Vegetable protein sources for carnivorous fish: potential and challenges

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Summary

In this review we describe how carnivorous fishes respond to vegetable protein feedstuffs. The amino acid profiles of gluten and soy products are complementary with respect to amino acid profile, and hold promise for further development of processing to increase protein contents and improve nutritional qualities. Soybeans are rich in antinutritional factors that disturb the digestion and/or physiology of carnivorous fish. Among these are heat–stable factor(s) inducing enteritis. Most soy antinutritional factors are removed by the thermal treatments followed by ethanol washing used to produce protein concentrates. Gluten products contain few antinutritional factors. As with most vegetable protein feedstuffs, gluten and soy protein concentrates do, however, contain phytic acid. Phytic acid–bound phosphorus is unavailable to fish, and phytic acid also binds essential divalent mineral elements, rendering them unavailable. The reduced availability of minerals has possible deleterious consequences and phytic acid should be eliminated by enzymic hydrolysis before feeding diets with high levels of plant protein to carnivorous fishes.

Keywords: carnivore, fish, feedstuffs, vegetable protein, antinutritional factors, processing

Introduction

Low temperature and steam dried fish meals are the commonly used protein sources in feeds for carnivorous fish. They are of high nutritional value, but are high–priced, and the supply is limited. Consequently there are major efforts to define and develop cost–effective protein sources that can, at least in part, be substitutes for fish meals in least–cost feed formulations. Problems related to bovine spongiform encephalopathy (BSE) have led restrictions on the use of animal by products in fish feeds. Thus, the main focus is on vegetable protein.

Legume seeds, such as peas, lupins, and soybeans (Hughes 1991; Watanabe *et al*. 1992; Shimeno *et al*. 1993; Kaushik *et al*. 1995; Olli *et al*. 1995; Robaina *et al*. 1995; Burel *et al*. 1998, 2000a), and low–glucosinolate rapeseeds (Hardy and Sullivan 1983; Burel *et al*. 2000a) have been used with success as partial substitutes for fish meal. Feeds for economically important cultured carnivorous fishes are, however, energy dense, and typically contain more than 40% protein, and at least 15% lipid. Because these fish digest starch and/or assimilate and metabolise glucose poorly (reviewed by Wilson 1994), the starch content of the diet is kept low. This calls for vegetable protein feedstuffs with high contents of protein and low contents of starch, sugars, and indigestible non–starch polysaccharides (NSP). Few seed crops meet these criteria, as legumes and oilseeds typically contain 25 to 40% protein and more than 30% carbohydrates. The seed commodity that comes closest is hulled and defatted (hexane extracted) soybean meal, but even this product is unsatisfactorily low in protein and high in NSP and sugars (Table 1).

Thus, the most feasible vegetable protein feedstuffs are the industrially manufactured protein concentrates. Corn gluten is already extensively used, and wheat gluten is being introduced. The soy industry has industrialised several processes that concentrate protein (reviewed by Lusas and Riaz 1995), and ethanol washed soy protein concentrates produced from hulled and defatted soy are of high nutritional quality for carnivorous fishes (Storebakken *et al*. 1998a; Berge *et al*. 1999; Mambrini *et al*. 1999; Kissil *et al*. 2000). Crude and refined soy products are reviewed in detail by Storebakken *et al*. (2000b). Extraction procedures to concentrate rapeseed protein are also developed (Jones 1979) but, although the resulting rapeseed concentrate is of high nutritional value (Higgs *et al*. 1994), this process is still not economically viable. Hulling and grinding followed by an air separation process may to some extent concentrate protein from legume seeds with low oil content (Booth *et al*. 2001).

Even when concentrated, the use of plant protein in feeds for carnivorous fish introduces several challenges. Compared with fish meal, the amino acid composition of vegetable protein is unbalanced, and excessive heating during industrial drying may reduce the protein quality of vegetable feedstuffs even further. More troublesome still is the fact that plants contain various antinutritional factors that disturb the digestion and/or physiology of fish. Carnivorous fishes have short intestines with little microflora (reviewed by Buddington *et al*. 1997), and therefore are very sensitive to such factors.

Protein quality

Compared to animal protein, plant protein is generally low in the indispensable amino acids arginine, lysine and methionine. For carnivorous fishes, the reported requirements range from 3 to 6% of crude protein (CP; N x 6.25) for arginine, 4 to 5% of CP for lysine, and 2 to 4% of CP for methionine (National Research Council 1993). As shown in Table 2, these requirements are well met by fish meal, but not by soy protein nor by gluten. The amino acid profiles of corn gluten and soy proteins are, however, complementary. Being a legume, soybean contains high levels of arginine and lysine in its protein, but little methionine. Corn gluten, on the other hand, is deficient in arginine and lysine, but has high methionine content. When combined, these vegetable protein feedstuffs produce a reasonably good partial substitute for fish meal (Watanabe *et al*. 1993; Akiyama *et al*. 1995; Yamamoto *et al*. 1995).

Most commercial vegetable protein meals are residues from industrial manufacture of vegetable oil or starch. Such industrial processes usually include repeated heating to soften the seeds, remove solvents, and/or dry the meal residue. Excessive heating reduces the protein quality by amino acid cross–linking, binding to other nutrients, and/or oxidation (Finley and Phillips 1988; Davidek *et al*. 1990). This destroys the amino acids involved, and reduces the general digestibility of the protein (Ljøkjel *et al*. 2000). The most vulnerable amino acids are lysine (Maillard reaction) and cysteine (formation of disulfide bonds). When selecting industrially manufactured feedstuffs, it is important to be aware of and consider potential heat damage of the protein. Heat damage is avoided or reduced by moderate, short and moist heating.

Vegetable antinutritional factors

Vegetable antinutritional factors may broadly be divided into heat–stable and heat–labile factors. Unless these components can be inactivated or removed, they constitute the major restriction on the use of vegetable protein in fish feeds.

Table 1 Typical composition of fish meal and vegetable protein ingredients currently used in commercial feeds for carnivorous salmonid fish (percent of dry matter).

Protein source	Protein	Oil	Starch	NSP	Sugars
Fish meal[1,2]	78	12	–	–	–
Full fat soy[3,4]	42	21	3	18	11
Hulled and defatted soy[3,4]	57	1	3	23	14
Soy protein concentrate[3,4]	68	1	7	19	2
Corn gluten[2,4]	67	2	21	3	1
Wheat gluten[5]	85	6	7	–	–

[1]Anderson *et al*. 1993; [2]Anderson *et al*. 1992; [3]Lusas and Riaz 1995; [4]Bach–Knudsen 1997: [5]Storebakken *et al*. 2000a

Table 2 Typical critical indispensable (essential) amino acids in protein ingredients currently used in commercial feeds for salmonid fishes as compared to fish meal (g/16 g N = percent of CP).

	Fish meal[1]	Soybean[1]	Corn gluten[2]	Wheat gluten[3]
Arginine	5.9	7.3	3.7	3.6
Lysine	8.1	6.1	1.8	1.5
Methionine	3.0	1.4	2.3	1.6

[1]Ljøkjel *et al*. 2000; [2]Anderson *et al*. 1992; [3]Storebakken *et al*. 2000a

As the term implies, heat–labile factors may be destroyed or inactivated by thermal processing. Glutens are practically free from such components, but legumes, and particularly soybeans, are rich in heat–labile antinutritional factors. The most significant of these are proteinase inhibitors and agglutinating lectins. Proteinase inhibitors are proteins capable of binding protein–hydrolysing digestive enzymes, thus restricting digestion and utilisation of dietary protein. Lectins are glycoproteins that bind (agglutinate) to receptors in the epithelium of the fish intestine (Hendriks *et al*. 1990), possibly with deleterious effects. Proteinase inhibitor–activity is commonly measured as mg bovine trypsin inhibited per g sample (TI–activity; Hammerstrand *et al*. 1981). Carnivorous fishes are sensitive to proteinase inhibitors (Krogdahl and Holm 1983; Takii *et al*. 1998), and both nutrient digestibility and growth by carnivorous fish are severely reduced if the dietary TI–activity exceeds 5 mg/g (Wilson and Poe 1985; Krogdahl *et al*. 1994; Olli *et al*. 1994). TI–activity may exceed 30 mg/g in raw soybeans. Certain beans, like navy or kidney beans, may also have a high TI–activity, whereas it is negligible in lupins and starch–rich legumes like peas and lentils (Table 3).

The most significant heat–stabile antinutritional factors in current vegetable protein feedstuffs for fish are antigens and phytic acid. Indigestible carbohydrates may also be troublemakers, and in particular the NSP.

Antigenicity is so far only investigated in salmonid fish, and only for soy products and wheat gluten. Soybeans contain antigenic factor(s) that induce enteritis in the distal intestine of salmonid fishes. This inflammation is characterised by widening and shortening of the mucosal foldings, loss of the supranuclear vacuolisation of the absorptive cells in the intestinal epithelium, widening of and increased amounts of connective tissue in the central stroma within the mucosal foldings, and infiltration of a mixed leukocyte population in the *lamina propria* and submucosa (Baeverfjord and Krogdahl 1996). It is, furthermore, reflected in elevated lysozyme and IgM levels in the mucosa (Krogdahl *et al*. 2000), an increased number of circulating leukocyte cells, elevated circulating concentrations of plasma proteins and immunoglobulins, and increased activity of circulating neutrophils, monocytes, and macrophages (Rumsey *et al*. 1994). The condition is associated with a reduced absorptive capacity for nutrients by the distal intestine (Nordrum *et al*. 2000). How much the condition actually contributes to the reduced absorption of nutrients seen in soy–fed salmonids (see Figure 1) is unclear, as the distal intestine is not recognised as a major absorptive site in fish (Buddington *et al*. 1997). Furthermore, salmonid fish suffering the condition appear to grow normally (Refstie *et al*. 2000, 2001). On the other hand, the large intestine of fish absorbs macromolecules throughout life, and has important enteric immune functions (Buddington *et al*. 1997). The antigen(s) inducing this inflammatory response are still not identified. It is also unclear whether other plant seeds have similar antigenic properties, and whether the antigens(s) affect fish in general. The antigen(s) are apparently soluble in alcohol, as alcohol washed soy protein concentrates do not induce enteritis, whereas

Table 3 Contents in raw and processed legumes of Kunitz trypsin inhibitor (TI), Bowman–Birk combined trypsin and chymotrypsin inhibitor (BB–TI), and agglutinating lectins, together with activities of functional TIs, lectins, and urease.

	Contents of			Activities of		
Protein source	**Kunitz–TI mg/g**	**BB–TI mg/g**	**Lectins mg/g**	**TIs mg/g***	**Lectins mg/g****	**Urease pH rise*****
Raw pea[1]				1.3–1.8		
Raw lentil[1]				1.6		
Raw sweet lupin[2]				0.1		
Raw navy bean[3]				11.5		
Raw kidney bean[3]				5.4		
Raw soybean						
Conventional[1,4,5,6,7]	30.3	10.7	8.3	17–31	2.3	2.1
Kunitz inhibitor–free[4,5]	0.04	11.4	8.0	5.6		2.0
Lectin–free[4]	28.4	13.0	<0.0002			
Toasted soybean meal[4,6,7,8,9]				3–9	0.01–0.2	<0.2
Soy protein concentrate[6,8]				2–7		

* mg bovine trypsin inhibited per g meal
** mg lectins agglutinating to brush border vesicles of chicken per g meal
*** pH rise in phosphate buffer as urease acts upon urea to produce ammonia
[1] Pisulewska and Pisulewski 2000; [2]Booth *et al.* 2001; [3]Dhurandhar and Chang 1990; [4]Han *et al.* 1991; [5]Douglas *et al.* 1999; [6]Anderson and Wolf 1995; [7]Maenz *et al.* 1999; [8]Refstie *et al.* 1999; [9]Waldroup *et al.* 1985

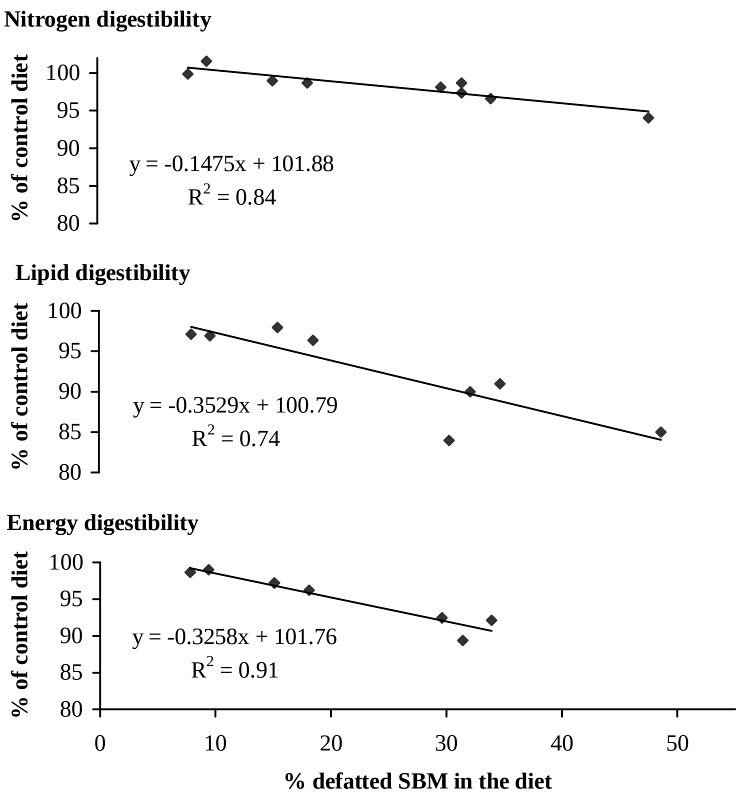

Figure 1 Reduced digestibility of nitrogen, lipid and energy by Atlantic salmon when replacing low temperature fish meal (LT–FM) by defatted and toasted soybean meal (SBM) in the diet. Fish oil was the only lipid source in all diets. Responses are presented relative to when feeding the LT–FM (control) diet. Data are taken from Refstie *et al.* 1998, 1999, 2000, 2001, and Storebakken *et al.* 1998b.

the alcohol extract (soy velasse) does (Ingh *et al*. 1996; Krogdahl *et al*. 2000). Wheat gluten, which may induce celiac sprue in humans, does not induce enteritis in salmon (Storebakken *et al*. 2000a).

Indigestible seed carbohydrates (Table 1) are largely in the form of soluble and insoluble NSP. Dietary soluble NSP may reduce lipid absorption by fish. For instance, indigestible starch (Grisdale–Helland and Helland 1997) and viscous guar gum (Storebakken 1985) restrict the absorption of protein and lipid by salmonids. Impaired lipid absorption when feeding soluble NSP is also a well–known and serious problem in chicken nutrition. Legumes and crucifers furthermore contain indigestible sugars (α–galactosides; Table 1) that produce diarrhoea in fish.

As shown in Table 4, the phosphorus content of fish meal is substantial. Thus, the concentration in diets for carnivorous fishes is high, and usually exceeds 10 g P/kg DM, but a major fraction of this is present in bone and thus is poorly available (Nordrum *et al*. 1997; Vielma *et al*. 1999). This results in a high P load to the aquatic environment, which may lead to algal blooms in freshwater systems (Vielma *et al*. 2000). Vegetable protein ingredients contain significantly less P than fish meal, and substitution of fish meal by vegetable feedstuffs thus enables the use of less, and more highly digestible, P sources in the diet. As shown in Table 5 this is, however, complicated by the presence of phytic acid which is abundant in all types of vegetable protein feedstuffs that have a potential for use in feeds for carnivorous fish.

Phytic acid–bound P is unavailable to fish. Phytic acid also binds essential divalent mineral elements like Zn, and reduces the intestinal hydrolysis and thus the utilisation of dietary protein (Spinelli *et al*. 1983; Caldwell 1992). Lowered availability of Zn cause eye cataracts, as demonstrated in Chinook salmon (*Oncorhynchus tshawytscha*; Richardson *et al*. 1985). Baeverfjord *et al*. (1998) demonstrated that P deficiency in Atlantic salmon (*Salmo salar*) is initially manifested as a reduction in whole body Ca and P levels and the development of abnormally soft bones, although growth is little affected. In later stages, growth is severely impaired, and mortality increases. Reduced body Zn concentration is overcome by dietary supplementation of this element (Ramseyer *et al*. 1999); however, as indicated by Table 5, reduced body concentrations of Ca and P, and thus bone ash, seem harder to overcome.

It follows that vegetable antinutritional factors and nutrient classes from different feed ingredients may

Table 4 Typical phosphorus (P) and phytic acid in dry matter of fish meal and vegetable protein meals.

Ingredient	Fish meal[1]	Soybean[2]	Pea[3]	Rapeseed[3]	Wheat gluten[1]
Phosphorus, g/kg	21.1	4.5	4.4	14.9	2.5
Phytic acid, g/kg	–	7.5	9.7	41.5	2.1
Phytic acid P as percent of total P	–	47	49	63	24

[1]Storebakken *et al.* 2000a; [2]Refstie *et al.* 1999; [3]Burel *et al.* 2000b

Table 5 Availability of nitrogen and phosphorus in and elemental composition of Atlantic salmon grown for 84 days on diets with 60% fish meal or 15% fish meal and 48% untreated or phytase–treated soy protein concentrate as dietary protein sources. Soy protein concentrate diets were supplemented with dicalcium phosphate to obtain similar dietary P contents. From Storebakken *et al.* (1998a).

Dietary phytic acid, g/kg DM*	Fish meal diet 0.8	Soy protein concentrate diet 9.3	Phytase treated soy protein concentrate diet 0.5
Nitrogen			
Digestibility, %	91[a]	85[b]	88[ab]
Retention, %	58[ab]	55[b]	62[a]
Excretion, g/kg gain			
Faecal	4.8[b]	8.2[a]	5.9[b]
Metabolic	17.0[a]	16.3[a]	13.8[b]
Phosphorus			
Digestibility, %	41[b]	30[c]	49[a]
Retention, %	30[a]	25[b]	32[a]
Excretion, g/kg gain			
Faecal	8.1[a]	8.7[a]	6.3[b]
Metabolic	1.4[a]	0.5[b]	1.9[a]
Whole body concentration of			
Ash, g/kg	21.0[a]	19.2[b]	21.5[a]
Ca, g/kg	4.2[a]	3.5[c]	4.0[b]
P, g/kg	4.4[a]	4.0[b]	4.4[a]
Mg, mg/kg	403[a]	389[b]	413[a]
Zn, mg/kg	61.6[a]	42.6[b]	60.6[a]
Ratio Ca : P	0.94[a]	0.85[b]	0.91[a]

[a,b,c] Within rows, values not followed by the same superscript are significantly different ($P < 0.05$)

* From dietary wheat

interact to affect the overall absorption of nutrients by fish. Although the contribution of each effect is small, the interactional sum may be significant. This is demonstrated in Figure 1, which shows how the digestibility of nutrients by Atlantic salmon is reduced by inclusion of toasted soybean meal in diets based on low temperature fish meal and fish oil. This appears to be a general response in carnivorous fishes, and inclusion of more than 20% soybean meal in the diet usually leads to reduced feed efficiency and slower growth (Watanabe *et al.* 1992; Shimeno *et al.* 1993; Kaushik *et al.* 1995; Olli *et al.* 1995).

From Table 3, it is apparent that raw soybean is the least acceptable protein feedstuff for carnivorous fishes. Defatted soy flakes are, however, typically toasted (steam–cooked) at 105°C for 30 minutes to remove solvent residues after the oil extraction procedure. This reduces the TI–activity down to levels tolerable by carnivorous fishes, and is accompanied by denaturation and inactivation of the lectins. Furthermore, feedstuffs in modern energy–dense fish feeds are subjected to a second moist heating during the high–pressure moist extrusion manufacturing of the diets. Thus, proteinase inhibitors and lectins are rarely problematic when using defatted soy and/or other vegetable protein feedstuffs in modern fish feeds.

Heat–stable antinutritional factors are, however, hard to inactivate, and if not removed they restrict the use of plant proteins in fish feeds. Oligosaccharides, soluble NSP, and soy antigenic factor(s) that induce distal enteritis in salmonids (Ingh *et al*. 1996) are all soluble in alcohol, and so they are removed by ethanol and water washing in the manufacture of plant protein concentrates. This leaves phytic acid, which should be hydrolysed if vegetable feedstuffs provide a major part of the dietary protein. It is hydrolysed by microbial phytases, which are usually produced from *Aspergillus niger*. Current phytases are heat labile and consequently susceptible to inactivation during the high–pressure moist extrusion of feeds for carnivorous fish. Furthermore, phytase from *A. niger* has a temperature optimum of more than 50°C, and its hydrolytic activity is less than 10% of maximum at 10°C (Hoppe 1992). When used as a feed enzyme supplement, the phytase activity will be low at ambient water temperatures where cold–water carnivorous fishes are farmed. An alternative solution is pre–incubation of the vegetable protein sources with phytase, a procedure that is highly effective as shown in Table 5. If culturing fish at warm ambient water temperatures, post–extrusion application of liquid feed enzymes may prove a useful procedure to avoid thermal phytase destruction (Hughes and Soares 1998; Oliva–Teles *et al*. 1998, Vielma *et al*. 1998).

Future

Vegetable protein feedstuffs for carnivorous fishes need to have high protein contents and, in that respect, the most feasible candidates are gluten products. Soy protein concentrates are also promising, and such products are already available commerciallly. As the amino acid compositions of vegetable proteins are generally imbalanced for fish, it is important to combine vegetable protein feedstuffs with complimentary amino acid profiles. This is achieved by combining soy protein and corn gluten. It is also critical to eliminate and remove antinutritional factors. Soybeans are rich in antinutritional factors, but most of these are removed by industrial thermal treatments followed by ethanol washing to produce the commercial protein concentrates. Such manufacturing processes must be optimised to avoid protein damage by excessive heating. It is also desirable to eliminate NSP in vegetable feedstuffs in order to enhance the concentration of digestible energy in the feedstuffs. Finally, phytic acid should be eliminated to avoid disturbances of mineral element absorption and utilisation by fish fed high levels of plant protein. If and when these goals are achieved by cost–effective means, vegetable protein feedstuffs may replace substantial amounts of the fish meal currently used in energy–dense feeds for carnivorous fishes.

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