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# Nutritional Evaluation of Some Species of Fishes in Khuzestan and Determination of the Amount of Soy Bean Meal Used in the Fish Food Formula on the Basis of its Isoflavone Content

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**Abstract:** Soy defatted meal which contains some useful phytochemicals other than high amounts of protein has been used in fish food formula. In this research four species of fish with local names of Shirbot (*Barbs grypsus*), Berzem (*Barbus barbilus*), Capoor (*Cyprinus carpio*) and Ghezel-ala (*Salmo trutta*) were analysed for their nutritional value. The protein contents were between 17.4 to 19.8% and the fat between 1.5 to 8.9%. Soy isoflavones in the fish formula were determined with HPLC. The amount of soy defatted meal used in the fish food formula was estimated on the basis of its soy isoflavone content, which is a new suggestion to do this for fish food formula and other soy containing food-stuff. Soy isoflavone metabolites were traced in fish meat as well.

**Key words:** Shir-bot (*Barbs grypsus*), Berzem (*Barbus barbilus*), Capoor (*Cyprinus carpio*), Ghezel-ala (*Salmo trutta*), nutritional value, soy isoflavones, HPLC

### INTRODUCTION

Ecological limitations, have seriously affected food production especially that of animal protein for the human beings in the world. Fish culture and in general, aquatic food production in areas which have favourable climate and with a lot of wastages or agricultural by-products, suitable for this purpose, has been a promising way to produce valuable animal protein. Khuzestan province with its natural resources such as expansive land, enough water and suitable climatic conditions, in addition to expanded food industry and a huge amount of agricultural by-products especially in connection with sugar cane industry, can be very promising for fish culture. According to the report of the Fishery Organisation affiliated with the Agricultural Ministry, more than 17000 tons of warm water fish in 2005 and 76 t of cold water fish and 21 t of prawn in 2004, produced in Khuzestan (Fishery Organization, 2006). In this research nutritional value of three cultured fish Cyprinus carpio, Barbus grypsus, Salmo trutta and one uncultured Barbus barbilus which are popular in Khuzestan, are compared. Soy bean defatted meal which is used in fish culture for its valuable protein, contains some phytochemicals such as isoflavones, saponins, trypsin inhibitors etc., which may affect directly or indirectly human nutrition. There have been numerous papers about soy bean and its

components in recent years (Crouse et al., 1999; De Kleijn et al., 2002; Richelle et al., 2002; Setchell et al., 2001; Slavin et al., 1998; Teixeira et al., 2000; Tikkanen et al., 1998; Wong et al., 1998). In this study soy content of trout food formula is estimated by the analysis of its isoflavones (this is the first report in the world). Isoflavon metabolites are also traced in trout flesh. As to importance and huge volume of researches done about soy bean and its isoflavones, a short review is presented about soy bean isoflavones.

Isoflavones belong to a group of compounds known as flavonoids. Soy isoflavones are twelve different isomers found in soya products. They are phenolic compounds which are present as simple glucosides or esterified with acetyl or malonyl groups. Three main isoflavones are genistein, daidzein and glycitein in order of their amounts present in the seed.

After consumption of these compounds, glucosidase enzymes of bacteria present in human intestine, hydrolyse them to free aglycones which may be absorbed and metabolised (Knight and Eden, 1995; Izumi *et al.*, 2000).

As to similar structure of soy isoflavones with 17  $\beta\text{-}$  estradiol (the main estrogen of human blood) they can be absorbed by  $\alpha$  and  $\beta$  estrogen receptors. Recent studies have shown that these phytochemicals may protect human beings against some diseases. These include different cancers like breast, prostate, colon and

cardiovascular diseases, osteoporosis, menopause problems etc. The basis of these effects is not yet clearly known, but weak esterogenic effect, antioxidancy, enzyme inhibitory effect and so on, may be of importance (Coward *et al.*, 1993).

#### MATERIALS AND METHODS

This study was conducted in Shahid Chamran University of Ahvaz, Iran during January to March 2007. Fish food formula containing soy defatted meal was prepared by Chineh Co. and its composition is shown in Table 1. Cultured and uncultured fishes were obtained through the Fishery Organization of Khuzestan and were identified scientifically in the University Lab. Soy isoflavone standards were purchased from Sigma and other chemicals from Merck Company, suitable for HPLC analysis. Determination of water, fat, protein and ash content of samples were done according to the AOAC (AOAC, 2000). HPLC analysis were done by a Cecil (England) gradient system composed of two pumps (high pressure mixing) and a UV visible detector. A Eurosphere -100 C18-5, column made by Knaver, Germany (125×4 mm) was used. The wavelength of the UV detector was set at 260 nm. A reverse phase gradient method was used which was a modified method of Franke et al. (1998).

Table 1: Proximate composition of fish food formula (%)

Food component	(%)
Crude protein	48.0
Fat	12.0
Carbohydrates	13.0
Ash	13.0
Fibre	2.5
Water	11.0

Gradient elution system was composed of two solvents. Solvent A (5% acetic acid) and solvent B (methanol: acetonitrile, 100:50). Elution starts with 90% A, which after 20 min reduces to 50%. After two minutes of 100% solvent B, it goes back to the original condition. Ten minutes of equilibration is necessary before the next run.

#### RESULTS AND DISCUSSION

The results shown in Table 2 that theses fishes are somewhat medium in fat and protein content. However, they can provide valuable amount of these nutrients in the diet, considering that the quality of the fat and protein content of the fish is generally so high. Figure 1 is the chromatogram of isoflavones found in fish food formula. Comparing these results with that of soy defatted meal (Fig. 2), the amount of 15% soy content of this formula was confirmed.

Direct extraction of fish meat could not reveal any isoflavone or known metabolites, but there was an unknown compound which might be an isoflavone metabolite (Fig. 3).

Further relevant, more detailed studies are needed to provide enough data about soy bean isoflavones and their metabolites in fish meat.

As it is clear from the results, protein is the main compound of fish meat. The protein content of the samples was between 17.4 to 19.8%. Except for the *Barbus grypsus* (Shirbot), other samples contained considerable amount of fat (4.6 to 8.9%). These results agree with the previous researches (Food Standard Agency, 2002).

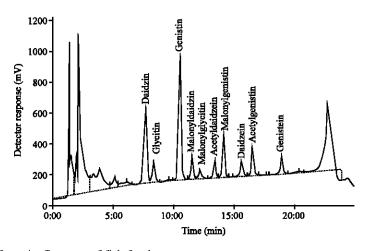


Fig. 1: Chromatogram of soy-isoflavones of fish food

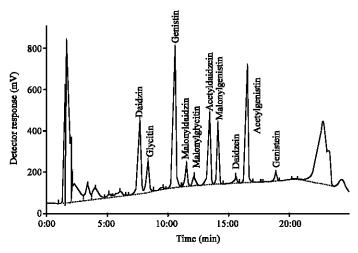


Fig. 2: Chromatogram of isoflavones of soy-defalted meal

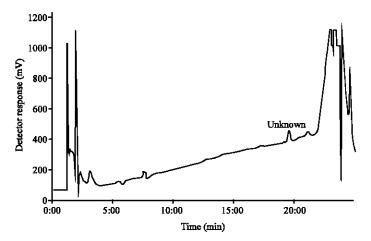


Fig. 3: Chromatogram of isoflavones and their metabolites in Ghezel-ala (Salmo trutta) fish flesh

Table 2: Proximate composition of the meat of the fish samples					
Sample's	Water	Fat	Protein	Ash	
scientific-local name			(%)		
Barbus grypsus-Shir-bot	78.3±0.72	1.5±0.21	19.5±0.39	0.70±0.08	
Barbus barbilus-Berzem	74.3±0.45	6.1±0.18	18.7±0.44	$0.75\pm0.10$	
Cyprinus carpio-Capoor	$73.1\pm0.63$	8.9±0.14	$17.4\pm0.18$	$0.62\pm0.08$	
Salmo trutta-Ghezel-ala	74.9±0.84	4.6±0.11	$19.8 \pm 0.21$	0.67±0.09	
Values are given as means $\pm$ SD (n = 3)					

The trout fat content was somewhat lower than the reported amounts, which might be due to the age, season, climatic conditions and the nutrition (4.6% compared to 6.6%)

Isoflavone content of the fish food formula was determined accurately and successfully. It could be concluded that the amount of 15% soy meal content of the fish food formula is correct. Determination of soy meal content of any food such as hamburgers or sausages can be done by the analysis of their isoflavone contents. This is a valuable message from this research which is for the first time in the literature.

Although the isoflavone and their metabolites in the fish flesh samples were not clearly shown, it is a hint for other researchers to make more efforts.

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