Evaluation of the Genetic Diversities and the Nutritional Values of the *Tra* (*Pangasius hypophthalmus*) and the *Basa* (*Pangasius bocourti*) Catfish Cultivated in the Mekong River Delta of Vietnam

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ABSTRACT : A total of 50 individual catfish, the *Tra* (*Pangasius hypophthalmus*) cultivated in either floating cages (*Tra*-c) or in ponds (*Tra*-p) and the *Basa* (*Pangasius bocourti*) raised in three floating cages, were collected in two of the Mekong Delta provinces. The caudal fin of each individual fish was used for protein electrophoresis employing the SDS-PAGE method. The one fillet sides were used as a representative sample to determine the dry matter (DM), crude protein (CP), ether extract (EE) and amino acids (AAs). The catfish oil was extracted from the belly fats, and the fatty acid (FA) composition was analyzed. There were 21 bands of the *Tra* and the *Basa*. Protein bands of the two varieties were 28.6-33.3% polymorphic, while polymorphic individuals of the *Tra* ranged from 80.0 to 100.0%, and the *Basa* was 90.0% polymorphic. The phenotypic diversity (Ho) of the *Tra* ranged from 1.71 to 1.80, while the *Basa* ranged as high as 2.14%. Diversity values (H_{EP}) for genetic diversity markers were equal in the *Tra* and the *Basa*. The sum of the effective number of alleles (SENA) of both varieties ranged from 3.40 to 3.83 for the *Basa* and the *Tra,* respectively. The lower values of Ho and SENA, as compared with those of the fresh water prawn (*Macrobrachium equidens*) in the area, would suggest that the species with the low values will become extinct due to inbreeding; the gene pools of each observed population were below a suitable threshold. Many of the differences in the nutritional values of the *Tra*-c, the *Tra*-p and the *Basa* were measured; their nutrient values were comparable to fishmeal or fish oil. Most of the DM, CP, and EE were higher in the *Tra,* especially in the *Tra*-c. The essential AA content, especially that of lysine, was highest in the *Tra*-c, next highest in the *Tra*-p, and lowest in the *Basa.* Therefore, the amino acid patterns were closer to the ideal patterns in the same sequences. In contrast, the essential FAs were concentrated in the *Basa* fish oil. It was found that suitable selection of parents for seed production is required to avoid inbreeding. Catfish may be valuable sources of nutrition for both humans and animals, and the differences in their nutritional values by variety and/or management must be taken into account. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 671-676)***

Key Words : SDS-PAGE, Animal Nutrition, Amino Acids, Fatty Acids, The Mekong Delta

INTRODUCTION

Aquaculture using floating cages began in the 1960s in the Mekong River Delta of Vietnam. Catfish species such as *He* (*Puntius altus*), *Bong Tuong* (*Oxyeleotris marmorata*), *Basa* (*Pangasius bocourti*), and *Tra* (*Pangasius hypophthalmus*), have traditionally and commonly been cultivated. In recent years, catfish variations in the area have been concentrated in the *Genus Pangasius*: the *Basa* and the *Tra*. The production of these *Pangasius* catfish has increased from 30,000 tons in 1994 to 150,000 tons in 2002 (Cantho University and the CIRAD, unpublished leaflet) as a result of the national open market economy and exportation to international markets.

Tra catfish may either live in rivers or be raised in ponds using traditional feed, such as rice bran, trash feed, vegetables and the like. *Basa* catfish*,* on the other hand is, raised mainly in river floating cages. Differences in both external and internal features between the two species have been reported (Kawamoto et al., 1972). The *Tra* and the *Basa* catfishes mature in three-to-four, and four-to-five years, respectively, in nature, and their habits of reproduction have been reported. Moreover, artificial reproduction has been rapidly pervasive. Artificial seed production met only 10% of the requirement for river and pond cultivation in 1999, but it has almost satisfied the requirement in the last few years (Khanh, 2003).

However, there is no information on the genetic diversities of the two varieties of catfish. Furthermore, interest in their nutritional values, not only for humans but also for domestic animals has increased with the rising production of catfish, including their residue, such as the head and the fat. Thus, this study aims to evaluate the genetic diversities of the *Tra* and the *Basa* and their nutritional values for pigs, the dominant livestock in the area.

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MATERIALS AND METHODS

Sampling sites and sample collection

The An Giang province (Area 3,406 km^2), situated

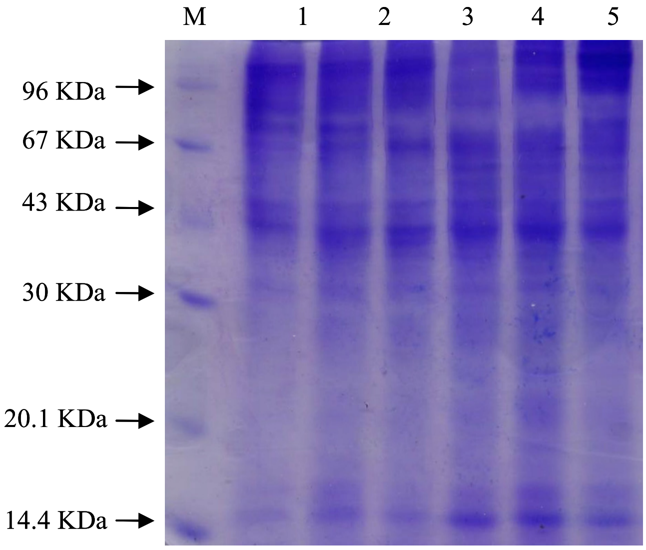

Figure 1. A profile of total protein variations of *Basa* and *Tra* catfish. M: Marker, 1-3: *Basa* catfish, 4-6: *Tra* catfish.

beside the border with Cambodia, is one of the two provinces where catfish cultivation using the floating cage is dominant. Can Tho province (Area 2,986 km^2) is not only the geographic but also the political and economic center of the Mekong Delta. Intensive farming systems, including paddy rice, fruits, animals and fish, are well developed in the province (Sanh, 1998; Son, 1998), and the two types of catfish cultivation, both in ponds and in floating cages, are prevalent.

In Chau Doc town of An Giang province, the 30 *Tra*-c catfish of 7 months old in three different cages were sampled. Within the samples, we chose randomly the 20 specimens with 0.9-1.0 kg live weight for detecting genetic diversity. Similarly, 20 specimens in 30 samples of the *Basa* catfish were taken in Chau Thanh district of Cantho province. The *Tra*-p catfish, as they were raised in small-scale families and difficult to collect; only a total of 10 sample of around 7 months old at four ponds in the Chau Thanh district of Can Tho province could be sampled and used for the analysis.

Genetic diversity

The 0.1 g of individual caudal fin was used for the analysis. The sample was grounded with liquid nitrogen, added to 500 μl extracted solution (0.1 M Tris-HCl/pH = 8.0) containing 1.5 M 2-mercaptoethanol, 0.01 M $MgCl_2$, 18% sucrose and 2% sodium dextran sulfate), then incubated at room temperature for overnight. Proteins were separated on a 5% stacking gel with 40 volts and 12% separating gel with 80 volts. Gel was stained with 0.2 M Coomassie Brilliant Blue R250 in methanol, acetic acid, and distilled water with a ratio of 44:6:50. The destained solution was commixture of acetic acid, methanol, and distilled water with a ratio of 5:28:67, respectively.

The genetic diversity was described on parameters, i.e., the phenotypic diversity value (Ho), genetic diversity value (H_{EP}) and sum of the effective number of alleles (SENA). The each parameter was measured by using formulae as following (Huh and Ohnishi, 2002; Thanh et al., 2003);

$$Ho = -\sum f_i \ln f_i, \quad H_{EP} = 1 - f_i^2, \quad SENA = (1/f_i^2 - 1)$$

Where f_i is the frequency of phenotype i. Frequency of all the bands visible to the naked eye were scored as present (1) or absent (0). The degree of polymorphism was quantified using the Hovalue. Thus, the Ho values were compared among different populations. If the Ho value is equal to zero, it would be proved that all individuals in a population is homologous, or inbreeding. In general, the higher Ho value means the more phenotypic diversity. H_{EP} value also ranges from zero to one, minimum value indicates that a population is completely inbreeding, while maximum value indicates maximum genetic diversity. SENA was calculated by determining the effective number of alleles for each locus. Band polymorphic or individual polymorphism is estimated when their bands are lower than 95% of total variation.

Nutritional values

The 20 *Tra*-c, 10 *Tra*-p and 20 *Basa* catfishes were cut off at one fillet side (250 g) and the fillets of the same groups were bulked together. The dry matter (DM), nitrogen, crude protein (CP), ether extract (EE), amino acid (AA) and fatty acid (FA) content of the catfish samples were determined. The DM of the fillet samples with six replications for each group was determined by using a two-step procedure and calculation, i.e., Total DM = Partial DM×Laboratory DM (Undersander, 1993). The CP, EE and AA content of the air-dried fillets were analyzed by standard methods (AOAC 2000). The CP and EE contents of the air-dry samples were analyzed with ten replications for each. The one air-dried sample of the each catfish group was sent to National Institute of Animal Husbandry in Hanoi capital to determine the amino acid contents. The one catfish oil sample extracted from the catfish belly fat of each group was sent to Experimental Analysis Service Center in Ho Chi Minh city to determine their fatty acids content and composition (Jacobs et al., 2000). The data was analyzed by ANOVA using the General Linear Model of Minitab Statistical Software version 13.2 (Ryan et al., 2000). Sources of variation were treatments in factors. The Tukey test for paired comparisons was used to separate means when the differences were significant at p = 0.01 or 0.05 levels.

Table 1. Genetic diversity parameters of the *Tra (Pangasius hypophthalmus)* and the *Basa (Pangasius bocourti)* catfish in the Mekong River Delta

	Tra-c	*Tra*-p	*Basa*
Polymorphic individual (%)	80.0	100.0	90.0
Polymorphic band (%)	33.3	28.6	33.3
Ho	1.71	1.80	2.14
H_{EP}	0.21	0.21	0.23
SENA	3.83	3.83	3.40

Tra-c: the *Tra* catfish in cage; *Tra*-p: the *Tra* catfish in pond; *Basa*: the *Basa* catfish in cage; Ho: phenotypic diversity; H_{EP}: the genetic diversity value; SENA: the sum of the effective number of alleles.

Table 2. The dry matter (DM), crude protein (CP), and ether extract (EE) contents of the *Tra* (*Pangasius hypophthalmus*) and the *Basa* (*Pangasius bocourti*) catfish fillets in the Mekong River Delta (%)

	Tra-c	*Tra*-p	*Basa*	Pooled SEM
Fresh matter basis				
DM	31.4^a	25.8^c	28.3^b	0.015
CP	17.5^a	14.9^c	16.1^b	0.131
EE	12.5^a	9.3^c	11.4^b	0.037
Dry matter basis				
CP	58.6^a	57.0^b	53.6^c	0.229
EE	39.9^b	36.1^c	40.4^a	0.127

$^{a, b, c}$ The means in the same row with the different letters are significantly different (p<0.01).
Abbreviations: see Table 1.

Table 3. The compositions and the patterns of amino acids of the *Tra (Pangasius hypophthalmus)* and the *Basa* (*Pangasius bocourti*) catfish fillets in the Mekong River Delta (dry matter basis)

	Tra-c	*Tra*-p	*Basa*
Compositions (%)			
Arginine	3.79	3.23	3.24
Glutamic	5.75	5.57	2.95
Glycine	2.41	2.66	3.10
Histidine	2.51	2.71	1.07
Isoleucine	3.35	3.26	3.25
Leucine	5.12	4.87	4.66
Lysine	4.50	4.13	2.37
Methionine	0.79	1.14	1.11
Phenylalanine	2.20	2.11	2.11
Threonine	2.07	2.68	2.92
Valine	2.82	2.80	2.96
Patterns relative to lysine (lysine = 100)			
Arginine	84	78	137
Glutamic	128	135	124
Glycine	54	64	131
Histidine	56	66	45
Isoleucine	74	79	137
Leucine	114	118	197
Lysine	100	100	100
Methionine	18	28	47
Phenylalanine	49	51	89
Threonine	46	65	123
Valine	63	68	125

Abbreviations: see Table 1.

RESULTS

Genetic diversity

The profiles of the total protein variations of the *Basa* and the *Tra* are shown in Figure 1. As many as 21 types of protein bands could be seen by SDS-PAGE electrophoresis. Table 1 shows the genetic diversity values of the *Tra* and the *Basa*. Protein bands of the two varieties were 28.6-33.3% polymorphic, while polymorphic individuals of the *Tra* ranged from 80.0 to 100.0%, and the *Basa* was 90.0% polymorphic. The values of the phenotypic diversity (Ho) of the *Tra*-c and the *Tra*-p were 1.71-1.80, lower than those of the *Basa* (2.14). There were small differences on the genetic marker H_{EP} between the *Tra* and the *Basa*, i.e., 0.21 of the *Tra* and 0.23 of the *Basa*. The sum of the effective number of alleles (SENA) of the *Tra* was 3.83, slightly higher than that of the *Basa* (3.40).

Nutritional values

The DM, CP and EE contents of the fillets are shown in Table 2. The average DM of the fillets of the *Tra*-c was 31.4%, significantly (p<0.01) higher than that of the *Tra*-p (25.8%) and the *Basa* (28.3%). The CP and EE contents on a fresh matter basis were also significantly (p<0.01) different among the *Tra*-c (17.5 and 12.5%), the *Basa* (16.1 and 11.4%) and the *Tra*-p (14.9 and 9.3%), respectively. The CP contents of the fillets on a DM basis were highest in the *Tra*-c (58.6%), next highest in the *Tra*-p (57.0%) and lowest in the *Basa* (53.6%) (p<0.01). In contrast, the EE contents were highest in the *Basa* (40.4%), next highest in the *Tra*-c (39.9%) and lowest in the *Tra*-p (36.1%) (p<0.01). Table 3 shows the compositions and the patterns of amino acids of the catfish fillets. The concentrations of arginine (Arg), glutamic (Glu), isoleucine (Ile), leucine (Leu), lysine (Lys), and phenylalanine (Phe) on a dry matter basis were highest in the *Tra*-c, second highest in the *Tra*-p and lowest in the *Basa*, though the composition of the Phe of the *Tra*-p and the *Basa* was the same. The composition of histidine (His) and methionine (Met) was highest in the *Tra*-p, while the composition of glysine (Gly), threonine (Thr) and valine (Val) was highest in the *Basa*. As for the amino acid patterns relative to lysine, the proportion of Glu and His was highest in the *Tra*-p, and the other amino acids were highest in the *Basa*. The total fat weight and lipid content of the catfish bellies and the fatty acid composition of the raw oils extracted from the belly fats are shown in Table 4. The belly fat weight per catfish was highest in the *Basa* (235 g), second highest in the *Tra*-p (61 g) and lowest in the *Tra*-c (34 g), though the total lipid extractions were nearly the

Table 4. The total fat weight and the lipid content of the *Tra* (*Pangasius hypophthalmus*) and the *Basa* (*Pangasius bocourti*) catfish bellies, and the fatty acid compositions of the raw oils extracted from the belly fats in the Mekong River Delta

	Tra-c	*Tra*-p	*Basa*
Total fat weight (g/catfish)	34	61	235
Total lipid (%)	95.2	95.9	94.3
Fatty acids (%)			
C12:0 (Lauric acid)	0.6	0.1	0.0
C14:0 (Myristic acid)	5.7	5.1	1.1
C16:0 (Palmitic acid)	34.4	31.1	27.0
C16:1 (Palmioleic acid)	1.0	0.7	0.8
C18:0 (Stearic acid)	8.0	7.5	7.6
C18:1 (Oleic acid)	38.1	38.9	44.4
C18:2 (Linoleic acid)	9.7	12.9	16.7
C18:3 (α-Linolenic acid)	0.4	0.6	0.6
C20:0 (Arachidic acid)	0.2	0.3	0.2
C20:1 (Eicosenoic acid)	1.0	0.8	0.4
Satuated fatty acid, total	48.8	44.1	36.0
Unsatuated fatty acid, total	50.2	53.8	62.9

Abbreviations: see Table 1.

same at 95.9, 95.2 and 94.3% for the *Tra*-p, the *Tra*-c and the *Basa*, respectively. The total content of poly-saturated fatty acids such as lauric, myristic, palmitic and stearic acid was higher in the *Tra*-c than in the others. In contrast, the total content of the poly-unsatuated fatty acids (PUFAs) such as oleic, linoleic (LA) and α-linolenic acid (α-LA) was highest in the *Basa* (62.9%), next highest in the *Tra*-p (53.8%) and lowest in the *Tra*-c (50.2%). LA and α-LA, the essential PUFAs of the catfish oils, were especially high in the *Basa* (16.72 and 0.59%), second highest in the *Tra*-p (12.92 and 0.56%) and lowest in the *Tra*-c (9.69 and 0.41%).

DISCUSSION

The protein profiles of the *Tra*-c and the *Tra*-p under electrophoretic analysis were similar in genetic parameters such as Ho, H_{EP} and SENA. These results were consistent with the report of Gepts (1990) that protein storage was unchanged in different environments and was reliable. The Ho and SENA values of the two varieties of catfish examined were lower than those of *M. equidens*, a species of freshwater prawns (*Macrobrachium spp.*) in the same area, although such values in the polymorphic individuals were very high. Freshwater prawns with values lower than 3 of Ho or 0.6 of SENA were found to be scarce in nature (Thanh et al., 2003). Species with such low values will become extinct due to inbreeding; the gene pools of each observed population were below a suitable threshold. It was supposed, therefore, that the seeds of the collected catfish had been delivered from several dominant seed companies in the area and that only several pairs of *Tra* or *Basa* parents would be used for the seeds or for young catfish production. Thus, most progeny had the same protein bands, and the values of genetic diversity parameters were small. It was necessary to employ mass production methods quickly for catfish seed production in order to reduce dependence on the natural habitat that crosses only several pairs of parents.

Table 5. The *Tra* (*Pangasius hypophthalmus*) and the *Basa* (*Pangasius bocourti*) catfish fillets' essential amino acid patterns relative to lysine in the Mekong River Delta compared with the ideal patterns of pigs[1]

	Tra-c	*Tra*-p	*Basa*
Piglets and growing pigs			
Histidine	169	199	137
Isoleucine	135	144	249
Leucine	114	118	197
Lysine	100	100	100
Threonine	77	108	205
Valine	90	97	178
Total	*684*	*765*	*1,067*
Pregnant sows			
Histidine	186	219	150
Isoleucine	87	92	159
Leucine	154	159	266
Lysine	100	100	100
Threonine	288	406	770
Valine	59	63	117
Total	*872*	*1,039*	*1,562*
Lactating sows			
Histidine	143	168	116
Isoleucine	106	113	196
Leucine	99	103	171
Lysine	100	100	100
Threonine	242	342	648
Valine	90	97	178
Total	*780*	*922*	*1,410*

[1] The proportions of amino acids relative to lysine in Table 4 were divided by the proportions of ideal amino acid pattern for the pigs (ARC 1981).

Raw mackerel (*Scomber scombrus*), in the western coutries, has been fed to pigs and can regularly be used with the other types of feed (Gohl, 1981). The DM, CP, and EE of the mackerel were 32.5, 18.0 and 13.0%, respectively. These values are nearly the same as those of the *Tra*-c. Moreover, all of these catfish values are comparable to those of commercial fishmeal (MAFF, 1995). Lys is most likely the first limiting amino acid in practical feed for pigs (NRC 1998). The *Tra* catfish, especially the *Tra*-c, are very good sources of Lys, though the *Basa* would be the source of Met and Thr. Lys, Met and Thr contents in fishmeal with a CP concentration of 56.7-58.1% were 4.16-4.25, 1.50-1.53 and 2.28-2.34%, respectively (Degussa, 1996). The AA composition of the catfish tested was comparable to that of the above fishmeal, except for the Lys of the *Basa*, and the Met and Thr of the *Tra*-c. Table 5 shows the fillets' essential amino acid patterns relative to Lys compared with the ideal patterns of pigs (ARC, 1981). Certain imbalances of amino acid patterns were observed, *e.g.,* the Thr of the *Tra*-c was deficient for piglets and growing pigs, but it exceeded the requirements of pregnant and lactating sows. The Var of the *Tra* catfish was either deficient or tended to be deficient, but that of the *Basa* exceeded the requirements of all types of pigs. Moreover, the Ile, Lue, Thr and Val of the *Basa* greatly exceeded the requirements of all pigs, especially when compared with the amino acid patterns of the *Tra*. These results demonstrate that supplementation with the *Basa* might easily cause amino acid imbalance, *i.e.,* Lys deficiency, and the *Tha* might have more potential as the source of amino acids. The fish oil supplies PUFA, and its approximate percentage in cod-liver oil was 80%, though that in whale oil was only 40% (Gohl, 1981). The concentrations of PUFA in the tested catfish oils were highest in the *Basa* (62.9%), next highest in the *Tra*-p (53.8%) and lowest in the *Tra*-c (50.2%). The values show that these catfish, even the *Tra*-c, would be optimal sources of PUFA, comparable to some types of fish oils. LA and α-LA can convert into short-chain fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), that become beneficial to the human consumer, as they are associated with a decreased risk of cardiovascular disease (Simopoulos, 2001). EPA and DHA are found mainly in fish oils and certain marine algae (McDonald et al., 1995), and high intake of EPA and DHA is expected to improve disease resistance in pigs (Calder, 1996), too, which, in turn, may beneficially affect growth performance. LA and α-LA of the catfish oils were highest in the *Basa* (16.7 and 0.6%), next highest in the *Tra*-p (12.9 and 0.6%) and lowest in the *Tra*-c (9.7 and 0.4%). These findings are consistent with the data of Nhu (2003): 12.6% of LA and 1.48% of α-LA. The LA and α-LA content of the catfish, especially that of the *Basa,* shows that the catfish could be effective sources of essential fatty acids for both humans and pigs.

The genetic diversities of the two varieties of catfish were delineated, and the importance of the selection of parents for seed production was stressed. The nutritional values of the catfish were ascertained, and it was clarified that the nutritional values of the catfish were comparable to those of fishmeal and some types of fish oils. Then, the usage of the catfish by-product in Vietnam would be expected to increase like the other aquaculture by-products such as shrimp by product meal (Linh et al., 2003). It is recommended that the differences in catfish nutritional values by variety and management should be taken account when the catfish are formulated as pig feed.

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