo & Shannon (1982) purified a galactose-binding protein which displays two activities: (a) an α-galactosidase activity and (b) a haemagglutinin activity. This protein is clearly distinct from soya bean agglutinin, apparently immunologically unrelated and displays different carbohydrate specificities.

Reducing content of lectins.

The literature contains very little information on soya bean lines with reduced lectin content, suggesting little research has been carried out in this area. However, Douglas & Parson (1997) conducted a nutritional evaluation comparing raw lectin-free soya beans with raw reduced trypsin inhibitor soya beans, raw conventional soya beans and commercial heat processed soya bean meal in the diets of broiler chicks. Analysed lectin values (mg/g) were 7.2, 7.1 and less than 0.00015 for the low trypsin inhibitor, conventional and lectin-free soya beans respectively. The results of the study indicated that the nutritional value of raw lectin-free soya beans is greater than raw conventional soya beans but is less than raw low trypsin inhibitor soya beans and soya bean meal.

CONCLUSIONS

It is unquestioned that the anti-nutritional factors in soya beans (and most other plant materials) are a serious impediment to the efficient use of these crops in diets for non-ruminants. Whilst there are research programmes designed to remove or minimize these factors, it should be stressed that they do have a fundamentally important structural and/or protective role in the plant.

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Table 1. ***Classification of the 4 BBI isoinhibitor subgroups***

Subgroup	Half-cysteine residues	Amino acid residues	Trypsin inhibition	Chymotrypsin inhibition
I	14	71	Good	Good
II	14	71	Poor	Very poor/non-specific
III	10	71	Very poor	
IV	2	200	Very good	

Table 2*. Effects of raw conventional soyabeans (RCS) and raw low-trypsin inhibitor soyabeans (LTS) on chick performance in a soyabean replacement assay*(Han* et al*., 1991).*

Amount of soyabean protein replaced	Weight gain (g)	Feed intake	Gain: feed
ControlH	223.5	323.0	0.692
25% by RCS	216.9	322.9	0.672
25% by LTS	219.7	324.0	0.678
50% by RCS	203.6	322.5	0.631
50% by LTS	211.6	319.0	0.672
75% by RCS	194.7	317.0	0.614
75% by LTS	208.2	320.5	0.650
100% by RCS	174.9	317.4	0.551
100% by LTS	198.6	319.4	0.622

Means of three groups of seven male crossbred chicks from 8 to 19 days post hatching.

H***Control diet was a 22% CP corn and soyabean meal diet in which 100% of the dietary soyabean protein was supplied by heat, dehulled soyabean meal.***

Table 3. ***Determination of protein quality of heated, dehulled soya bean meal, raw conventional soya beans and raw, low trypsin inhibitor soya beans in diets fed to laying hens (Zhang*** **et al.,** ***1991).***

Treatment	Egg yield (g egg/hen/day)	Feed intake (g/hen/day
Heated, dehulled soya bean meal	54.4^{a}	119.9^{a}
Raw, conventional soya beans	36.5^{d}	102.6^{c}
Raw, low trypsin inhibitor soya beans	46.4^{c}	114.1^{b}

$^{a-d}$ Means within columns with no common superscripts differ (P<0.05).

Table 4. ***Effect of raw, conventional and raw, low trypsin inhibitor soya bean diets on growth, feed efficiency and nutrient digestibility in broiler chickens (Chohan*** **et al.,** ***1993).***

	Raw, conventional soya bean	Raw, low trypsin inhibitor soya bean
21 day weight (g)	463.4^{d}	453.7^{d}
7-21 day gain (g)	333.1^{d}	325.9^{d}
7-21 day feed intake (g)	579.8^{d}	565.7^{d}
Feed / gain	1.75^{c}	1.76^{c}

$^{a-d}$ Means within rows with no common superscripts differ (P<0.05).

Table 5. ***Protein efficiency ratio (PER) and pancreas weights of rats fed raw conventional (Williams 82) and low trypsin inhibitor (L81-4950) soya bean flours (Friedman* et al*. 1991).***

	PER	Pancreas weight (g/kg body weight)
Williams 82	-0.14^{f}	8.06^{a}
L81-4950	0.46^{e}	7.21^{b}

$^{a-f}$ Means within colums with no common superscripts differ (P<0.05).

Table 6. ***Concentration of di- and oligosaccharides in soya beans (Hymowitz*** **et al.,** ***1972).***

Carbohydrate	Concentration in soya beans (g/kg)
Sucrose	25-82
Raffinose	1-9
Stachyose	14-41
Verbascose	Trace

Table 7. ***True metabolisable energy (TME) of diets containing unincubated and incubated soya flakes in diets of roosters (Angel*** **et al.,** ***1988).***

Test material	TME of diets (kJ/kg)
Soya bean meal	881[a]
Buffer-added-unincubated soya flakes	891[a]
Buffer-added-incubated soya flakes	912[ab]
Soya milk	931[b]
Standard error of means	10.8

[a-b] Means followed by different superscripts are different (P<0.05) on the basis of Tukey's multiple-range test.

Table 8. ***Weight gain and feed efficiency from 7-14 days of chicks fed diets containing unincubated or soya flakes incubated with 0.1 M sodium acetate buffer solution (Angel* et al., *1988).***

Test material	Dietary inclusion level (g/kg)	Chick weight gain (g)	Gain:feed ratio	Relative index of oligo-/mono-saccharide ratio*
Soya bean meal	300	154	0.76	100
Water-added-unincubated soya flakes	300	140	0.75	92
Buffer-added-unincubated soya flakes	300	145	0.76	86
Buffer-added-incubated soya flakes	300	152	0.73	6
Buffer-added-incubated soya flakes	200	146	0.73	6
Buffer-added-incubated soya flakes	100	158	0.74	6
Soya milk	150	168	0.79	81

* Relative index obtained by calculating the ratio of oligosaccharide to monoSaccharide peak areas and then assigning a value of 100 to the ratio obtained for raw soya flakes. The relationship between the peak area and the weight of each sugar was not determined.

Table 9. ***Performance of broilers and true metabolisable energy (TME) of differently treated soya bean meal (Irish*** **et al.,** ***1995).***

	Weight gain (g)	Gain:feed ratio	Coefficient of apparent digestibility	TME (MJ/kg DM)
Soya bean meal	365	0.706	0.91	12.39
Ethanol extracted soya bean meal	272	0.622	0.85	11.63
Water incubated soya bean meal	343	0.685	0.92	11.46
Water + a-galactosidase incubated soya bean meal	345	0.696	0.90	11.31

Table 10. ***Alteration of soya bean oligosaccharide content (g/kg DM) by conventional plant breeding (Kerr, 1996).***

Lines	Sucrose	Raffinose	Stachyose
Normal	51	10	47
stc1	60	4	13
stc1 + mod1	70	1	5
stc1 + mod3	115	1	0