Grain legumes produce high-energy, medium-protein seeds that can be used as feed ingredients. High levels of antinutrient factors present in the raw seeds diminish the nutritive value of the grain legumes. Shrimp fed with raw legumes were found to have severe tissue anomalies in the gut and hepatopancreas. The problem could be overcome by using a combination of processing methods to remove part or all of the antinutrient factors.

# Processed grain legumes:
## potential plant ingredients in shrimp feeds

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Fishmeal replacement has spurred exploration for less expensive and more sustainable plant protein sources for a number of years now. Much research has been done on solvent-extracted oilseed meals as protein sources in aquaculture feeds. In omnivorous fish species, a number of oilseed meals have been found suitable as feed ingredients. But, in shrimp and carnivorous fish species, soybean meal is by far the only major oilseed meal that is used in significant quantities. Other plant protein sources that have been found acceptable for use in shrimp feeds include grain legumes such as feed peas, and lupins.

**Grain legumes**

Grain legumes are plants within the Fabaceae family that produce high-energy, medium-protein seeds (Table 1). These include peas such as feed peas, chickpeas and cowpeas; lentils and grams such as black grams and horse grams; and beans such as mung beans and faba beans. On an average, they contain 20-40% protein. Generally, they are high in lysine (approximately 7% of protein). Unlike oilseeds that also belong to the legume family, grain legumes have lower levels of fat (1-2%). They are comparable to oil-extracted oilseed meals in terms of fat content. Legumes are rich in starches and significant sources of some vitamins and trace minerals.

The nutritive value of grain legumes is much less than that is calculated from the chemical composition because of the presence of various antinutritional substances. Antinutritional factors (ANFs) have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals. Antinutrients in feed ingredients may be classified according to their ability to withstand thermal processing, the most commonly employed treatment for destroying them, as heat labile and heat stable ANFs. Most grain legumes are generally associated with three common ANFs such as trypsin inhibitors (TI), phytates, and tannins. Whereas the first two ANFs are destroyable by heat, the effect of thermal treatment on substances such as tannins remains uncertain. Most well known among these is the trypsin inhibitor occurring in almost all raw legume seeds. The mode of action of above mentioned ANFs has not been studied in shrimp. However, recent studies showed severe histological abnormalities in hepatopancreas and midgut of shrimp fed raw grain legumes (see box). Nutritive value of grain legumes can be improved by different processing methods.

*Table 1: Chemical composition of some leguminous seeds (% as fed)*

| Ingredients | Crude Protein | Crude Fat | Crude Fiber | Ash | Calcium | Phos-phorus | Lysine | Meth-ionine |
|---|---|---|---|---|---|---|---|---|
| Bean, Mung (*Phaseolus aureus*) | 23.9 | 1.3 | 3.9 | 3.8 | 0.13 | 0.35 | 1.10 | 0.26 |
| Chickpea (*Cicer arietinum*) | 19.1 | 4.1 | 7.0 | 2.9 | 0.15 | 0.33 | 1.25 | 0.24 |
| Cowpea (*Vigna sinensis*) | 21.7 | 1.3 | 10.8 | 4.7 | 0.06 | 0.01 | 2.10 | 0.20 |
| Lentil (*Lens culinaris*) | 24.4 | 1.0 | 3.4 | 2.6 | 0.08 | 0.38 | - | - |
| Field Pea (*Pisum sativum*) | 23.2 | 1.3 | 5.9 | 2.9 | 0.16 | 0.38 | 1.44 | 0.23 |
| Lupin (*Lupinus luteus*) | 54.7 | 8.7 | 14.0 | 4.4 | 0.23 | 0.39 | 2.30 | 0.40 |
| Soybean meal (44% protein) | 44.4 | 1.5 | 6.2 | 6.4 | 0.35 | 0.64 | 2.85 | 0.59 |

## Processing of grain legumes

A number of processing techniques are used to remove antinutrients from grain legumes. These include dehulling, soaking (in water, alkali and acid), autoclaving, radiation, germination, fermentation, and recently extrusion cooking. The influence of above processing methods on the nutritional and antinutritional factors of legume seeds is specific to the nature of the seed and the variety.

*Dehulling*: A traditional processing technique which removes the outer shell or hulls of the seeds. The legume seeds could be dehulled in rubber roll sheller and segregated from the un-decorticated ones. Removal of low protein hulls covering the seeds leads to concentration of protein and results in a slight increase in protein content. Dehulling improves protein quality and digestibility, most likely due to the removal of the seed coat tannins and indigestible fiber.

*Soaking*: Simply soaking the seeds in either distilled or alkali (< 2% sodium bi-carbonate) water for 12-20 h in room temperature has shown a moderate reduction in ANFs such as trypsin inhibitors, tannin and phytic acid in grain legumes. Proximate composition is little affected by soaking.

*Autoclaving*: Autoclaving is a familiar moist-heat treatment method which cooks the seeds under pressure. It efficiently removes almost all heat labile ANFs, particularly the trypsin inhibitor. In addition, some of the heat stable ANFs have also been found to be removed partially. Sometimes prolonged heating may end with loss of nutritional quality of feed ingredient. Hence, optimum exposure duration for each legume grain should be established based on ANF content and the tolerance limit of the target animal.

*Irradiation*: Irradiation is a physical process involving an energy-input that does not induce radioactivity in foods. Irradiation causes chemical changes in foods in amounts directly related to the radiation (treatment) dose. It reduces both heat-stable and heat-labile antinutrients. In particular, irradiation levels up to 10 kGy, which are admissible for the irradiation of foods, seem to be effective in inactivating antinutrients such as protease inhibitors, lectin, phytic acid, non-starch polysaccharides and oligosaccharides without altering the nutritional quality of the food.

*Germination*: Germination is an ancient and popular practice which enhances the nutritive value of legumes by inducing the formation of enzymes that eliminate or reduce the antinutritional and indigestible factors in legumes. Germination of legume seeds increased the protein content and total essential amino acid and decreased the lipid and total carbohydrate content.

**Histological anomalies in shrimp fed unprocessed legume seeds**

Shrimp fed test diets formulated to contain 50% unprocessed grain legumes (chick peas) were found to suffer from severe mortality in the middle of a trial. Histopathological investigations revealed that the hepatopancreas and midgut of the shrimp showed some common abnormalities.*(Kumaraguru vasagam et al. In Press)*

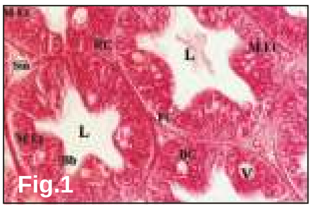
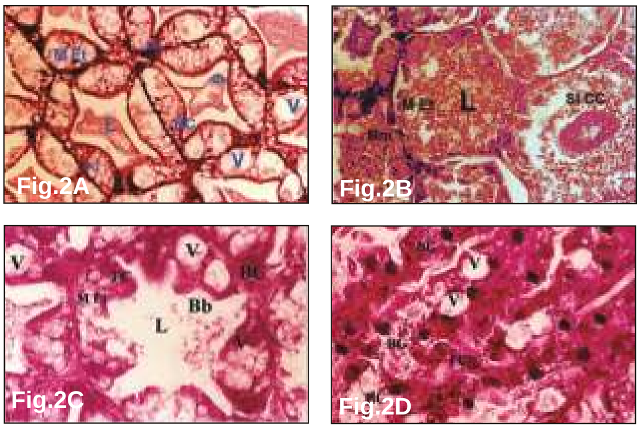



*Figure 1 shows the transverse section through hepatopancreas of shrimp fed reference diet showing normal tubules with star-shaped lumina (L). The cell types (B, F and R) are well preserved. The tubules are surrounded by a basal lamina separating the glandular cells from the haemal sinuses between the tubules. A brush border (Bb) is evident on the luminal surface of the cells. A thin myo-epithelial layer (M Et) surrounds the tubules. Between tubules haemal sinuses (Sin) are seen.*




*Figures 2 (A-D) show hepatopancreas from shrimp fed diet containing raw legume seeds showing abnormal tubules. [2A] hepatopancreas showing severe vacuolization (V) in B-cells of tubules taking a foamy appearance; [2B] hepatopancreatic tubules showing sloughing of myo-epithelial layer (M Et) and sloughed cellular contents (SL CC); [2C] hepatopancreatic tubules showing disorganized acinar structure because of the sloughing off of the cell lining and tubular lamina lost their star-like outline; [2D] Longitudinal section through hepatopancreas of shrimp fed test diet showing basophilic granules (BG) in basal lamina of glandular cells.*

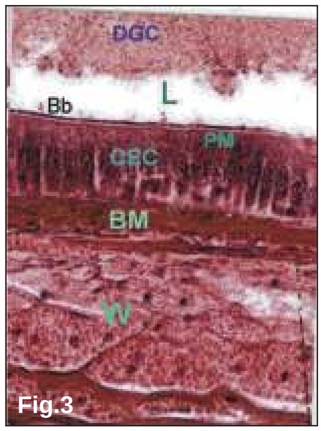
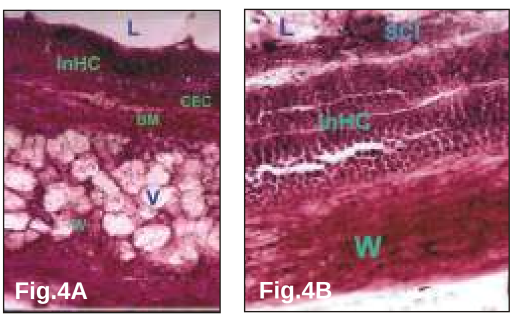



*Figure 3 shows the longitudinal sections of the midgut from shrimp fed reference diet showing midgut wall (W). Note that it is uniformly lined with tall, quite narrow columnar epithelial cells (CEC). The columnar epithelial cells rest on a basement membrane above the gut wall. Tubular lumen (L) is dispersed with digested remains of feed material. The free border of the CEC is conspicuously striated with a brush border (Bb). Goblet cells which secretes mucous are interspersed among the CEC. A thin chitinous periotrophic membrane (PM) is evident on luminal surface of the Bb.*




*Figures 4 (A&B) show that the midgut of shrimp fed diet containing raw legume seeds showing abnormalities like vacuolation and swelling in gut wall [4A]; detachment of columnar epithelial cells, necrosis of epithelial cells of mucosal lining and occurrence of inflammatory hemocytes [4B].*

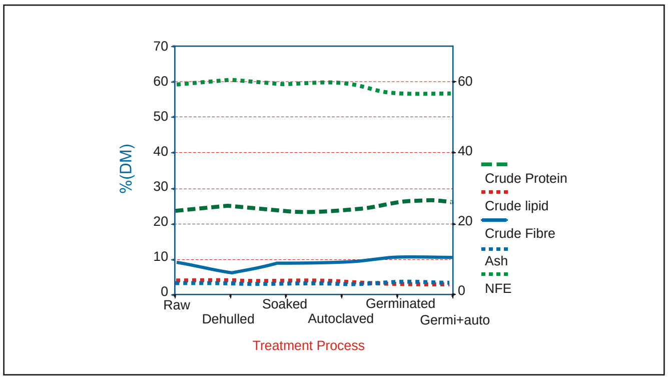



*Figure 1: Influence of different processing methods on proximate composition of chick pea*

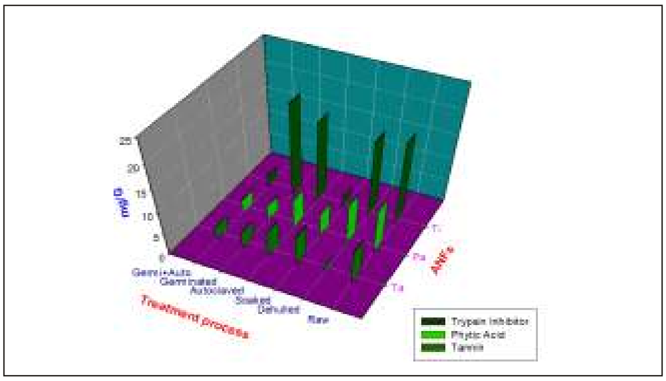



*Figure 2: Influence of different processing methods on antinutrient factors in chick pea*

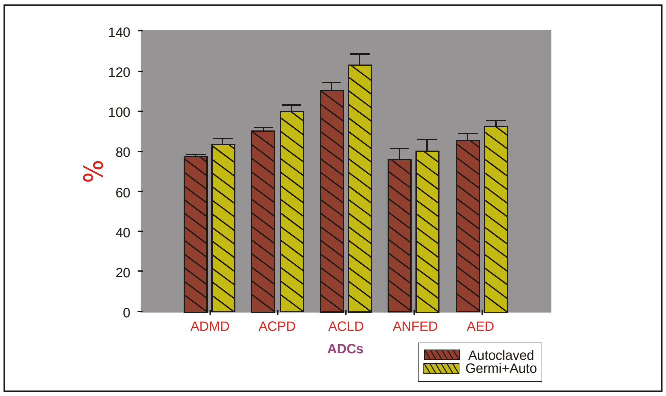



*Figure 3: Influence of processing methods on digestibility of chick pea in black tiger shrimp (ADMD=Apparent Dry Matter Digestibility; ACPD=Apparent Crude Protein Digestibility; ACLD=Apparent Crude Lipid Digestibility; ANFED=Apparent Nitrogen Free Extract Digestibility; AED=Apparent Energy Digestibility)*

Though most ANFs get reduced, trypsin inhibitor alone showed an increase on germination. However subsequent heat treatment could completely remove the above ANF.

*Fermentation*: Solid state fermentation is a proven bioprocess technology for protein enrichment and reducing the phytic acid content of legume meals. Certain selected strains of fungi and/or bacteria could significantly enrich the protein content of the substrate by utilizing the carbohydrate and fiber content. Phytase produced by the microbes destroys the phytate and increase the bioavailability of phosphates to the animal.

*Micronization*: Micronization, an infrared heat, semi-moist processing method, reduces the cooking time of seeds while maintaining their protein digestibility and nutritional quality. Though this processing found improves the digestibility of feed ingredients, its effect on ANFs is unknown.

*Extrusion cooking*: Reduces antinutritional factors and therefore improves the nutritional quality at a cost lower than other heating systems due to the more efficient use of energy and better process control with greater production capacities. It improves nutritional value through starch gelatinization, denaturation of protein, modification of lipid and inactivation of enzymes, microbes and many antinutritional factors in grain legumes.

Though there are many processing techniques showing varying degree of beneficial influence on the nutritional and antinutritional factors of grain legumes, no single method may completely remove all antinutrients and improve nutrient quality. Therefore, a combination of processing methods may be required. For example, chick pea processed by germination in combination with autoclaving resulted in a better quality feed ingredient than by either methods alone (Figures 1, 2 & 3).

## Practical recommendations

Grain legumes have potential as a practical feed ingredient in shrimp feeds provided that undesirable forms of carbohydrate and deleterious anti-nutrients are removed. Bautista-Teruel et al. (2003) has demonstrated whole feed pea meal can be used as an ingredient in the feeds for the black tiger shrimp. An inclusion level of up to 42% did not result in any adverse effect on growth, feed intake, FCR, survival, body composition, and digestibility of the shrimp. Cruz-Suarez et al. (2001) observed improvement in feed conversion and protein efficiency ratio when extrusion-cooked feed pea was included in feeds for blue shrimp. In the same study the highest feed intake and growth rate was obtained with shrimps fed micronized pea. Yet another recent study (Kumaraguru vasagam et al., 2006) with cow pea and mung bean showed that the digestibility of the legumes could be enhanced significantly by germinating the seeds and then autoclaving. Eusebio (1991) also found significant improvement in nutritive value of rice beans when the legumes were dehulled. The study found that there was no need to dehull the seeds if extrusion cooking is used. Another good scope with grain legumes is production cultivars with low ANFs through selective breeding efforts (Castell et al., 1996). These results show that grain legumes offer interesting possibilities as practical ingredients in shrimp feeds.

*References in page 26*

Dr K.P. Kumaraguru vasagam received MSc, MPhil and PhD degrees in Marine Biology from the Center of Advanced Study in Marine Biology, Annamalai University, Tamilnadu, India. His doctoral research was focused on fish meal replacement in shrimp feed with special reference to low cost vegetable sources. Recently he has joined Mega Prawns, Brunei as the Manager of its Shrimp Nutrition Program.