Indian Journal of Animal Sciences **85** (2): 212–215, February 2015/Article

Nutritional and anti-nutritional contents of alternative plant feed ingredients for fish feed formulation

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Received: 12 September 2014; Accepted: 2 October 2014

ABSTRACT

The present study evaluates the importance of plant materials as alternative feed ingredients for fish feed preparation. Commonly available plant materials i.e., sweet potato leaf meal, banana leaf meal, soybean leaf meal, salvinia leaf meal, papaw leaf meal, gliricidia leaf meal, habarala leaf meal and jack seed powder were subjected to proximate analysis and mineral analysis. Anti-nutrients such as saponin, phytic acid, cyanide and tannin were also analyzed. The crude protein content of the ingredients was ranged from 9.66 to 29.32 g/ 100 g and the crude lipid contents were recorded as 0.8 to 11.6 g/100 g. Crude fiber contents were between 2.2 and 53.05 g/100 g and ash contents ranged from 5.21 to 19.24 g/100 g. The ingredients had trace amounts of Na, K and P which ranged between 0.49 to 2.71 g/100 g. All the tested ingredients contained saponin (0.79 to 5.58 g/100 g), phytic acid (0.07 to 0.81 g/100 g), cyanide (10.82 to 24.17 mg/100 g) and tannin (2.19 to 9.04 g/100 g). The present study revealed that above ingredients contained nutrients in appreciable amounts and anti- nutrients in low levels and could be used for fish feed preparation. Further studies are needed to find the effect of those ingredients on fish growth.

Key words: Anti nutrient analysis, Crude lipid, Crude protein, Plant based ingredients

In aquaculture industry, feed cost accounts for about 40 to 50% of the total operational cost (Shang 1992) and fishmeal is the major protein source used in many commercial fish feed preparation. Fish meal is known to contain essential amino acid (EAA) profile that is needed to meet the protein requirement of most fish species (Wilson *et al*. 1989) and is also a good source for vitamins and growth factors (Tacon 1993). However, the high cost of fish meal coupled with short supply has made the necessity to substitute it with alternative low cost ingredients.

Plant proteins are potential ingredients as alternatives for fishmeal in fish feed preparation. Many plant sources are fairly rich in protein and contain favorable essential amino acid profiles, but they are deficient in one or more essential amino acids (Soltan *et al*. 2008). Some studies have emphasized that a mixture of plant protein sources is more appropriate to obtain adequate amino acid profile compared to the incorporation of a single plant protein sources (Watanabe *et al*. 1995, Regost *et al*. 1999). Most of the plant based protein sources contain anti-nutritional factors such as cyanide, saponin, phytic acid etc. (NRC 1993, Becker and Makkar 1999, Francis *et al*. 2001) and these compounds interfere in food utilization and negatively affect growth and other physiological activities of fish (Francis *et al*. 2001). Therefore, focussing on alternative plant ingredients which are nutritionally compatible, readily available and which are not used for human consumption and animal feed industry is important for development of fish feed industry.

In Sri Lanka there are large numbers of plant based matter which are not currently used for any industrial use. Few examples of such plant based ingredients are plant leaves of banana (*Musa paradisiaca*), sweet potato (*Ipomoea batatas*), manioc (*Manihot esculenta*), fruit peels from canaries, molasses and baggases, sugar cane tops. However, at present only limited information is available on the utilization prospects of those alternative ingredients. Some countries such as India, Bangladesh have analyzed various ingredients to explore the possibility of using them as fish feed (Bhosale *et al*. 2010, Mahmud *et al*. 2012). To develop low cost, nutritious fish feed, information about the availability and proximate composition of locally available feed ingredients is essential. Therefore, the present study was conducted to evaluate the nutritional and anti-nutritional contents of locally available plant based ingredients to find their suitability as alternatives for fish meal in fish feed preparation.

MATERIALS AND METHODS

Plant materials: Leaves of *Ipomoea batatas* (sweet potato), *Musa paradisiaca* (banana), *Glycine max* (soybean), *Salvinia molesta* (salvinia), *Carica papaya*

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(papaw), *Gliricidia sepium* (gliricidia), *Colocasia esculenta* (habarala) and *Artocarpus heterophyllus* (jack seeds)) were collected from the southern part of the country. Above Plant materials were cut into small pieces, kept in an oven pre-heated to 60°C for 24 hours, ground using a kitchen grinder and sieved using a 1.000 mm mesh sieve. The powdered sample was packed in air tied polythene bags and kept in the refrigerator (4°C) until taken for further analysis and feed preparation.

Proximate analyses of the ingredients were done using following standard methods.

Moisture content: The moisture content was calculated as loss in weight of the original sample and expressed as percentage moisture content (AOAC 1990).

Ash content: Dried sample was incinerated overnight at 550°C. The weight of the residual ash was calculated (AOAC 1990).

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)} \times 100}{\text{Weight of sample (g)}}$$

Crude protein: The crude protein was determined by the Kjeldahl method. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (AOAC 1980).

Crude lipid: The fat content was determined quantitatively by extraction with a mixture of chloroform: methanol (2:1). Mixture was partitioned with 0.88 % KCl solution. Organic layer was recovered and left overnight at room temperature to remove chloroform (Folsch *et al.* 1957).

$$\text{Fat \%} = \frac{\text{Weight of fat (g)} \times 100}{\text{Sample weight (g)}}$$

Crude fiber: Powdered sample was defatted using petroleum ether. Defatted sample was used to determine the fiber content according to AOAC (1995) method.

$$\text{Crude fiber} = \frac{\text{loss in weight of sample on incineration (g)} \times 100}{\text{Initial weight of the sample (g)}}$$

Elemental analysis: Potassium and sodium contents of the samples were determined using flame photometer (Jeffery *et al*. 1989). P content was determined using UV spectrophotometer at 830 nm according to Jeffery *et al.* (1989) method.

Anti- nutrient analysis was done according to following methods.

Saponin content: Ground sample was soaked in 20% aqueous ethanol and extracted into *n* butanol. The saponin content was calculated as percentage (Obadoni and Ochuko 2001)**.**

Phytic acid: Dried sample was soaked in 2% HCl solution and filtered. The solution was titrated against a standard iron III Chloride solution containing 0.00195 g of iron per milliliter in the presence of 0.3% ammonium thiocyanate. The percentage phytic acid was calculated (Lucas and Markakas 1975).

$$\text{\% Phytic acid} = \gamma \times 1.19 \times 100$$

$$\gamma = \text{Titre value} \times 0.00195$$

Cyanide content: Powdered sample was soaked in a mixture containing distilled water and orthophosphoric acid for 24 h. The resulting sample was distilled and the distillate was titrated against 0.02 N $AgNO_3$ in the presence of 5% potassium iodide (1 mL of 0.02 N $AgNO_3$ = 1.08 mg HCN) (AOAC 1990).

Tannin content: Powdered sample was extracted into 70% acetone. Sample extract aliquot was mixed with folin-cioalteau reagent, 10% Na_2CO_3 and incubated for 40 minutes at room temperature. The absorbance was measured at 700 nm. Tannin content was expressed as g/ 100 g Tannic acid equivalents (Makkar *et al.* 1993).

RESULTS AND DISCUSSION

Sri Lanka is endowed with many by-products from agricultural processing, which are usually not utilized for human consumption, but may have a high potential as fish feed. The ingredients used in the present study are easily available in bulk amounts in Sri Lanka throughout the year and might be possible sources for fish feed preparation. However, information on the nutritional composition of them is scarce. The proximate composition of the plant based ingredients used in the present study are given in Table 1. Protein is the major growth promoting factor in feed (Mahmud *et al*. 2012). The protein requirement of fish varies according to size and age of the fish, and environmental conditions etc. (Mahmud *et al*. 2012). The

Table 1. Proximate composition of plant based ingredients in dry matter basis

Ingredient	g/ 100 g				
	Moisture (wet basis)	Crude protein	Lipid	Fiber	Ash
Papaw leaves	83.45±2.02	30.11±1.12	0.64±0.04	14.07±0.13	10.30±0.21
Banana leaves	79.49±1.97	20.97±0.78	3.25±0.06	53.05±0.19	8.55±0.17
Habarala leaves	85.17±4.00	19.33±0.93	0.73±0.06	8.34±0.43	12.27±0.11
Gliricidia leaves	80.63±0.66	22.88±0.12	3.39±0.07	10.12±0.29	7.60±0.07
Salvinia leaves	90.75±0.71	9.90±0.29	1.23±0.08	34.13±1.24	11.66±0.33
Soybean leaves	81.65±0.57	27.50±0.62	3.98±0.09	9.56±0.4	9.01±0.13
Sweet potato leaves	86.04±1.36	20.45±0.28	2.98±0.06	8.36±0.4	11.67±0.16
Jack seed powder	46.31±1.28	28.01±0.27	0.75±0.07	2.2±0.08	3.20±0.06

Table 2. Mineral composition of plant based ingredients (dry matter basis)

Ingredient	g/ 100 g		
	K	Na	P
Papaw leaves	0.66±0.08	0.09±0.01	0.19±0.0
Banana leaves	3.19±0.15	0.02±0.00	0.16±0.01
Colocasia esculenta leaves	0.60±0.04	++	0.44±0.01
Gliricidia sepium leaves	2.80±0.13	0.13±0.01	0.37±0.00
Salvinia molesta leaves	2.49±0.08	0.89±0.01	0.16±0.00
Soybean leaves	0.51±0.02	0.34±0.01	0.03±0.00
Ipomoea batatas leaves	0.46±0.08	0.53±0.01	0.10±0.01
Jack seed powder	1.24±0.04	++	++

++ = Present in trace amounts, but could not be quantified.

aquaculture feeds is the bioavailability of phosphorous (NRC 1993) as it will cause environmental eutrophication when released with feces. In the present study phosphorous content in all the studied ingredients were less than 0.5 g/ 100 g. Na content of the ingredients were less than 1 g/100 g (Table 2) and K content was found in between 0.46 to 3.19 g/100 g.

Nutritive value of plant ingredients influenced the growth of fish which is constrained by anti nutritional factors. Although plant derived materials analyzed in the present study have the potential to be used as fish feed ingredients; those ingredients contain more than one of the anti nutrients. Francis *et al*. (2001) reviewed that the presence of anti nutrients causes to reduce the nutritional quality of the fish

Table 3. Level of anti- nutrient content in plant based ingredients (dry matter basis)

Ingredient	Dry matter basis			
	Saponin (g/ 100 g)	Phytic acid (g/ 100 g)	Tannin (g/ 100 g)	Cyanide (mg/ 100 g)
Papaw leaves	1.98±0.05	0.87±0.08	6.03±0.01	30.02±2.35
Banana leaves	4.81±0.16	0.42±0.04	4.15±0.53	28.22±3.04
Colocasia esculenta leaves	2.85±0.08	0.31±0.03	5.63±0.58	29.05±1.82
Gliricidia sepium leaves	3.94±0.12	0.34±0.04	9.04±0.01	25.42±1.28
Salvinia molesta leaves	0.85±0.08	0.15±0.06	4.32±0.19	20.94±0.98
Soybean leaves	0.89±0.05	0.18±0.02	2.19±0.00	16.23±0.04
Ipomoea batatas leaves	2.89±0.05	0.49±0.04	8.22±0.01	16.95±1.02
Jack seed powder	5.68±0.15	0.08±0.007	3.77±0.01	20.14±2.70

protein contents of ingredients used in the present study were in the range of 19.33 to 30.11 g/100 g except for salvinia leaves (9.90 g/100 g), but comparatively lower when compared with fishmeal which contains 50 to 70 % protein content (FAO 2013). Protein content of papaw leaves (30.11 g/100 g) and sweet potato leaves (20.45 g/ 100 g) are compatible with the previous studies (Oduro *et al*. 2008, Asmah *et al*. 2014).

Lipid content of the ingredient is an important factor in fish feed formulation as lipids supply essential fatty acids (EFA) and serves as transporters for fat-soluble vitamins (Craig and Helfrich 2009). Crude lipid content was ranged from 0.64 to 3.98 g/100 g and the highest lipid content was recorded from soybean leaves (3.98 g/100 g). Fiber plays a nutritional role in moving the bolus and absorbing toxins. In fact, the amount of crude fiber in fish feeds is usually less than 7 % of the diet to limit the amount of undigested material entering the culture system (Delbert 2010). Except for jack seed powder all the other plant ingredients used in the present study exceed the limit and banana leaves showed the highest crude fiber content (53.05 g/100 g). Highest amount of ash content was from habarala leaves (12.27 g/ 100 g) and ash contents in other ingredients were ranged from 3.20 to 12.27 g/100 g. High ash content reduces the digestibility of ingredients in the diet resulting in poor growth of the fish and ash is not desirable to exceed 12% (De Silva and Anderson 1995).

One of the drawbacks of plant-based ingredients in feeds. All the plant materials in the present study showed the presence of anti- nutrients such as saponin, phytic acid, cyanide and tannin (Table 3). Dietary saponins above a level of 0.15 g/100 g can retard growth and damage intestinal mucosa in fish (Francis *et al*. 2001) and all the plant ingredients tested contained saponins above this level. Phytic acid level in fish feeds above the concentration of 0.5 % was detrimental to the growth of fish (Francis *et al*. 2001) and in the present study saponins in papaw leaves exceeds this level. Becker and Makkar (1999) has shown that tannins interfere with the digestive processes by inhibiting protease and also forming indigestible complexes with dietary protein at inclusion rate of 2 g/100 g. All the analyzed ingredients consisted of tannins above this inclusion rate. All the ingredients contained < 30 mg/100 g of cyanide. Cyanides derive from hydrolysis of cyanogens that suppress natural respiration and cause cardiac arrest (Davies 1991). No exact limit of toxicity of cyanide for fish has been recorded yet and more studies are required to determine the cyanide level of tolerance for fish (Francis *et al*. 2001).

Sweet potato and papaw leaves were used in fish feed formulation (Munguti *et al*. 2012; Wickramasekara *et al*. 2011). No research were conducted to test the potential of using most of the other plant materials in fish feed preparation. Therefore, further studies are needed to evaluate the suitability of using these materials in fish feed preparation.

The results indicated that all the above investigated ingredients have the potential as feed ingredients. However all the plant materials contained various amounts of anti nutrients and further studies are needed to evaluate the effect of those in fish growth by conducting growth trials.

ACKNOWLEDGEMENTS

Financial support provided by TURIS (Transforming University of Ruhuna into International Status) grant (RU/DVC/Pro 149) to conduct this research was greatly acknowledged.

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