Grain legumes and human health

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Abstract

Since early civilisation in different continents, grain legumes have been a part of balanced diets together with cereals. In general the seeds from those two plant families give together a good source of essential amino acids and may have given an evolutionary advantage. A sustainable diet for vegetarians may not be possible without the protein-rich legumes. Consumed alone, legumes can be a mixed blessing because of their deficiency of some essential amino acids. This deficiency can be balanced in a varied diet containing components richer in tryptophan and the sulphur amino acids methionine and cysteine. Many legume species are exceptionally rich in secondary metabolites, some of which are beneficial to human health while without adequate processing through heating or leaching many can be detrimental to the health of the consumer. Soybean (Glycine max) is one of the most popular and also one of the more healthy species. The presence of genistein and other isoflavones makes it famous as an anti-cancer nutraceutical. Isoflavones and other phytoestrogens, occurring mainly in legumes, might be a factor promoting longevity. Other beneficial
secondary metabolites are found among the non-protein amino acids. Legumes are very rich in an enormous variety of non-protein amino acids that can be beneficial or toxic. A beneficial non-protein amino acid occurs in high concentration in fenugreek seeds (Trigonella foenum-graecum L.), a component of curry, while a variety of toxic amino acids occurs in the genera Lathyrus and Vicia. Legume seeds also contain a variety of anti-nutritional factors that can be reduced by post-harvest processing such as fermentation or germination. Fermentation also improves the balance of essential amino acids. Numerous dietary products such as soy sauce or tempeh are traditional food ingredients derived from legumes.

Popular advice for healthy diets that may promote health and longevity include the daily consumption of at least three servings of fruits or vegetables and the variation of foods to include items derived from different plants and those plants should belong to different botanical families (Thompson et al., 1999).

Ancient civilisations in the Middle East and in America included grain legumes and cereals in well-balanced diets. In the funeral offerings found in the Egyptian pyramids various legume seeds were present, including lentils and grasspea. Apparently, legumes were a food of special consideration to be offered to kings, in contrast to the present day reputation of being the meat of the poor.

Legumes are important factors in the natural cycle of nitrogen, being able to reduce atmospheric nitrogen in symbiosis with Rhizobium bacteria. This enables the leguminous plants to thrive on poor soil, which makes them essential partners in the maintenance of soil fertility, and to produce protein-rich seeds, however, maintenance of optimum rates of nitrogen fixation requires continued attention by plant breeders (Provorov and Tikhinovich, 2003).

Legumes are also unusually diverse in their defence against predators by producing a large array of secondary metabolites forming their chemical armoury. The variability in these secondary metabolites allows the classification of genera and subgenera on the basis of chemical taxonomy. Those metabolites include anti-nutrients such as inhibitors of digestion and compounds interfering with human metabolism reaching as far as brain function and hormonal control. Some of these metabolites are beneficial by their inhibition of cancer cells or by antioxidant activity that can delay ageing.

Although legumes have many beneficial properties, they are not a well balanced food by themselves because of deficiencies in some essential amino acids, and should not be the sole component of the food basket. In combination with cereals that are richer in those essential amino acids which are deficient in legumes such as methionine, cysteine and tryptophan, legumes are beneficial for human health and for the world’s ecology. The optimum protein intake is approximated when 60-70% cereals are mixed with 30-40% cooked legumes. This would produce a combined quality of protein comparable with meat (Bressani and Elias, 1974).

Both germination and fermentation have positive effects on the nutritional quality of legumes. Anti-nutritional factors such as trypsin and chymotrypsin inhibitors and the
galactosidases as flatulence factors are reduced by germination or fermentation. Germination also reduces the level of fats and carbohydrates while increasing the dietary fibres and vitamins (Vidal-Valverde et al., 2002). Fermentation can increase the amino acid score as the micro-organisms involved can also produce essential amino acids. In some cases toxins present in the seeds can be reduced by fermentation (Kuo et al., 2000).

Proteins that act as anti-nutritional factors such as trypsin inhibitor, chymotrypsin inhibitor and alpha-amylase inhibitor are destroyed by simple cooking (moist heat treatment) and only pose a problem when the seeds are consumed raw or are cooked insufficiently due to lack of fuel, water or after prolonged storage. Soybean seedlings (or more often mungbean seedlings) are consumed raw in salads in Western cuisine, while in the East, from where the practice originated, seedlings are always heated before consumption.

Just a few examples of grain legumes with beneficial properties and with toxic properties are described below.

**Soybean, the Healthiest Legume**

Soybean (*Glycine max*) is the legume with the highest amino acid score and closest to the standard set by the FAO and WHO. The content of the sulphur amino acids methionine and cysteine is double when compared to grasspea (*Lathyrus sativus* L.), the legume with the lowest content of these essential amino acids. Nevertheless, farmers are spending an estimated 100 million dollars each year to supplement the soybean based feed with methionine for optimal performance by their domestic animals (Imsande, 2001). Compared to other grain legumes, soybean produces the highest yields of seeds per ha. The reason for this may in part be the allocation of better land for soybean than for other pulses.

The main advantage of soybean for human health, besides the nutritional value provided by its energy and protein content, is the high level of isoflavones genistein and daidzein present in the seeds. These secondary metabolites play a role in the symbiosis of the plant with *Rhizobium* but also have beneficial effects on human health. The concentration of these isoflavones in soybean is several orders of magnitude higher than in other commercial legumes. This includes the mungbean seedlings that are marketed as soybean sprouts in some European countries.

Soybean isoflavones are used in medicine at daily doses of 50 mg for the prevention of prostate cancer and breast cancer. This is the equivalent of about 50 g of soybean products or about 30 kg of other legumes (Liggins et al., 2000). Isoflavones are also antioxidants that can regenerate vitamins E and C, and are considered as a factor in the longevity of people who regularly consume soybean products in various forms. These include the seeds used in various recipes, soybean milk, many products made from precipitated protein, fermented foods such as soy sauce, tempeh and also the germinated seeds.

Considerable efforts are underway to further improve the nutritional quality of this healthy grain legume by increasing the content of sulphur-containing amino acids in the seed. Genetic transformation seems to be the method of choice for this endeavour. A
successful approach is the introduction of cereal storage proteins rich in methionine and cysteine (Li et al., 2005).

**Fenugreek: A Little Known Legume**

Fenugreek (Trigonella foenum-graecum L.) is perhaps best known as a component of curry. It also seems to be favoured by elderly Ethiopians who consume fenugreek for strength (Getahun, Pers. comm.). It is the original source of trigonelline, the smallest alkaloid and a multi-functional plant hormone. In plants it is known as the cell-cycle arrest hormone and it also plays a role in various stress situations. Recent investigations with human cells indicate that this compound may support the regeneration of neuronal networks and prevent memory loss (Tohda et al., 2005). Trigonelline was a ubiquitous compound present in all legume seedlings we examined (Rozan et al., 2000).

Fenugreek also contains an unusual amino acid, 4-hydroxyisoleucine that stimulates the production of insulin in non-insulin-dependant diabetes or diabetes I patients (Sauvaire et al., 1998). Besides this beneficial effect on insulin production, consumption of fenugreek also lowers the cholesterol in human blood (Sowmya and Rajyalakshmi, 1999). This has been ascribed to the action of saponin and the high fibre content. Fenugreek extracts also seem to have a chemopreventive effect against breast cancer (Amin et al., 2005) and against oxidative damage (Kaviarasan et al., 2004). No toxicity has been linked to fenugreek consumption in monogastric animals including humans or to the use of high doses of 4-hydroxyisoleucine (Flammang et al., 2004). However, ataxia and deaths in sheep grazing a pure stand of mature fenugreek have been observed (Allden and Geytenbeek, 1980). One may expect a rise in popularity of fenugreek with health conscious consumers in the coming years. Biotechnological research on this crop seems to have just started and initial results are promising (Khawar et al., 2004).

**Vetch: A Fodder, a Green Manure or a Food?**

The genus Vicia has an unusual aberration in its amino acid metabolism that leads to the accumulation of gamma-glutamyl-beta-cyanoalanine (Glu-BCA). Beta-cyanoalanine is a by-product of ethylene biosynthesis which in other plants is converted to the protein amino acid asparagine.

It is an inhibitor of cystathionase, an enzyme involved in the conversion of methionine to cysteine, leading to the urinary excretion of cystathionine (Ressler et al., 1964; Ressler, 1969). Common vetch (Vicia sativa) also contains the pyrimidine glycosides vicine and convicine. Common vetch has been implicated in a human neurologic disease comparable to neurolathyrism that was published as “lathyism without Lathyrus” (Shah, 1939). The people suffering from that disease had been consuming large quantities of vetch seed during a period of food insecurity and the vetch toxin BCA was considered a potential factor in neurolathyrism (Ressler, 1962). However, in contrast to neurolathyrism the neurological symptoms from vetch over-consumption were reversible.

Vavilov (1922) used the close similarities between some types of common vetch and
lentils as an illustration, published as colour plate, for his law on homologous series of variation in plants. The close resemblance between red lentils and the *Vicia sativa* cultivar ‘Blanchefleur’ led to its marketing for human consumption during the early 1990s.

With the toxic properties of common vetch known for decades, the considerable amounts of common vetch (cv ‘Blanchefleur’) destined for substituting split red lentils in markets of the Middle East and the Indian Subcontinent were a serious cause for concern (Tate and Enneking, 1992).

The pyrimidine glycosides vicine (0.75 % DW) and convicine (0.08% DW) (Ritthausen, 1881; Pitz *et al*., 1980) present in common vetch are factors implicated in the aetiology of favism (Chevion *et al*., 1982; Arbid and Marquardt, 1986). Their hydrolysis products are unstable and form radicals which can cause a depletion of reduced glutathione (GSH) in red blood cells deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD). A reduced activity of this enzyme provides insufficient NADPH to replenish GSH and predisposes the red blood cells to oxidative damage which can, ultimately, result in a haemolytic crisis (Chevion *et al*., 1982; Arbid and Marquardt, 1988). The antioxidant vitamins C and E or iron chelators can reduce the toxic effects of the favism factor divicine in rats (Marquardt and Arbid, 1988). Following the pioneering work of Fowden and Bell (1965), it would seem logic to reduce the toxicity of common vetch by preventing the sequestration of BCA into its gamma-glutamyl dipeptide glu-BCA. After all, it only requires the addition to BCA of a single molecule of water to convert a toxic compound into a nutrient, and this process apparently occurs in all other plants except for some *Vicia* species.

**Grasspea: A Controversial Lifesaver**

Grasspea (*Lathyrus sativus*) has been cultivated since the Neolithic era. It is a very drought tolerant grain legume that thrives on poor soil and is appreciated by farmers for its easy cultivation and for its production of tasty seeds when other crops fail due to drought. The plant is also more resistant to biotic and abiotic stress than other legumes and needs little inputs. These agronomic properties make it the cheapest pulse available in Bangladesh and Ethiopia during times of drought and food insecurity. It often is a lifesaver. Farmers in Ethiopia and in Bangladesh consider grasspea an insurance crop (Campbell, 1997). The number of people whose life was saved during famine thanks to grasspea must be considerable, but this is never mentioned in reports on neurolathyrism, the crippling disease caused by over-consumption of grasspea during a prolonged period. Indeed, grasspea is a mixed blessing. The crippling disease strikes with little or no warning after several months of nearly exclusive consumption of grasspea preparations. Up to 6 % of the population can be affected with the highest incidence among young males. Popular believes do not link the disease with the prolonged over-consumption of this life saver (Getahun *et al*., 2002).

While earlier researchers considered that neurolathyrism might be caused by the deficiency of an essential factor (Ressler, 1962), after the discovery of a neuro-excitatory amino acid beta-ODAP (beta-N-oxalyl-L-alpha,beta-diaminopropionic acid) (Rao *et al*., 1964) all attention has focussed on the elimination of this neurotoxin from the plant and on
studies of its pharmacological properties. While outcrossing is a potential threat to the stability of low toxin strains in areas with indigenous grasspea cultivation, an overlooked fact is also that, in situations of environmental stress and food insecurity, grasspea is the only legume consumed as a staple in monotonous diets, and that the content of sulphur amino acids in grasspea is the lowest of all commercial pulses. Recent epidemiological research has linked the occurrence of neurolathyrism in Ethiopia to poverty, illiteracy and the use of traditional clay utensils for cooking. Protective factors were identified as literacy, the use of at least one third of cereals in the diet and the addition of antioxidant-rich condiments such as onion, garlic and ginger to the grasspea preparations (Getahun et al., 2003, 2005). A fresh look at detailed earlier studies on lathyrism victims in a prisoner of war camp also indicate the relative safety of diets containing sufficient cereals mixed with grasspea (Lambein et al., 2001). Beta-ODAP is a specific excitant on the AMPA-receptors of the neuronal cells (Kusama et al., 2000), giving rise to the formation of nitric oxide (NO) and reactive oxygen species, but besides this neuroexcitatory potential, beta-ODAP is also responsible for a range of other biochemical and physiological activities, some of which also contribute to oxidative stress, and it can be metabolised by humans (Rudra et al., 2004). Considering all this, the low level of sulphur amino acids methionine and cysteine may be at least equally important in the aetiology of neurolathyrism as the presence of beta-ODAP. Efforts to improve the nutritional quality of grasspea should not exclusively be directed at the lowering or elimination of beta-ODAP but also at the improvement of the amino acid score by increasing methionine and cysteine in the seeds (Vaz-Patto et al., 2005) or in foods or food supplements which can be made available to balance diets containing large proportions of grasspea.

Recent preliminary neuro-physiological experiments with isolated neurons have shown an increased sensitivity to beta-ODAP when the medium is depleted of cysteine and methionine. This increase in sensitivity is more pronounced in motoneurons than in other neuronal cells. If the cysteine provided in the medium is the oxidised cystine form, where the -SH groups are oxidised to -S-S-bridges, then the addition of beta-ODAP gives no decrease in the survival rate of the neuronal cells (Kusama-Eguchi, pers. comm.). This experiment corroborates the view that the redox potential of the environment surrounding the motoneurons is of primary importance and suggests that intake of methionine and cysteine, and perhaps of other antioxidants that help to counter oxidative stress, may indeed help in the prevention of neurolathyrism.

Seedlings of grasspea and other members of the genus Lathyrus are a very rich source of the labile heterocyclic isoxazolinone compounds. Members of this class of natural products are the precursors for beta-ODAP, beta-aminopropionitrile (BAPN) and other toxins specific for the genus Lathyrus. The consumption of plant material containing the osteo-lathyrogen BAPN before adulthood may be responsible for the rare cases of human osteo-lathyrism occurring in Bangladesh (Haque et al., 1997).
Discussion

We can safely propagate that the presence of grain legumes in a food basket containing also a variation of cereal products and vegetables belonging to other plant families is beneficial for human health and for the world ecology. While some grain legumes have particular and proven advantages on human health and longevity, some of the so-called toxic grain legumes are probably harmless when consumed by well-informed people in limited amounts in a varied diet.

Sulphur: A Key to the Health of Legume Consumers

Grain legumes are well known to contain deficient levels of sulphur amino acids. While this fact is widely appreciated, a closer examination of several groups of antinutritional factors reveals that many of these factors target metabolic systems in their predators which also involve sulphur amino acids.

The initial stimulus to investigate these relationships stems from the widely publicised vetch/lentil substitution problem during the early 1990s.

Clearly, the deficient level of sulphur amino acids in the seeds of \textit{Vicia sativa} L. and \textit{Lathyrus sativus} L. is further exacerbated by the activity of their toxic amino acids beta-cyanoalanine and beta-ODAP, respectively, while a third component of its predator defence armoury reacts with reduced sulphur amino acids, thus attacking a pivotal metabolic system in the predator from an additional angle.

There are several other factors in grain legumes associated with the depletion of sulphur amino acids in the predator’s metabolism. A significant portion of the cysteine content in grain legume seeds can be found in proteins designed to be active in hostile extracellular environments, where the cysteine functions to stabilise these proteins by disulfide bridges. Lectins, protease and amylase inhibitors can be found in this group. Generally, these stabilised proteins can be destroyed by moist heat during food preparation. However, depending on the effectiveness of the heat treatment [heat, moisture, time], some activity, particularly for the chymotrypsin inhibitors, may remain.

Untreated grain legume seeds can contain significant levels of digestive enzyme inhibitors to cause harm in the predator. This manifests itself by an enlargement of the pancreas (pancreatic hypertrophy) and a depletion of sulphur amino acids, since digestive enzymes also require elevated levels of cysteine for stabilisation in a hostile extra-cellular environment.

Hence, here we have an additional group of anti-nutritional factors which have evolved as a component of a concerted attack on the predator’s sulphur amino acid metabolism. In fact, \textit{Vicia sativa} L. and \textit{Lathyrus sativus} L. seeds also contain trypsin inhibitor activity, albeit at relatively low levels (Aletor \textit{et al.}, 1994; Wang \textit{et al.}, 1998).

Cyanogenic glycosides can also be found in some grain legumes, such as linamarin in the seeds of the Lima bean (\textit{Phaseolus lunatus} L.), a species often encountered in Greek cuisine as a substitute for the historical kyamos (\textit{Vicia faba} L.). While the seeds of the vetch
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cultivar Blanchefleur contain no cyanogenic glycosides, other genotypes of *Vicia sativa* L. contain very high levels in the seeds and also in the foliage (Hanelt and Tschiersch, 1967). The toxicity of cyanogenic glycosides is a result of cyanide (HCN) being released through the action of hydrolytic enzymes which cleaves off the sugar moiety and hence destabilises the compound. Protection against cyanide poisoning is afforded by the liver enzyme rhodanase in a reaction dependent on the availability of sulphur amino acids and producing the less toxic thiocyanate that is excreted in the urine.

The seeds of *Vicia sativa* L. and *Lathyrus sativus* L. therefore contain a small battery of antinutritional factors which additively lead to a depletion of sulphur amino acids, coupled with a limited supply of sulphur amino acids from other sources, and hence neurotoxic symptoms can develop in the predator. In the case of a vetch/lentil substitution, or an adulteration with grasspea, the predator is the unsuspecting human consumer.

The cool season food legumes all contain proteinaceous enzyme inhibitors and all are low in nutritional sulphur amino acids. In the case of grasspea, the neurotoxin beta-ODAP is a glutamate analogue. Recent studies suggest that the uptake of beta-ODAP into nerve cells is inhibited by the presence of cysteine (La Bella *et al*., 1996) and that mitochondrial dysfunction as a consequence of thiol oxidation by reactive oxygen species is the primary cause of neurodegeneration by beta-ODAP (Ravindranath, 2002).

The above leads to the hypothesis that sulphur amino acid depletion by different active principles acting together is the cause of low nutritive value and even toxicity of diets containing large proportions of insufficiently processed grain legumes or those containing heat stable antinutrients. Supplementation of such diets with additional sources of sulphur amino acids, or thiol antioxidants such as alpha-lipoic acid or N-acetyl-cysteine, can act as antidotes and enhance the nutritive value of otherwise unwholesome diets.

“A direct application of these ideas may very well be a feeding experiment with low beta-ODAP strains of grasspea, with *Vicia sativa* L. mixed in various proportions with high and low beta-ODAP seeds to determine the sulphur amino acid depleting activity of such mixtures and hence to simulate conditions of malnutrition which lead to the onset of neurolathyrism. A safe grasspea cultivar would not increase the toxicity of a diet containing vetch at a no-effect level (i.e. 10-20% in pig diets)”.

While there are other toxic systems in legumes, i.e. lupin alkaloids, selenium analogues in *Astragalus* and canavanine, an arginine analogue in alfalfa, etc., the sulphur metabolism in legume predators appears to be one of the major targets for legume antinutrients. Interestingly, the biosynthesis of several toxic amino acids in legumes is also part of sulphur amino acid pathways (Ikegami *et al*., 1992). Looking for hitherto unknown factors affecting the sulphur system, and the utility of dietary supplementation to overcome potential toxicities, may prove a productive avenue for research to improve the health of legume consumers.
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