Fishmeal replacers: Review of antinutrients within oilseeds and pulses - A limiting factor for the aquafeed Green Revolution?

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Fishmeal replacers: Review of antinutrients within oilseeds and pulses - A limiting factor for the aquafeed *Green Revolution*?

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SUMMARY - The paper reviews the endogenous antinutritional factors or antinutrients present within plant feedstuffs, and in particular those protein-rich feedstuffs with potential for use as `fishmeal replacers' within compound aquafeeds. The toxicity of the major antinutritional factors for farmed fish and shrimp are discussed, and information presented concerning the processing and/or biotechnological methods commonly used to destroy or inactivate them.

Key words: Antinutrients, pathology, nutrition, aquafeeds.

RESUME *- "Remplacement des farines de poissons : Passage en revue des facteurs antinutritionnels des oléagineuses et légumineuses - Est-ce un facteur limitant pour la Révolution Verte en Alimentation Aquacole?" Cet article passe en revue les facteurs antinutritionnels endogènes présents dans les aliments végétaux, et en particulier les matières riches en protéines ayant une utilisation potentielle en remplacement des farines de poisson dans les aliments composés. La discussion portera sur la toxicité des principaux facteurs antinutritionnels pour les poissons et crevettes d'élevage, en présentant des informations concernant l'élaboration et/ou les méthodes biotechnologiques utilisées couramment pour détruire ou neutraliser ces derniers.*

Mots-clés *: Facteurs antinutritionnels, pathologie, nutrition, aliments pour poissons.*

INTRODUCTION

Plant oilseeds and pulses constitute a readily available source of dietary protein for use within compound aquafeeds; total world production of plant oilseeds and pulses in 1993 being over 287 million metric tonnes (mmt) and oilseed cakes and meals (derived after oil extraction) totalling about 126 mmt (Figure 1, 2). By contrast, total world fishmeal production in 1993 was reported to be 6.2 mmt, of which only 3.62 mmt was available for export (FAO, 1995).

However, despite the ready market availability of most oilseeds and pulses and their lower cost compared with fishmeal (Table 1), their use within compound aquafeeds is usually restricted by the presence of one or more endogenous antinutritional factors or antinutrients (Hendricks & Bailey, 1989; Kaushik, 1989; Krogdahl, 1989; NRC, 1993). In fact it is generally believed that the presence of naturally occurring antinutrients within oilseeds and pulses is the single most

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important factor limiting their use as `fishmeal replacers' at high dietary inclusion levels within compound aquafeeds; the inherent essential amino acid deficiencies of most plant proteins being readily rectified by dietary supplementation with the limiting free amino acids or by mixing complementary protein sources (Tacon & Jackson, 1985).

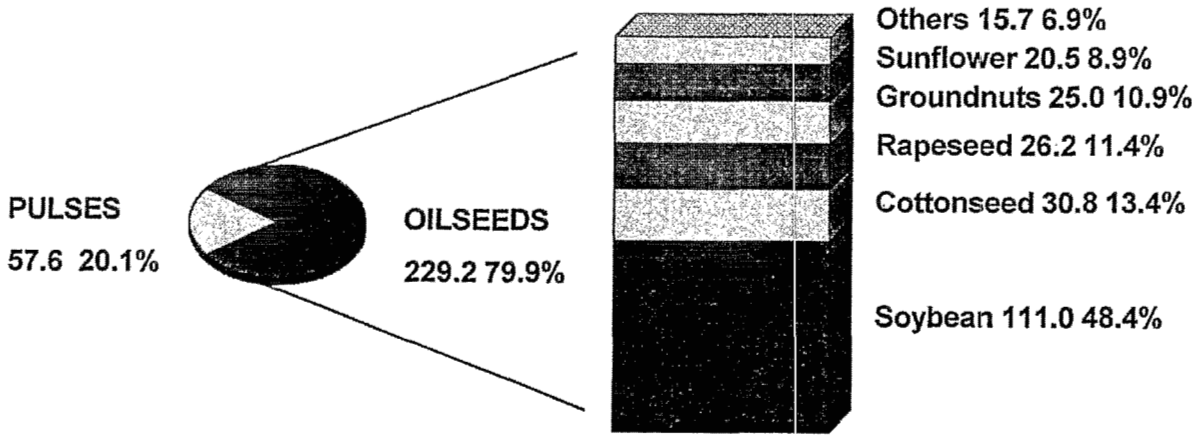

Fig. 1. Total world production of pulses and oilseeds in 1993 (values given in million metric tonnes, mmt). Total world production of pulses and oilseeds in 1993 was 286.86 million metric tonnes (FAO, 1994). Pulses include (mmt) Haricot beans 16.2, Peas 16.0, Chick peas 6.6, Broadbeans 4.0, Lentils 2.7; Other oilseeds (mmt) Copra 4.6, Palm kernel 4.4, Sesame 2.5, Linseed 2.2, Castor 1.2, Safflower 0.8.

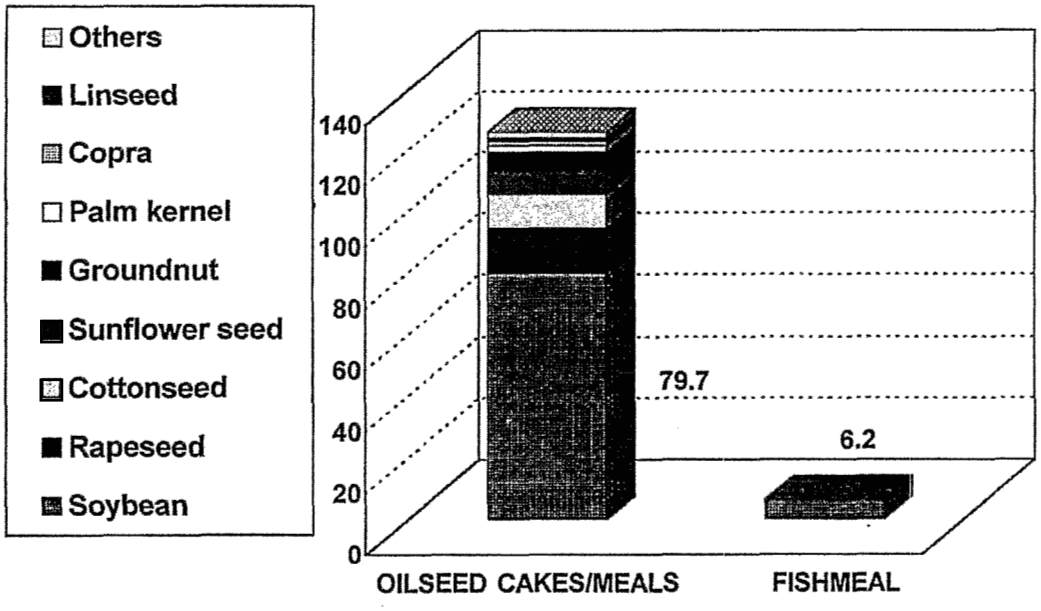

Fig. 2. Total world production of plant oilseed cakes and meals in 1993 (values given in million metric tonnes, mmt). Oilseed cake/meal production in 1993 was reported as Soybean cake/meal 79.71 mmt, rapeseed cake/meal 14.69 mmt, cottonseed cake/meal 11.09 mmt, sunflower seed cake/meal 7.96 mmt, groundnut cake/meal 5.69 mmt, palm kernel cake/meal 2.37 mmt, linseed cake/meal 1.11 mmt, others 1.93 mmt (FAO, 1994).

Table 1. Average international market price of selected oilcakes and meals in 1994

OILSEED/MEAL	US$/MT	US$/KG PROTEIN
SOYBEAN MEAL (45-46% protein)	193	0.424
SUNFLOWERSEED MEAL (38% protein)	119	0.313
RAPESEED MEAL (34% protein)	148	0.435
GROUNDNUT CAKE (48-50% protein)	168	0.343
COTTONSEED CAKE (43% protein)	155	0.361
COPRA CAKE (23-24% protein)	131	0.504
PALM KERNEL CAKE (18% protein)	111	0.617
LINSEED CAKE (36% protein)	228	0.633
FISHMEAL (65% protein)	376	0.578

FAO (1995a): International market price information. Report of the FAO Intergovernmental Group on Oilseeds, Oils and Fats, 27th Session, Rome 9-12 May 1995. CCP:OF 95/CRS 3; 12p. FAO, Rome, Italy

Soybean meal - Argentina, pellets, c.i.f. Rotterdam; Sunflowerseed meal - Argentina/Uruguay, pellets,c.i.f. Rotterdam; Rapeseed cake - f.o.b., ex-mill Hamburg; Groundnut cake - Argentina, c.i.f. Rotterdam; Cottonseed cake - expellers, China, c.i.f. Denmark/UK; Copra cake - Philippines, pellets, c.i.f. Hamburg; Palm kernel cake - expeller, Malaysia, c.i.f. Hamburg; Linseed cake - expellers, c.i.f. Rotterdam; Fish meal - any origin, wholesale, c.i.f. Hamburg.

This paper attempts to review the major antinutrients present within the most promising plant `fishmeal replacers' and to highlight their toxicity for farmed fish and shrimp, as well as the prospects for their removal or destruction.

MAJOR ANTINUTRIENTS

Figure 3 summarizes the major groups of antinutritional factors present in plant feedstuffs and Table 2 provides specific examples of the antinutrients present within selected oilseeds and pulses commonly used within farm-made or commercially compounded aquafeeds. For general review see Huisman & Tolman (1992), Jansman & Poel (1993), and Liener (1989).

Protease inhibitors

Protease inhibitors (PIs) are protein-based substances widely distributed within the plant kingdom, including the seeds of most cultivated legumes (Table 2), which have the ability to inhibit the activity of proteolytic enzymes within the gastro-intestinal tract of animals. In fact PIs are widely believed to be the most important of the antinutrients present within soybeans and commercial soybean processing methods have generally been targeted almost entirely on the destruction of PI activity (through denaturation by heat processing techniques) rather than the removal or destruction of any other of the numerous antinutrients present within raw soybeans (Table 2; Krogdahl et al. 1994; Lim & Akiyama, 1991). For example, the PIs of raw soybeans (which account for about 6% of the total protein) can be broadly divided into two main types; the heat-labile Kunitz inhibitors with a molecular weight of 20,000-25,000 and possessing relatively few disulfide bonds and a specificity

directed mainly toward trypsin, and the more heat-stable Bowman-Birk inhibitors having a molecular weight of 6000-10,000, a high proportion of disulfide bonds and capable of inhibiting trypsin and chymotrypsin at independent sites (Liener, 1980, 1989).

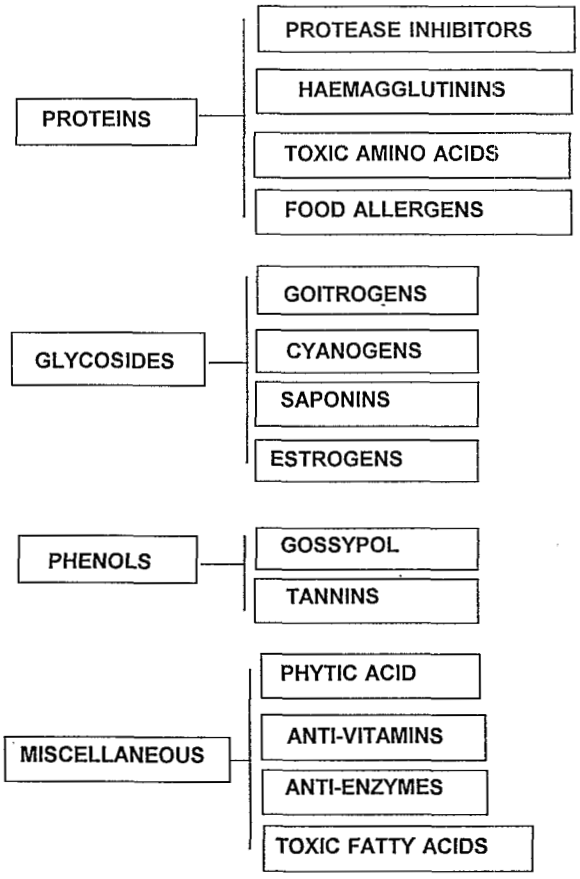

Fig. 3. Classification of endogenous antinutrients within plant feedstuffs (adapted from Liener, 1980).

Although relatively few feeding trials have been conducted to date concerning the effect of purified PIs in the diet of fish and shrimp, results with rainbow trout *Oncorhynchus mykiss* fed purified soybean protease inhibitors (SPIs) reported a negative effect of SPI on protein and amino acid digestion by inhibiting intestinal protease activity (Berg Lea et al., 1989; Krogdahl et al. 1994) and a possible increased dietary requirement for sulphur-containing amino acids over and above that which would normally be required to remedy the normal methionine deficiency of soybean protein (Krogdahl, 1989; Vohra & Kratzer, 1991). The latter is believed to be due to the high cystine content of the protease digestive enzymes (the production of which is stimulated by SPIs) and the increased conversion of methionine to cysteine in the liver of fish fed SPI supplemented diets (Krogdahl et al., 1994). By contrast, although shrimp *Penaeus japonicus* fed a fishmeal-based diet supplemented with 3.5% SPI reportedly displayed pancreatic hypertrophy at the end of a 28-day feeding period, the growth of shrimp fed the SPI supplemented ration was greater (142%) than that of shrimp fed the control fishmeal diet devoid of SPI (123%; Van Wormhoudt et al. 1986). According to these authors the shrimp

apparently had the capacity to compensate for SPI activity through intestinal hormone action by generating gastrin-like peptides to stimulate higher trypsin production.

Table 2. Endogenous anti-nutritional factors reportedly present within plant oilseeds and pulses commonly used within aquafeeds for farmed fish and shrimp (adapted from Liener, 1980 and Tacon, 1992)

LEGUMES	ANTI-NUTRITIONAL FACTOR[1/]
Broad/faba bean *Vicia faba*	1 (T,C,Th,Pr,Pa,Mc),2,5,7,22
Chick pea/bengal gram *Cicer arietinum*	1 (T,C),4,5,6,8,11
Cow pea *Vigna unguiculata*	1 (T,C),2,5,11
Grass pea *Lathyrus sativus*	1 (T,C),9
Haricot/kidney bean *Phaseolus vulgaris*	1 (T,C,E),2,4,5,6,11,12,18
Hyacinth/field bean *Dolichus lablab*	1 (T,C,Th),2,4
Lentil *Lens culinaris*	1 (T),2,6
Lima bean *Phaseolus lunatus*	1 (T,C),2,4,5,7
Lupin *Lupinus albus*	1 (T),6,8,25
Mung bean/green gram *Phaseolus aureus*	1 (T,En),5,6,11,13
Field pea *Pisum sativum*	1 (T),2,4,5,6,12
Pigeon pea/red gram *Cajanus cajan*	1 (T),2,4,5
Runner bean *Phaseolus coccineus*	1 (T,C),2
Jack bean *Canavalia gladiata/ensiformis*	1 (T,C,S),2,4,6,26
Velvet bean *Stizobolium deeringianuum*	1 (T),22
Winged bean *Psophocarpus tetragonolobus*	1 (T),2
Alfalfa/lucerne *Medicago sativa*	1 (T),6,8,12
Black gram *Phaseolus mungo*	1 (T,C,S),5
Sesbania *Sesbania* spp.	6,26
Ipil Ipil *Leucaena leucocephala*	23
OILSEEDS	
Groundnut *Arachis hypogaea*	1 (T,C,Pl),2,5,6,8
Rapeseed *Brassica campestris napus*	1 (T),3,5,7
Indian mustard *Brassica juncea*	1 (T),3,13
Sunflower *Helianthus annuus*	1 (T),6,7,20
Cottonseed *Gossypium* spp.	5,8,10,12,24
Linseed *Linum usitatissimum*	4,5,8,13,15
Sesame *Sesamum indicum*	5
Crambe *Crambe abyssinica*	3
Soybean *Glycine max*	1 (T,E,C,Pa,In),2,3,5,6,8,11,12, 14,16,17,27

1/1-Protease inhibitors (T-trypsin, C-chymotrypsin, Pl-plasmin, Pr-pronase, Th-thrombin, S-subtilisin, En-endopeptidase, In-insect proteases, Pa-papain, E-elastin, Mc-microbial proteases), 2-Phytohaemagglutinins, 3-Glucosinolates, 4-Cyanogens, 5-Phytic acid, 6-Saponins, 7-Tannins, 8-Estrogenic factors, 9-Lathyrogens, 10-Gossypol, 11-Flatulence factor, 12-Anti-vitamin E factor, 13-Anti-thiamine factor, 14-Anti-vitamin A factor, 15-Anti-pyridoxine factor, 16-Anti-vitamin D factor, 17-Anti-vitamin B12 factor, 18-Amylase inhibitor, 19-Invertase inhibitor, 20-Arginase inhibitor, 21-Cholinesterase inhibitor, 22-Dihydroxyphenylalanine, 23-Mimosine, 24-Cyclopropenoic acid, 25-quinolizadine alkaloids, 26-canavanine, 27-allergens

It follows from the above that individual fish and shrimp species may differ considerably in their tolerance and response to PIs (Hendricks & Bailey, 1989; NRC, 1993); the reported sensitivity of farmed species ranging from species said to be very sensitive such as salmonids (the inhibition of rainbow trout *O. mykiss* proteases by PI being 15 times greater than that of the same amount of human enzymes *in vitro*; Beckmann & Pfeffer, 1989; Krogdahl and Holm, 1983; Krogdahl et al. 1994;

Olli et al. 1989; Van den Ingh et al. 1991) to less sensitive species such as yellowtail *Seriola quinqeradiata* (Shimeno et al. 1992, 1994), European seabass *Dicentrarchus labrax* (Amerio et al. 1989), common carp *Cyprinus carpio* (Viola et. al. 1982, 1983; Pongmaneerat & Watanabe, 1993), tilapia *Oreochromis mossambicus* (Davies et al. 1989), *Oreochromis niloticus* (Shu, 1992; Tacon et al. 1983; Wee & Shu, 1989), channel catfish *Ictalurus punctatus* (Lovell, 1991; Webster et al. 1992; Wilson & Poe, 1985) and penaeid shrimp *P. vannamei* (Sessa and Lim, 1992), *P. japonicus* (Van Wormhoudt et al. 1986).

However, with the exception of the studies of Krogdahl & Holm (1983) and Van Wormhoudt et al. (1986) no comparative studies have been conducted to date so as to ascertain the precise sensitivity and tolerance of individual fish and shrimp species to graded levels of the different major types of purified PIs (including both the Kunitz and Bowman-Berk Inhibitors) either *in vitro* or *in vivo*. Moreover, analysis of PI data from the majority of published studies using graded levels of processed oilseed meals is complicated by the use of different oilseed processing methods and feed manufacturing techniques (Rumsey et al. 1993), and more importantly by the possible presence of other endogenous antinutrients within the oilseed meal in addition to PI (Table 2). For example, the abnormalities reported within the mucosa of the distal intestine of Atlantic salmon *Salmo salar* fed a diet containing 30% full-fat soybean meal could have been due to one or more of a variety of different antinutrients present within the soybean meal tested and not only due to the presence of PIs (Van den Ingh et al., 1991).

With the exception of the more heat-resistant Bowman-Birk inhibitors, most PIs are readily destroyed by heat; the degree of destruction or inactivation depending upon the temperature, duration of heating, particle size and moisture conditions (Bates, 1994; Beckmann & Pfeffer, 1989; Jansman & Poel, 1993; Lim & Akiyama, 1991; Poel, 1989; Viola et al. 1983; Vohra & Kratzer, 1991). In general the destruction or inactivation of PIs, together with any other heat-labile antinutrients (ie. lectins, goitrogens, anti-vitamins, phytates) is accompanied by a marked improvement in the nutritive value of the protein source (Akiyama, 1988; Amerio et al. 1989; Lim & Akiyama, 1991; Rumsey et al. 1993; Shimeno et al. 1992; Shu, 1992; Viola et al. 1983; Wee & Shu, 1989; Wilson & Poe, 1985). The optimum level of destruction of PI activity has been reported to be between 80-90% (equivalent to a dietary Trypsin Inhibitor (TI) activity of 1-5mg/g diet or a cresol red value of 3.8-4.3); excessive heat treatment reducing the availability of heat-sensitive amino acids, and in particular that of lysine (Akiyama, 1988; Lim & Akiyama, 1991; NRC, 1993; Viola et al. 1983). For example, the studies of Rumsey (1991) with rainbow trout *O. mykiss* fed diets with TI activities ranging from 2.6 to 51mg TI/g reported that TI levels below 5 had little effect on the growth and feed efficiency of trout. Similarly, Sessa & Lim (1992) reported no growth depressing effect of diets containing processed soybean meal with trypsin inhibitor levels ranging from 0.77 mg to 6.14 mg TI/g diet in laboratory reared *P. vannamei* over a 10-week period; in fact shrimp fed the ration containing the lightly toasted soybean flour (which had the highest TI content of 6.14 mg/g diet) displayed the highest final weight gain and best feed efficiency. Moreover, Wilson & Poe (1985) reported that channel catfish *I. punctatus* fed a 35% crude protein diet tolerated soybean meal with a much higher TI activity (ie. TI 8.9mg/g diet) than fish fed a 25% crude protein diet (ie. TI 3.6 mg/g diet). On the basis of these and other studies it would appear therefore that the

nutritive value of these oilseed proteins is not just dependent upon the destruction or removal of PI activity but also upon the removal or inactivation of other antinutrients present within these plant proteins (Table, 2; NRC, 1993; Olli et al. 1989; Rumsey et al. 1993). Despite this, TI activity has been demonstrated within most commercially available fish feeds (Dawson & Houlihan, 1993). Clearly, considerable further research is required concerning the overall role played by dietary PIs in fish and crustacean nutrition.

Phytohaemagglutinins

Phytohaemagglutinins or lectins are glycoproteins widely distributed in legumes and some certain oilseeds (including soybeans; Table 2) which possess an affinity for specific sugar molecules and are characterised by their ability to combine with carbohydrate membrane receptors; lectins *in vitro* causing the agglutination of vertebrate red blood cells (for general review see Freed, 1991; Huisman & Tolman, 1992; Liener, 1989; Lord & Robertus, 1994; Pusztai, 1989). Although lectins have been found to occur naturally in the blood serum of some fish species (Dash et al. 1993) there is no published information concerning the dietary effect of purified lectins from legumes or oilseeds on fish and shrimp. By contrast, studies with humans and laboratory animals have reported toxicity symptoms ranging from reduced growth, alterations in gut microflora, to death (for review see Liener, 1980, 1989; Huisman & Tolman, 1992). Recent studies with rats fed purified lectins isolated from the red kidney bean *Phaseolus vulgaris* (lectins constituting 10% of the total seed protein; Confalonieri et al. 1992) have shown lectin directly binding to the intestinal mucosa (Almeida et al. 1991; Santiago et al. 1993), interacting with the enterocytes and interfering with the absorption and transportation of nutrients (ie. carbohydrates) during digestion (Santiago et al. 1993) and causing epithelial lesions within the intestine (Oliveira et al. 1989). Interestingly, similar intestinal lesions or abnormalities were reported within the gut mucosa of Atlantic salmon *S. salar* fed a diet containing 30% full-fat soybean meal (Van den Ingh et al., 1991).

Although lectins are usually reported as being heat-labile, their stability varies between plant species; many lectins being resistant to inactivation by dry heat and requiring the presence of moisture for more complete destruction (Almeida et al. 1991; Ayyagari et al. 1989; Coffey et al. 1993; Poel et al. 1990). Moreover, although it has also been reported that lectins are readily inactivated by proteolytic enzymes (*in vitro*) during digestion (Coffey et al. 1992; Lim & Akiyama, 1991; NRC, 1993) the fact that raw legumes or partially processed meals may still confer residual lectin activity and therefore toxicity suggests that these antinutrients warrant careful attention and toxicological study within farmed fish and shrimp.

Glucosinolates

Glucosinolates or thioglucosides are glycosides containing J-D-thioglucose and occur naturally in cruciferous plants, and in particular to the genus *Brassica*, including the oilseeds rapeseed (*B. napus, B. campestris*), mustard seed (*B. nigra*) and crambe (*Crambe abyssinica*). Although glucosinolates are not harmful *per se*, on enzymatic hydrolysis (ie. due to the release of the associated enzyme

myrosinase when the raw material is crushed) they yield toxic products which are goitrogenic (ie. antithyroid agents) and growth inhibitory, including goitrin (5-vinyloxazolidine-2-thione), isothiocyanates, thiocyanate ions, and nitriles. Goitrin is the most potent of the antithyroid agents and acts by inhibiting the thyroid gland to bind iodine; a process which cannot be reversed by dietary supplementation within iodine (for review see Duncan, 1991; Duncan & Milne, 1989; Huisman & Tolman, 1992; Liener, 1980, 1989).

Feeding studies concerning the toxicity of purified glucosinolates on fish and shrimp have only been reported for one species, namely the common carp (*C. carpio*). Fish were fed purified allyl isothiocyanate (ie. the major glucosinolate present in mustard seed) and displayed marked thyroid hyperplasia and reduced growth at glucosinolate levels above 0.4 mg/g diet (Hossain & Jauncey, 1988). Similar toxicity signs have also been reported in fish fed semi-purified and practical diets containing high dietary levels of rapeseed meal and mustard seed meal; the severity of the toxicity depending upon the overall level of glucosinolate in the diet fed (Abdou Dade et al. 1990; Davies et al. 1990; Gomez et al. 1993; Hasan et al. 1991; Higgs et al. 1989; Hossain & Jauncey, 1989; Leatherland & Hilton, 1988; Mays & Brown, 1993; NRC, 1993; Shimeno et al. 1993; Teskeredzic et al. 1992). However, although it would appear that individual fish species may vary in their sensitivity and toxicological response to glucosinolates (ie. as in the case of TIs, carnivorous fish species generally being more to susceptible to glucosinolate toxicity than omnivorous/herbivorous fish species), analysis of data is complicated by the presence of other antinutrients within the meals tested (see Table 2). Moreover, although the reported glucosinolate content of traditional rapeseed meals may vary from 3 to 8%, selective breeding programmes have yielded rapeseed varieties (ie. canola) with glucosinolate levels below 0.2 mg/g (Higgs et al. 1989; NRC, 1993; Pelletier et al. 1991). Until further information is available it is therefore recommendable that total dietary glucosinolate levels be kept below 0.2 and 0.4 mg/g diet for salmonids and warmwater fish species, respectively.

Although the enzyme myrosinase is readily destroyed by heat, the glucosinolates are more heat resistant and are not always completely destroyed (unless the meal is extruded; Gomez et al. 1993) and so there is a risk that they may be hydrolyzed within the gastrointestinal tract by the gut microflora (Liener, 1989; NRC, 1993). However, glucosinolates are reported to be readily removed by extraction with hot water, dilute alkali, or organic solvent mixtures (Liener, 1989; Shahidi & Gabon, 1990). Clearly, considerable further research is required concerning the long-term toxicity of dietary glucosinolates (and their purified derivatives) in feeds for the major cultivated fish and shrimp species. For example, at present no published information exists concerning the toxicological effect of glucosinolates in shrimp.

Gossypol

Gossypol is a naturally occurring polyphenolic compound ($C_{30}H_{30}0_8$) present in the pigment glands of cottonseed (*Gossypium spp)*; the average gossypol content varying from 0.4-2.4% within glanded cottonseeds to less than 0.01% free gossypol within some low-gossypol cottonseed meals (ie. new glandless varieties; Castaldo, 1995; Liener, 1980; NRC, 1993; Robinson & Brent, 1989).

Feeding studies using purified derivatives (ie. gossypol acetate) have reported a negative effect of free gossypol on fish growth and feed efficiency at dietary gossypol levels of 0.1% in rainbow trout *O. mykiss* (Roehm et al. 1967; dietary levels tested 0.025%, 0.1% & 0.2%) and 0.14% in channel catfish *I. punctatus* (Dorsa et al. 1982; dietary levels tested 0.03%, 0.09% and 0.14%). However, the dietary studies of Herman (1970) with rainbow trout using either purified gossypol acetate (0.033-0.1% diet) or cottonseed meal (0-0.0531% free gossypol in diet) reported liver and kidney abnormalities (including thickening of glomerular basement membrane, liver necrosis, and ceroid deposition in the liver, spleen and kidney) in rainbow trout fed diets containing 0.0095% or more free gossypol, reduced growth in fish fed 0.0290% or more free gossypol, and a severe reduction in haematocrit, haemoglobin, and plasma protein levels, in addition to reduced growth in fish fed diets containing 0.0531% or more free gossypol. By contrast, the feeding studies of Robinson et al (1984) with tilapia *Tilapia aurea* using either purified gossypol acetate (0.1-0.2% diet) or cottonseed meal (0.005-0.012% free gossypol in diet) found that whereas fish tolerated high levels (0.18%) of free gossypol in purified form with no loss in growth, feed efficiency or survival, fish fed rations containing cottonseed meal with lower levels of free gossypol display reduced growth and feed efficiency compared with fish fed soybean and/or groundnut based diets. Similar results have also been reported by Ofojekwu & Ejike (1984) and more recently by El-Sayed (1990) with tilapia *Oreochromis niloticus* fed cottonseed meal based diets; poor growth and feed efficiency being observed with fed cottonseed meal based diets compared with fish fed control diets.

It follows from the above that interpretation of the data reported from feeding trials using cottonseed meals (as opposed to purified gossypol derivatives) will be complicated by the presence of other antinutrients within cottonseed meal tested (ie. PIs, cyclopropenoic fatty acids, phytic acid, anti-vitamin E factors; Table 2) or other nutritional imbalances. For example, reduced lysine availability has been reported with cottonseed protein due to the ability of gossypol to bind with the reactive epsilon amino group of lysine during heat/feed processing (Küiken, 1952; Robinson, 1991; Wilson et al. 1981). In fact one of the aims of cottonseed processing is to bind the free gossypol pigments to the protein within in the processed meal; current pelleting and cottonseed processing methods reducing the level of free-gossypol by 50-99% (Barraza et al. 1991; Castaldo, 1995; Hendricks & Bailey, 1989; Liener, 1980; McCurdy & March, 1992). Although the phenomenon of binding allows for the detoxification of the gossypol pigments, the disadvantage is that it also results in a lowering of the biological value of the protein component of the meal. However, this effect will be minimised with the production and use of low-gossypol glandless varieties of cottonseed.

In the absence of information on the precise mechanism of gossypol toxicity in fish (for a review of the mode of action and toxicity of gossypol in terrestrial farm animals see Randel et al. 1992, and Segal & Ueno, 1989), and the possibility of gossypol accumulation within the edible tissues of fish fed high-gossypol containing cottonseed meals (Dorsa et al. 1982; Robinson & Brent, 1989; Robinson, 1991), it is therefore recommendable that levels of free-gossypol be kept to below 0.01% within the finished diet.

Tannins

Tannins, like gossypol, are a diverse group of polyphenolic compounds but in contrast to gossypol on hydrolysis they yield sugar residues, phenolcarboxylic acids, and condensed tannins (for review see Liener, 1980; Mueller-Harvey & McAllan, 1992). Widely distributed in nature, food crops and legumes which have been reported to contain significant quantities of tannins include sorghum (containing up to 5% condensed tannins), faba bean, lima bean, sunflower seed meal (containing 1.2-2.7% chlorogenic acid), and rapeseed (Table 2). Although no information is available concerning their toxicity within feeds for fish or shrimp, in higher animals tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity (Helsper et al. 1993 reported that condensed tannins were responsible for the testa-bound TI activity in faba beans) and/or by binding to digestive enzymes or by binding directly with the dietary protein (Elkin & Rogler, 1990; Hagerman et al. 1992). Tannins are also have the ability to complex with vitamin B_{12} (Liener, 1980). Although the toxic effects of chlorogenic acid can be counteracted by dietary supplementation with methyl donors such as choline and methionine, chlorogenic acid is reported to be readily removed from sunflower seeds using aqueous extraction methods (Dominguez et al. 1993); see also Poel et al. (1991) for the effect of different processing methods on the tannin content of faba beans.

Phytic acid

Phytic acid or the hexaphosphate of myo-inositol occurs naturally throughout the plant kingdom, and is present in considerable quantities within many of the major legumes and oilseeds, including soybean, rapeseed and cottonseed (Table 2); 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds being in form of organically bound phytin phosphorus, respectively (Matyka et al. 1993). Apart from the major part of the phosphorus contained within phytic acid being largely unavailable to animals (due to the absence of the enzyme phytase within the digestive tract of monogastric animals), phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability (Davies & Gatlin, 1991; Hossain & Jauncey, 1993; Erdman, 1979; Ketola, 1985; NRC, 1993; Spinelli et al. 1983). For example, phytic acid is reported to chelate metal ions such as calcium, magnesium, zinc, copper, iron, and molybdenum to form insoluble complexes that are not readily absorbed from the gastrointestinal tract (Hendricks & Bailey, 1989; Liener, 1980).

It is perhaps not surprising therefore that feeding studies with diets containing high levels of purified phytic acid have reported a negative effect on fish and shrimp, including rainbow trout *O. mykiss* (0.5% phytic acid - reduced growth and feed efficiency: Spinelli et al. 1983), tilapia *O. aureus* (1.5% phytic acid - reduced zinc bioavailability: McClain & Gatlin, 1988), common carp *C. carpio* (0.5% phytic acid - reduced growth, feed efficiency, protein efficiency ratio and protein digestibility, reduced calcium and zinc bioavailability, hypertrophy and vacuolization of intestinal epithelium: Hossain & Jauncey, 1993), channel catfish *I. punctatus* (1.5% phytic acid - reduced zinc bioavailability: Gatlin & Phillips, 1989; 2.2% phytic acid - reduced growth and feed efficiency, reduced zinc bioavailability: Satoh et al. 1989),

chinook salmon *O. tshawytscha* (2.58% phytic acid - reduced growth, feed efficiency, protein efficiency ratio and thyroid function, increased mortality, promotion of cataract formation at low dietary zinc levels, abnormal pyloric caeca structure: Richardson et al. 1985), white shrimp *P. vannamei* (1.5% phytic acid - reduced phosphorus and zinc bioavailability: Davis et al. 1993; 1.5% phytic acid phosphorus - reduced growth and feed efficiency, reduced phosphorus, calcium and zinc bioavailability, reduced muscle ash content and increased calcium ash content: Civera & Guillaume, 1989), and tiger shrimp *P. japonicus* (1.5-2.0% phytic acid phosphorus - reduced muscle ash content and increased calcium ash content: Civera & Guillaume, 1989).

Studies using oilseed meals containing `natural' phytic acid have also reported reduced mineral bioavailability; Gatlin & Wilson (1984) reporting that the zinc requirement of channel catfish *I. punctatus* fed a diet containing 50% soybean meal was about five times the normal requirement for growth. However, the negative effects of phytic acid on protein and mineral bioavailability can be reduced by the removal or extraction of phytic acid from the pulses or oilseed meals by the use of appropriate feed processing techniques (Beleia et al. 1993; Han, 1988; Prendergast et al. 1994). For example, Hossain & Jauncey (1990) reported the loss of 48-72% and 51-74% of the original phytic acid content of linseed and sesame seed meal by aqueous extraction (18h) and autoclaving (120°C/2h), respectively. Another, and possibly more promising technique, has been the recent use of dietary phytase supplements within farm animal feeds as a means of breaking down the phytate protein/mineral complexes and thereby increasing protein and mineral bioavailability for digestion (Gibson & Ullah, 1990; Lei et al. 1993; Naesi, 1990).

Interestingly, Cheng & Guillaume (1984) reported a beneficial effect of purified phytic acid on growth and exoskeleton development in shrimp *P. japonicus*. Similarly, although McClain & Gatlin (1988) reported reduced zinc bioavailability in tilapia *O. aureus* fed 1.5% phytate, fish growth was improved compared with control fish fed no phytate. Moreover, recent studies with mammals have suggested that phytic acid may have a beneficial effect within the animal body by suppressing the incidence of colonic cancer by acting as a food antioxidant and forming iron-chelates that inhibit iron-catalyzed hydroxyl radical formation and lipid peroxidation (Empson et al. 1991; Graf & Eatin, 1993).

Saponins

Saponins are a heterogenous group of naturally occurring foam-producing triterpene or steroidal glycosides that occur in a wide range of plants, including pulses and oilseeds such as kidney bean, lentil, pea, chickpea, alfalfa, soybean, groundnut, lupin and sunflower (Table 2: Liener, 1980; Jenkins & Atwal, 1994; Price et al. 1987). Although no information exists concerning their toxicity within feeds for fish or shrimp, studies with chicks have reported reduced growth and feed efficiency, and interference with the absorption of dietary lipids, cholesterol, bile acids, and vitamins A and E (Jenkins & Atwal, 1994).

Moreover, because of their ability to lower surface tensions and haemolyze red blood cells saponins are widely used within aquaculture as a `pond cleaner' to

eradicate unwanted fish from ponds prior to stocking or during cultivation; teaseed cake, a commonly used `fish eradicator' containing 5.2-7.2% saponin (for review see Cruz-Lacierda, 1993; Liang et al. 1992; Minsalan & Chiu, 1986). Although fish have been found to be much more susceptible to saponin poisoning than shrimp, recent studies of Cruz-Lacierda (1993) reported that a concentration of 100 mg/l of saponin resulted in a 66% incidence of shell softening in tiger shrimp *Penaeus monodon*.

In view of the frequent use of saponins by aquaculturists for the removal of unwanted fish from ponds and the widespread presence of saponins within feedstuffs used within aquafeeds clearly long-term feeding trials are required so as to ascertain the toxicity of these compounds for different fish and shrimp species; the latter species group being particularly important bearing in mind the potential dietary effect of saponins on cholesterol metabolism and the dietary essentiality of cholesterol for crustaceans. In general, saponins are readily removed from plant tissues by extraction with hot water or ethanol (Liener, 1980).

Alkaloids

Alkaloids represent a large and structurally diverse group of compounds present within angiosperm plants; the majority of alkaloids usually containing nitrogen in the form of a heterocyclic ring (Hendricks & Bailey, 1989). Some of the more well known alkaloids include coniine (hemlock), nicotine (tobacco), atropine (deadly nightshade), cocaine (leaves of coca plant), quinine (cinchona bark), strychnine (seeds of *Nux vomica*), morphine (dried latex of opium poppy) and solanine (unripe potatoes and potato sprouts). However, the only seed legume likely to be used within aquafeeds which contains alkaloids, in the form of quinolizidine alkaloids, is the lupin *Lupinus spp* (Table 2; Cheeke & Kelly, 1989).

Although no information is available concerning the toxicity of quinolizidine alkaloids in fish or shrimp, reported toxicity signs in higher animals have included reduced feed palatability and growth, impaired function of the central nervous system, and even death (Cheeke & Kelly, 1989; Kingsbury, 1964; Pastuszewska et al. 1988). However, Hendricks et al (1981) have investigated the effect of dietary pyrrolizidine alkaloids (extracted from the tansy ragwort *Senecio jacobaea*) in rainbow trout *O. mykiss*; fish fed 100 mg/kg diet of pyrrolizidine alkaloids displaying markedly reduced growth and mortality from the fourth month onwards. Fish fed lower dietary concentrations (20 mg/kg diet) displayed marked liver abnormalities, including liver megalocytosis, necrosis, fibrous tissue scarring, and venoocclusion of hepatic veins. At present the only practical way of reducing the toxic effect of alkaloids is either by removing them by aqueous extraction (ie. solanine extraction from potatoes by hot water) or by the genetic selection and use of low-alkaloid varieties (Farrell, 1992).

Cyanogens

Cyanogens or cyanogenetic J-glycosides occur in several important food plants and legumes, including cassava, chickpea, kidney bean, lima bean, hyacinth bean, field pea, pigeon pea, jack bean, and the oilseed linseed (Table 2; Liener, 1980).

Although cyanogens are not toxic *per se*, on hydrolysis they liberate the toxin hydrogen cyanide (HCN); hydrolysis usually being facilitated after physical damage/crushing and soaking with water through the action of associated extracellular J-glucosidase enzymes. For example, linseed, lima beans and cassava all contain the cyanogen linamarin, which on hydrolysis with the associated enzyme linamarase yield glucose, acetone and HCN. However, although cyanogens are generally heat stabile and only sparingly soluble in water, the associated enzymes are readily destroyed by heat and cooking. Although no experiments have been reported with fish or shrimp fed purified cyanogens, feeding studies in fish using linseed meal (expeller cake) as a dietary protein source have generally reported reduced growth and feed efficiency; Indian major carp *L. rohita* (Hasan et al. 1991) and common carp *C. carpio* (Hossain & Jauncey, 1989). Similar results have also been found with tilapia *O. niloticus* fed cassava leaf meal (Ng & Wee, 1989); the nutritive value of cassava leaf meal for fish being markedly improved by soaking the leaves for 24h prior to drying to remove HCN (Borlongan & Coloso, 1994).

Lathyrogens

Lathyrogens are toxic compounds found in certain *Lathyrus* plant species, including the flat-podded vetch (*L. cicera*), spanish vetch *(L. clymenum*), and the grass pea (*L. sativus*; Table 2). Lathyrogens include J-amino propionitrile and the neurotoxic amino acid J-N-oxalyl-L-I,J-diaminopropionic acid. Consumption of lathyrogens in humans causes a disease called lathyrism; the toxicity symptoms including skeletal lesions, retarded sexual development and paralysis. However, the toxins are reported to be readily removed by cooking the pulse in water and draining off the excess water, by soaking the pulse overnight in cold water, by steeping the dehusked seeds in hot water, or by dry roasting at elevated temperatures (for review see Liener, 1980).

Cyclopropene fatty acids

Cyclopropene fatty acids (CPFA) are toxic fatty acids found in cottonseed and baobab seed (*Adansonia digitata*), and include malvalic acid and sterculic acid. Reported toxicity signs in rainbow trout *O. mykiss* fed cyclopropene fatty acids (CPFA) include the inhibition of fatty acid desaturase systems and consequent altered lipid metabolism, and histological abnormalities, including hepatocycte necrosis, unusual liver glycogen deposition, appearance of `fibres'in the hepatocyte cytoplasm, proliferation of bile ducts, and fibrosis (Hendricks & Bailey, 1989). More recently, Chikwem (1987) has suggested that CPFA may impair amino acid uptake in rainbow trout; fish fed 300 mg/kg CPFA displaying impaired lysine uptake compared with fish fed the control or a 50 mg/kg CPFA diet. In addition, there is considerable evidence to suggest that CPFA are carcinogenic, inducing hepatomas in the presence or absence of aflatoxins (Hendricks & Bailey, 1989; Hendricks et al. 1980; Roehm et al. 1970; Scarpelli et al. 1974; Sinnhuber et al. 1976; Taylor et al. 1973). However, although CPFA are generally removed from oilseeds by lipid extraction, residual levels within the extracted meal (ca. 0.01%) may persist and be cause for concern. Moreover, since specific histological effects have been reported in rainbow trout fed as little as 10 mg/kg sterculic acid and CPFA can accumulate

within tissue lipids, it is not generally recommended to use cottonseed meals within starter or broodstock feeds for rainbow trout (Hendricks & Bailey, 1989). Clearly, in view of the recent renewed interest in the possible use of full-fat cottonseed meals within aquafeeds considerable attention should be given to the CPFA content of the meals and their possible toxicity to the cultured fish or shrimp.

Erucic acid

Erucic acid or *cis*-13-docosenoic acid (20:1n-9) is a fatty acid present in rapeseed oil, at levels ranging from less than 1% (low-erucic rapeseed varieties) to as high as 55% of the oil (high-erucic varieties; Hendricks & Bailey, 1989). Although erucic acid has been reported to be cardiotoxic to rats (causing lipid accumulation and focal necrosis of the muscle fibres; Kramer et. al. 1992; Slinger, 1977), unpublished studies by Parker and Hendricks (as cited by Hendricks & Bailey, 1989) with coho salmon (*O. kisutch*) fed high-erucic acid (50%) rapeseed oil (6% for 6 months and 12% for 4 months) have reported reduced growth, increased mortalities, and histopathology of the skin, gills, kidney and heart, including fat accumulation in the kidney and epicardial connective tissue. However, recent trials with Atlantic salmon (*S. salar*) fed low and high-erucic acid rapeseed oil supplemented diets (2.6% to 13.%) reported no deteriorative changes in growth of fish over a 18-week trial period compared with fish fed capelin oil supplemented diets, although elevated erucic acid levels were observed in the heart and muscle lipids (Thomassen & Røsjø, 1989). Although no erucic acid-related pathologies have been reported in fish or shrimp fed diets containing rapeseed meal to date (NRC, 1993), the possible effects of long-term accumulation of erucic acid within the tissues of fish or shrimp deserves further attention.

Allergens

Allergens are heat-stable proteins with the ability to stimulate antigenic challenge or immunological activity on ingestion and consequently causing gastrointestinal hypersensitivity within the animal (Liener, 1980; Porter & Barratt, 1993; Rumsey et al. 1993). For example, soybean is reported to contain specific allergens (globular proteins) which have been found to orally sensitize pre-ruminant calves, baby pigs, guinea pigs and rabbits; the sensitivity being manifested in gastrointestinal hypersensitivity reactions and digestive disturbances, including mucosal inflammation and luminal osmolality (Pedersen, 1989). Although no feeding trials have been reported concerning the sensitivity of fish or shrimp to soybean antigens, interestingly Rumsey et al. (1993) found that the best growth response and feed efficiency was observed with rainbow trout (*O. mykiss*) fed processed soybean meals containing the lowest antigenic activity. Clearly, considerable further work is required concerning the sensitivity of individual fish and shrimp species to different potential food allergens.

Phytoestrogens

Phytosterols are compounds exhibiting estrogenic activity and have been found in a wide variety of food plants and legumes, including wheat, rice, chick-pea, alfalfa, lupin, groundnut, linseed and soybean (Table 2; Gagnon et al. 1992; Liener, 1980). For example, compounds exhibiting estrogenic activity in soybean have been identified as isoflavones, including genistein, daidzein, and coumestrol, of which genistein (4',5,7-trihydroxyisoflavone) is the most prominent (Pelissero et al. 1991; Wang et al. 1990). Apart from it's estrogenic activity, when genistein was fed to male rats at a level of 0.5% it also inhibited growth and produced elevated zinc in the liver and bones and increased the deposition of calcium, phosphorus and manganese in the bones (Magee, 1963). Recent studies with the sturgeon *Acipenser baeri* have also demonstrated the estrogenic activity of phytosterols (ie. genistein, daidzein, biochanin A, equol, coumestrol) when injected by the intraperiotoneal route; coumestrol being the most potent with fish displaying induced vitellogenin secretion (Pelissero et al. 1991a; Pelissero & Sumpter, 1992). However, although genistein is reported to be heat stabile, and no dietary studies have been reported to date with fish or shrimp, these compounds are not thought to pose a serious threat to fish or shrimp health; the level of these compounds with processed soybean meal being quite low (0.1-0.25%; Hendricks & Bailey, 1989; Liener, 1980). Interestingly, genistein has been found to act as a powerful antioxidant by inhibiting tumour promoter-induced hydrogen peroxide formation *in vitro* and *in vivo* and as such holds particular promise as a candidate for the prevention of human cancers (Herrera et al. 1992).

Miscellaneous antinutrients

Toxic amino acids: a variety of naturally occurring toxic amino acids have been identified with plant feedstuffs, including:

Mimosine [J-N-(3-hydroxy-4-pyridone)-I-amino propionic acid]: found in the leaves of ipil-ipil (*Leucaena leucocephala*), and acts as an inhibitor of pyridoxal-containing transaminases, tyrosine decarboxylase, several metal-containing enzymes, and both cystathionine synthetase and cystathionase (Liener, 1980). Reported toxicity signs in fish and shrimp have included reduced growth and feed efficiency, and increased mortality (tilapia *O. mossambicus/niloticus* - Jackson et al. 1982; Santiago et al. 1988; Wee & Wang, 1987; Indian major carp *L. rohita* - Hasan et al. 1994; shrimp *P. monodon* - Penaflorida et al. 1992; Vogt, 1990). In the case of shrimp, marked histological alterations were evident within the midgut gland-cells of post-larvae fed mimosine-containing diets, including the progressive destruction of the mid-gland epithelial cells (Vogt, 1990). Histopathological signs of mimosine toxicity were also reported by Hasan et al. (1994) within the liver tissues of Indian major carp *L. rohita* fed diets containing ipil-ipil leaf meal; liver lesions included congestion of the blood vessels and fatty changes in the hepatocytes. However, although heat stable, mimosine can be readily extracted (by removal or by endogenous enzymatic conversion to a less toxic form) from ipil-ipil leaf meal by soaking in water with a marked improvement in nutritive value (Borlongan & Coloso, 1994; Penaflorida et al. 1992; Wee & Wang, 1987a);

. Canavanine: found in the seeds of the legume sesbania *Sesbania* spp and jack bean *Canavalia* spp (Table 2), and acts as an arginine antagonist. Although no dietary studies have been reported in fish or shrimp fed purified canavanine, feeding trials with tilapia *O. mossambicus* fed diets containing *Sesbania grandiflora* and *Canavalia ensiformis* reported anorexia, erratic swimming, food rejection, lethargic behaviour, and high mortality (Martinez et al. 1988; Olvera et al. 1988); the toxicity of the seed proteins increasing with increasing dietary inclusion level, and being reduced with water extraction (canavanine being soluble in water);

. Dihydroxyphenylalanine: found in the faba/broad bean *Vicia faba* and velvet bean *Stizobolium deeringianuum* (Table 2), and believed to be responsible for the occurrence of haemolytic anaemia in humans fed faba bean; a condition known as favism (Liener, 1980);

. Selenoamino acids methylselenocysteine and selenocystathionine present in selenium accumulating plants of the genera Astragalus and Machaeranthera (Liener, 1980); and

. Indospicine, present in the creeping indigo *Indigofera endecaphylla* (Liener, 1980).

Anti-vitamin factors: a wide variety of compounds exhibiting anti-vitamin activity have been isolated from plants (Table 2), including 1) anti-vitamin A factor present in soybeans, which destroys carotene and is not readily destroyed by heat, 2) anti-vitamin D factor present in soybeans, which interferes with calcium and phosphorus absorption in chicks, and is destroyed by autoclaving, 3) anti-vitamin E factor present in kidney beans, soybeans, alfalfa and field pea, causing liver necrosis and muscular dystrophy in chicks and lambs, and is destroyed by autoclaving, 4) anti-vitamin K factor present in sweet clover, 5) anti-thiamine factor called thiaminase present in cottonseed, linseed, mung bean, and mustard seed, 6) anti-niacin factor present in sorghum, 7) anti-pyridoxine factor present in linseed, which is destroyed by water extraction and autoclaving, and 7) anti-vitamin B_{12} factor present in raw soybeans (Liener, 1980).

Anti-enzymes: several compounds exhibiting anti-enzyme activity, in addition to the PIs, have been isolated from plants (Table 2), including 1) the cholinesterase inhibitor solanine present in green potatoes, which is heat stable and water soluble, 2) amylase inhibitors present in wheat, oats and rye, and 3) an arginase inhibitor present in sunflower seeds, which is a derivative of chlorogenic acid (Liener, 1980).

CONCLUDING REMARKS

On the basis of the above review it is clear that considerable further research is required concerning the toxicity and metabolic fate of the major plant antinutrients for farmed fish and shrimp. In particular, information is urgently required concerning the toxicity of graded levels of the `purified forms' of the individual antinutrients for the major cultivated fish and shrimp species; the interpretation of research results from feeding trials using diets containing more than one antinutrient being

speculative rather than definitive. For example, although the analysis of growth and histological data from a feeding experiment using full-fat soybean meal may of practical value (from a feed compounder's or farmer's viewpoint) it is difficult to ascertain with any certainty which of the twelve antinutrients known to be present within soybean (Table 2) is the most limiting factor or major antinutrient for the species concerned. Moreover, for research results to be applicable to practical farming conditions it is essential that experimental diets be of sufficient quality to elicit a maximum growth response over the different growth phases of the cultured species (ie. fry, fingerling, grow-out, brood) and that tissue histological samples be taken on a routine basis over the course of the feeding trials; histological abnormalities generally being evident before overt toxicity signs (ie. reduced growth and feed efficiency, mortality) and also indicating possible sub-clinical effects. In addition, further information is required concerning the destruction and availability of the major antinutrients within feed ingredients and finished feeds using different feed processing techniques, including mechanical extraction, solvent extraction, aqueous extraction, heat processing, enzymatic processing, and feed manufacturing techniques.

Despite the presence of antinutrients within raw pulses and oilseeds the prospects for the increased use of `processed' oilseed meals and pulses as `fishmeal' replacers within aquafeeds is very encouraging (Hughes & Handwerker, 1993; Rumsey, 1994; Tacon, 1993, 1993a, 1994). This is particularly so for those omnivorous/herbivorous farmed species feeding low on the aquatic food chain within semi-intensive and intensive farming systems; these species generally being more tolerant of plant antinutrients than their carnivorous counterparts and better digestively equipped to cope with the carbohydrates present within plant feedstuffs. However, even these carnivorous species will succumb to the aquafeed *`green revolution'* with the use of genetically improved low-antinutrient varieties (Bond & Smith, 1989; Helsper et al. 1993; Prendergast, 1994), and the use of improved feed processing techniques (Castaldo, 1995; McCurdy & March, 1992; Poel, 1989; Prendergast, 1994; Rumsey et al. 1993; Watanabe & Pongmaneerat, 1993) and feed formulation techniques, including the use of `protected' free amino acids (Murai, 1992; Swick, 1994) and exogenous dietary enzyme `cocktails' (Carter et al. 1994; Coelho, 1994; Farrell, 1992). However, although plant protein sources may be a cheaper and more sustainable source of dietary protein for use by the aquaculture industry, their success or not will rest upon the shoulder and skills of the feed ingredient processor, the aquafeed formulator, the aquafeed manufacturer, and last but not least, the farmer!.

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