Plant proteins in fish feed: An additional analysis

Protein is the vital and expensive ingredient of formulated fish feeds. Quality and quantity of proteins in formulated fish feeds are of paramount importance in promoting marketable fish growth[1]. Fish meal protein is being used globally as dietary protein in formulated fish feeds. However, the major problems are its rising cost, uncertain availability and variations in quality. Thus research efforts are continuing in search of cheap and alternative protein sources to minimize feed expenditure, especially cost of dietary protein. The utility of plant protein sources (PPS) to exclusively or partly replace the fish meal protein is being investigated globally. Research efforts in this avenue have revealed both positive and negative results. In this context, two comprehensive and critical analyses[2,3] of global data over the last two decades were published on the role of PPS in formulated fish feeds.

The first analysis[2] scrutinized quantitative data from 87 standard papers and revealed a low positive correlation (r = 0.177; n = 377; 40 species) between dietary crude protein (DCP in %) of PPS-based fish feeds and specific growth rate (SGR in %), and high negative correlation (r = –0.990; n = 332; 38 species) between DCP of PPS-based fish feeds and protein efficiency ratio (PER) of farmed fishes. It was inferred that SGR did not show any significant increase at higher levels of DCP of PPS-based feeds. Conversely, increase in DCP of PPS-based fish feeds decreases the PER of tested fishes. Since SGR and PER are conversion (= growth)-based indices of fish nutrition, it was concluded that conversion of assimilated energy into the body growth was low in tested fishes on PPS-based fish feeds.

In bioenergetics, absorption of food precedes conversion into growth. Thus, the second analysis[3] assessed the degree of absorption of PPS-based fish feeds by estimating correlation between absorption efficiency (Ae in %), an index of food absorption in animals and DCP of PPS-based fish feeds using the dataset of the first report[2]. A highly significant positive correlation (r = 0.939; n = 125; 53 species) between Ae of fishes and DCP of PPS-based fish feeds was obtained. Moreover, by following another impressive contribution to fish bioenergetics[4] on the relationship between food nitrogen (N in %) and Ae of fishes, the correlation between Ae of fishes and N of PPS-based fish feeds at both low (range 1.07–4.9; r = 0.988; n = 46; 22 species) and high (range 1.07–9.79; r = 0.984; n = 125; 53 species) levels was also estimated. It was inferred that both DCP and N of PPS-based feeds were significantly and positively correlated with Ae of tested fishes. But this assimilated energy is not converted into body growth as revealed by first analysis[2]. Table 1 summarizes the results of these two earlier reports.

Here an additional analysis is attempted to verify the conclusion of the first report[2], by estimating the correlation between the DCP of PPS-based fish feeds and feed conversion ratio (FCR) of cultivable fishes. FCR is also a conversion-based measure and is being interpreted as an index of feed efficiency in fish nutrition research[1]. It is calculated as a ratio between total feed intake and wet weight gain by the fish. From the earlier dataset[2], estimates of DCP of PPS-based fish feeds and FCR of tested fishes were mined and pooled. The final dataset included 282 estimates for 33 species from 51 standard papers, which appeared during the period 1978–97. Among these, 74 estimates for seven species are new additions. This dataset can be obtained from the author. It was analysed for correlation and regression using the software Sigma stat, version 6.5.

Results of the present analysis are given in Table 2. In the present dataset, FCR ranged from 0.25 to 39.5, with a mean of 3.28. DCP of PPS-based fish feeds ranged from 6.7 to 57.13%, with a mean of 34.08%. FCR of tested fishes is negatively (r = –0.391) and significantly (P = 0.001) correlated with DCP of PPS-based fish feeds. This inverse relationship between DCP and FCR is confirmed by the negative sign of the b value. The estimated r value is significantly (P = 0.001) deviated from zero and also significant at the level of population. The b value is also statistically significant (P = 0.001), as revealed by t as well as F test for its SE. However, the percentage of variation (r^2 = 15.29%) explained by DCP of PPS-based fish feeds in FCR of tested fishes is low. The result clearly indicates that FCR declines sig-

Table 1. Summary of two earlier reports

Variables correlated	No. of papers	No. of species	n	r	Reference
DCP–SGR	64	40	377	0.1772*	2
DCP–PER	60	38	332	–0.9822*	2
DCP–Ae	87	53	125	0.9398**	3
High–Ae	87	53	125	0.9844**	3
Low N–Ae	21	22	46	0.9880**	3

Significant at *P < 0.05 and **P < 0.001.

Table 2. Correlation and regression matrix for selected dataset

Statistics	Estimate
Correlation coefficient (r)	–0.391*
Coefficient of determination (r^2)	0.1529
Standard error (SE) of r	0.0550
Probable error (PE) of r	0.0371
Limits of population r	–0.3539–0.4281
t for SE of r	7.109*
Regression coefficient (b)	–0.172
SE of b	0.0271
t for SE of b	7.098*
F for SE of b	50.388*
Intercept (a)	9.835
SE of a	0.963
t for SE of a	10.211*

*Significant at P < 0.001.

nificantly on PPS-based fish feeds with increasing levels of DCP and confirms the conclusion of the first report[2] that conversion is low on PPS-based feeds in tested fishes. Generally, FCR decreases with increasing DCP levels of formulated fish feeds[5,6]. Remarkably, FCR declined almost inversely on higher DCP levels of PPS-based fish feeds[7–9]. In some species, no significant differences in FCR values between PPS supplemented and control diets were reported[10,11].

Inclusion of PPS instead of fish meal protein is recommended, with a condition that it should accompany the essential amino acids[10].

In contrast, the second report[3] revealed highly positive correlation between Ae of fishes and DCP of PPS-based fish feeds. When all these inferences are taken together, it appears that PPS-based fish feeds were absorbed well, but not efficiently converted into body growth of fishes. These assimilated amino acids could be used in the following ways instead of turnover into fish body proteins: (i) losses as such via integument and intestine, (ii) mainly deamination and oxidation as source of energy and (iii) conversion into N-compounds through synthetic pathways. However, the flow of assimilated amino acids to synthetic pathways of N-compounds is low compared to that channelled in oxidation or protein turnover; much of it, in fact, is utilized as an energy source[12]. When the rate of ingestion of amino acids exceeds the rate of their utilization for body protein synthesis, the excess amino acids are deaminated and used for energy production[13]. It was also established that during conversion, a substantial fraction of assimilated energy, particularly from protein-rich feeds, is lost in the form of heat in fishes, a process termed specific dynamic action (SDA). Its magnitude is dependent on the quality and quantity of feed, especially quality of protein[14,15]. A traditional interpretation of SDA suggested that a diet inducing a high SDA will reduce the amount of food energy available for growth. According to Pandian *et al.*[1], information on protein requirement of fishes will be of limited practical value, unless the optimum requirements of ten essential amino acids are known and one-third of dietary proteins should contain the essential amino acids. It is equally remarkable that Cowey[12], in his review on amino acid requirements of fishes, concluded that expression of amino acid requirements in terms of metabolizable energy is a difficult task. Generally, fishes conserve essential amino acids for growth at the expense of non-essential amino acids as energy currenty[16].

The low magnitude of r suggests that the inverse relationship between DCP and FCR is not too strong. This indicates that a considerable quantity of assimilated energy is converted into body growth in tested fishes. Some reasons in relation to PPS-based feeds are: (i) removal/inactivation of antinutritive factors and toxins by special methods; (ii) control of other conversion-influencing factors in experiments; (iii) inclusion of fish meal protein as secondary source of dietary protein; (iv) supply of adequate energy nutrient (fats) to reduce the diversion as metabolic fuel; (v) inclusion of one more PPS, some of which may supply essential amino acids and (vi) constant experimental conditions which may reduce the energy expenditure on metabolism; surplus energy is always diverted to growth[17].

Conversion is measured in terms of rate of efficiency. Estimation of gross (K_1) and net conversion (K_2) efficiencies may reveal exactly how much food assimilated energy is allocated to growth in terms of K_2 on PPS-based fish feeds. Such attempts are rare in contemporary researches on fish nutrition[11]. Therefore, future studies need to focus on: (i) evaluation of PPS-based fish feeds in terms of efficiencies of conversion, especially K_2; (ii) preference to PPS, which resulted in maximum conversion in terms K_2 (water hyacinth)[18] or conversion rate (*Amaranthus spinosus*)[19]; (iii) intensive research on PPS that resulted in maximum weight gain (cabbage, cassava, leucaena leaves)[10,20], which is a measure of net conversion; (iv) sources that act synergistically with animal proteins and promote better conversion (slaughter-house waste + groundnut-oil cake + rice bran)[21] and (v) sources that fulfil other nutritional requirements like vitamins and minerals (single-cell proteins)[22].

The foregoing discussion also revealed the importance of amino acid profile of PPS. Therefore, future research on fish-feed formulation using PPS should consider the following aspects together with conclusions of earlier reports[2,3]: (i) qualitative and quantitative data on essential and non-essential amino acids of PPS; (ii) ratio between essential and non-essential amino acids, e.g. evaluation of protein sources in terms of ratio of a given essential amino acid to the total essential amino acids[23]; (iii) energy density of the feed in terms of protein–energy ratio; (iv) supplementation of essential amino acids to enhance protein turnover, and (v) collection and analysis of supplementary data in addition to growth data to confirm the growth-promoting efficiency PPS-based feeds, e.g. measurement of plasma-free amino acids to confirm the requirement of some essential amino acids[24,25], and (vi) proper feeding schedules to reduce loss of energy from PPS-based feeds.

1. Pandian, T. J., Mohanty, S. N. and Ayyappan, S., In *Sustainable Indian Fisheries* (ed. Pandian, T. J.), National Academy of Agricultural Sciences, New Delhi, 2001, pp. 145–157.
2. Krishnankutty, N. and Sujatha, T. R., *Curr. Sci.*, 2003, **85**, 247–249.
3. Krishnankutty, N., *Curr. Sci.*, 2005, **88**, 865–867.
4. Pandian, T. J. and Marian, M. P., *Mar. Biol.*, 1985, **85**, 301–311.
5. Jauncey, A., *Aquaculture*, 1982, **27**, 43–54.
6. Steffens, W., *Aquaculture*, 1981, **23**, 337–345.
7. Jackson, A. J., Copper, B. S. and Matty, A. H., *Aquaculture*, 1982, **27**, 97–109.
8. Ng, W. K. and Wee, K. L., *Aquaculture*, 1989, **83**, 45–48.
9. Applier, H. N. and Jauncey, K., *Aquaculture*, 1983, **30**, 21–30.
10. Borlogan, I. G. and Coloso, R. M., In *Fish Nutrition Research in Asia* (ed. De Silva, S. S.), Asian Fisheries Society, Manila, 1994, pp. 63–67.
11. Manju, K. G. and Dhevendran, K., *J. Aquat. Trop.*, 2002, **17**, 221–230.
12. Cowey, C. B., *Aquaculture*, 1994, **124**, 1–11.
13. Cowey, C. B. and Sargent, J. R., In *Fish Nutrition* (eds Russel, F. S. and Yonge, M.), Academic Press, London, 1972, pp. 383–492.
14. Jobling, M., In *Fish Energetics: New Perspective* (eds Tytler, P. and Calow, P.), Croom Helm, London, 1985, pp. 213–230.
15. Pandian, T. J., In *Fish Nutrition Research in Asia* (ed. De Silva, S. S.), Asian Fisheries Society, Singapore, 1989, pp. 11–22.
16. Cowey, C. B. and Walton, M. J., In *Fish Nutrition* (ed. Halver, J. E.), Academic Press, New York, 1989, pp. 260–329.
17. Pandian, T. J. and Vivekanandan, E., *Bioenergetics*, Madras Science Foundation, Chennai, 1990, pp. 105–119.
18. Niamat, R. and Jafri, A. K., *Curr. Sci.*, 1984, **53**, 338–340.
19. Hanifa, M. A., Murugesan, A. G. and Fleming, A. T., *Curr. Sci.*, 1987, **56**, 846–848.

20. Hasan, M. R., Roy, P. K. and Akand, A. M., In *Fish Nutrition Research in Asia* (ed. De Silva, S. S.), Asian Fisheries Society, Manila, 1994, pp. 69–76.
21. Nandeesha, M. C., Devaraj, K. V. and Sudhakara, N. S., Proceedings of the First Asian Fish Forum, Manila, 1986, pp. 25–31.
22. Pantastico, J. B., In *Fish Nutrition Research is Asia* (ed. De Silva, S. S.), Heinemann, Singapore, 1988, pp. 71–87.
23. Penaflorida, V. D., *Aquaculture*, 1989, **83**, 319–330.
24. Santiago, C. B. and Lovell, R. T., In *Fish Nutrition Research is Asia* (ed. De Silva, S. S.), Asian Fisheries Society, Manila, pp. 1–9.
25. Wilson, R. P., Robinson, E. H. and Poe, W. B., *J. Nutr.*, 1978, **108**, 1595–1599.

ACKNOWLEDGEMENTS. This series of papers would never have been written without inspiration, constant guidance and support from Prof. T. J. Pandian, Madurai Kamaraj University, Madurai. I thank Dr S. Chandrasekaran, Madurai for help and the Board, Principal, HOD and colleagues in the Department of Zoology, Madura College, Madurai.

Received 13 July 2005; accepted 25 July 2005

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Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes

Jatropha, a drought-resistant, photo-insensitive[1] perennial plant belonging to the family Euphorbiaceae is attracting increasing attention as an important source of bio-diesel. The seeds of *Jatropha* plant contain a viscous, non-edible oil, which besides being a source of bio-diesel can also be used for manufacturing other useful products such as candles, high quality soaps and cosmetics as well as for healing several skin disorders. Because of its above-mentioned industrial and medicinal uses, Central and State Governments have drawn ambitious programmes for its large-scale cultivation.

Two species of *Jatropha* that are grown include *J. curcas* and *J. glandulifera*. Although only *J. curcas* is being promoted for bio-diesel, *J. glandulifera* is known for its beautiful flowers. The seeds of *J. curcas* contain 48% oil, while that of *J. glandulifera* contain 27% oil. Both the species need to be studied in detail for their fat and oil content. *Jatropha* plants grow well on poor stony soils[2–4]. *Jatropha* is a multipurpose tree with a long history of cultivation in tropical and subtropical regions of the world[2,5–7]. It is a native of Central America and occurs mainly at lower altitudes (0–500 m) in areas with annual temperatures of well above 20°C. The seeds are toxic due to the presence of cursive and curative ingredients, but after treatment the seeds or seed cakes can be used as animal feed[8]. *Jatropha* is grown as a boundary fence to protect fields from grazing animals and as a hedge to prevent erosion[2,9]. The problem of great concern regarding this plant is the rate of vegetative growth of plants and seed yield. The plants have profuse vegetative growth, but the number of seeds produced per plant is very low. Besides, the plants produce seeds after approximately 2–3 years depending on environmental conditions and seeds have a limited viability, they lose almost 50% viability within 15 months[10]. In spite of all these properties, research on cultivation and propagation of *Jatropha* is limited. Thus it was considered useful to undertake a systematic study on the vegetative propagation of *Jatropha* through stem cuttings as rooting is a crucial step in the propagation of woody plants and there is a great variability in the rooting ability of different species. While propagation through seeds leads to genetic variability and makes the crops prone to diseases, propagation through vegetative means offers an advantage in developing true-to-type, disease-free varieties of economically and commercially important plants for clonal multiplication[11].

We report here the results of our trials on the vegetative propagation of two species of *Jatropha*, namely *J. curcas* and *J. glandulifera* through stem-cuttings and the accompanying biochemical changes. This is a report of the effect of auxins in rooting and sprouting behaviour of the two *Jatropha* species. It is observed that during the rooting process many changes take place both at the physiological and biochemical levels and activities of many enzymes are up- and down-regulated. The initial levels of endogenous auxin and its oxidation enzymes – IAA-oxidase and peroxidase play a significant part in the process, these being more in *J. glandulifera* than in *J. curcas*. IAA-oxidase activity is involved in triggering and initiating the roots/root primordia, whereas peroxidase is involved in both root initiation and elongation of roots. Position of the cuttings on the mother branch also plays a significant role in rooting and sprouting. Cuttings made from the middle portions of the mother branches exhibit better rooting as compared to the most apical or most basal cuttings. These results are supported by the peroxidase isoenzyme analysis in the cuttings.

Healthy and uniform stem-cuttings (8–10 inches in length) of *J. curcas* and *J. glandulifera* were obtained from both the apical, middle and basal portions of branches of 2–3 year-old *Jatropha* plants. They were pre-treated with 10 and 100 mg/l indole-3 butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) for 24 h, with one group of untreated cuttings serving as control. After 24 h pre-treatment with IBA or NAA, the cuttings were transferred to the field. Observations on the number of cuttings rooted, number of roots and shoots/sprouts produced on each cutting and their length were recorded in each treatment at biweekly intervals up to 45 days. Samples from sprouted portions and from rooted portions were also collected till 45 days for biochemical analysis. Endogenous auxin content and changes in the activities of IAA-oxidase and peroxidase were determined[12–14]. Records of growth were maintained even after 45 days. The isoenzyme pattern of peroxidase was also studied by electrophoresis[15]. The protein was determined by the method of Lowry *et al.*[16].