Nutritional analysis of freshwater fish and shellfish for improving human nutrition in rural area in Laos

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Abstract

The nutritional composition of nine species of fish and three types of shellfish found in Nameuang Village and the capital city of Laos, Vientiane, were analysed to investigate their value as sources of food with the aim of improving nutritional status in rural Laos. These fish, except the Flying fish, had a high protein content (19–20 g/100 g sample) and quality (e.g., lysine content of 1,750–1,870 mg/100 g sample and 93.8– 97.9 mg/g protein). Their protein qualities were endorsed by amino acid score (amino acid score 100). In addition, the "small" group tended to have higher protein content than the "large" group across the fish species. In contrast, the fat content was higher in the "large" group than in the "small" group. Seasonal fluctuations in fat contents in three species, except the Walking catfish, were relevant to the reproductive cycle of the species. These results suggest that aquatic animals have high nutrient quality and are efficient/indispensable food sources for improving the nutritional status of the rural population in Laos.

Introduction

Freshwater aquatic animals such as fish and shellfish are reliable sources of essential nutrients for humans in inland countries. In the southeast Asian inlands, the major source of animal-food is obtained from freshwater aquatic animals in the Mekong River basin, which provide essential nutrients for people that subsist on cereal crops (Garaway et al. 2013; Halwart and Bartley 2014; Hortle 2007; Jams 2006). They are an excellent source of protein, fat, and micronutrients such as vitamins and minerals in an otherwise rice-based diet.

The Lao People's Democratic Republic (PDR) is one of the countries that are most dependent on these sources for daily nutrition (Pedersen et al. 2014; Phonvisay 2013). However, health challenges, such as malnutrition, are pervasive in Laos; one in four children (27%) under the age of 5 years is moderately underweight and 7% are severely underweight; of these, nearly half (44%) are moderately stunted and 19% are severely stunted (United Nations Children's Fund [UNICEF] 2012). People living in rural Laos account for more than 75% of the total population and depend on freshwater fish and other aquatic animals as a reliable source of animal proteins (Phonvisay 2013). Since there is little information about the nutritional value of the typical diet

in rural Laos, nutritional information of the available fish and shellfish is required. This study had two major objectives: to estimate the nutritional value of these foods based on previously published reports and food composition databases and to obtain preliminary information on the nutritional status of the rural population of Laos, including amino acid score.

Materials and Methods

Sample collection

This study about basic information on macro-nutritional values in major-freshwater fish and shellfish of Laos was conducted in a village, Nameuang, and two local markets in Vientiane, the capital of Laos. We selected eight groups of fish and shellfish based on the following criteria: common species found all over Laos, available throughout the year /or seasonally, and major species in the village (Table 1). The size of the samples depended on the feeding habit and supply to the village. The weight of samples varied with the species but was approximately 1 kg (on a wet weight basis) for each pooled sample from the markets. In the village, it was difficult to collect large amounts of a single pooled sample, and approximately 400 g was used for the analysis (Fujita et al. 2019).

There were differences in sizes of fish during growth. Species of fish studied included: Walking catfish (*Clarias batrachus*), Broadhead catfish (*Clarias macrocephalus*), Climbing perch (*Anabas testudineus*), and Striped snakehead (*Channa striata*). These were divided into three groups for analysis over four seasons (March, June, September, and January) throughout the year from March 2017 to January 2018. The seasons covered were the rainy season (beginning of May to end of September) and the dry season (beginning of October to end of April). Sampling was conducted at three local markets in Vientiane, which were selected due to abundance of natural fish traded directly between villagers and traders (Fujita et al. 2020). The target sample size was divided into three, and the number of fish was dependent on the sample size for each species but a minimum of 600 g (wet weight) was collected from samples pooled from each market.

Sample pre-treatment

Fresh or live samples were collected and transported on ice to the Living Aquatic Resources Research Center and stored frozen at -30 °C. Thereafter, the frozen samples were transported to the Japan International Research Center for Agricultural Sciences (JIRCAS) for nutrient composition analysis. The edible parts of fish may or may not include the head, viscera, scales, bones, and other parts according to traditional practices and depending on the species. Fillets with skin were analysed in all species except the small fish Such as, Flying barb (Pa siew) and Swamp barb C (Pa khao) which were analysed whole. Clam (Hoy kii) was analysed without the shell. Snails were prepared as follows: River snail (Hoy choup) was analysed after removal of the shell and eggs if present whereas, the Apple snail (Hoy pakkouang) was analysed after removal of the shell and internal organs (viscera).

Table 1. Name of the nutrition analysis sample[1].

No	Group name (in Lao)	Lao	English	Scientific name
Fish group				
1	Pa duk	Pa duk en	Walking catfish	*Clarias batrachus*
2		Pa duk oui	Broadhead catfish	*Clarias macrocephalus*
3	Pa keng	Pa keng	Climbing perch	*Anabas testudineus*
4	Pa khao	Pa khao	Swamp barb A	*Henicorhynchus siamensis*
5		Pa khao	Swamp barb B	*Puntius brevis*
6		Pa khao	Swamp barb C	*Rasbora aurotaenia*
7	Pa kor	Pa kor	Striped snakehead	*Channa striata*
8		Pa kor kan	Dwarf snakehead	*Channa gachua*
9	Pa siew	Pa siew	Flying barb	*Esomus metallicus*
Shell fish (Snails and Clams) group				
10	Hoy pakkouang	Hoy pakkouang	Apple snail	*Pomacea spp.*
11	Hoy choup	Hoy choup	River snail	*Viviparidae spp.*
12	Hoy kii	Hoy kii	Margaritiferidae (Freshwater bivalve)	*Unionidae spp.*

1) Sources: Fujita et al. (2019)

Proximate composition analysis

Essential food properties, such as moisture, protein, fat, carbohydrates, and ash contents, were evaluated according to the Standard Tables of Food Composition in Japan (STFCJ) (2015) based on the Association of Official Agricultural Chemists methods (Fujita et al. 2019). Each measurement was performed in duplicate. The protein content was analysed using the Kjeldahl method, and the quantity was calculated as 6.25 times the N content. Energy was calculated using Atwater's coefficient as a conversion factor: 4 for proteins and carbohydrates and 9 for fat. The total carbohydrate was calculated by subtracting measured moisture, protein, fat and ash from total weight while content was calculated from the total weight of each of the other components with the following formula: Carbohydrate (g/100 g)=100-(total moisture [g/100 g]+protein [g/100 g]+fat [g/100 g]+ash [g/100 g]).

Amino acid analysis

The amino acid composition of each sample was determined according to STFCJ (2015) using an automated amino acid analyser except for tryptophan, which was analysed by HPLC with a RF-20Axs spectrofluorometric detector (Fujita et al. 2019). The contribution of each species to the recommended nutrient intake of amino acids at each age was determined by comparisons to reference data. The amino acid score determines the effectiveness with which absorbed dietary N meets the essential amino acid requirement at a safe level of protein intake. It is based on the amount of limiting amino acid in 1 g of protein in a sample relative to the required amount (FAO/WHO/UNU 2007) using the following formula: Amino acid score = (mg of amino acid in 1 g test protein)/ (mg of amino acid requirement pattern) × 100.

Results and Discussion

Overview of proximate composition of fish and shellfish in Laos

Table 2 shows the proximate food composition, including crude protein, fat, carbohydrate, ash, moisture, and energy content of the edible parts in the 12 species studied. Total protein contents in the species of fish ranged between 19–20 g/100 g, except in Swamp barb A and Flying barb. The latter two had the lowest protein content among all fish due to inclusion of all parts of the fish, such as the head, bones, and viscera in the analysis. In contrast, the protein contents in the three types of shellfish ranged between 7.8–12.3 g/100 g, with no significant differences among species.

Focussing on specific species of fish, Climbing perch, Swamp barbs, and Flying barb, had lower water and protein contents and higher fat content (2.5–10.1 g/100 g) than the other species. With reference to previous data (James 2006; ASEAN FCD 2014), the fat content in these fish were two to seven times lower than was observed in the present study. Although the reason is not clear, previous research may have used cultured fish known to have more fat than the wild fish used in this study. Total energy varied widely among species from 79 kcal/100 g to 168 kcal/100 g according to their fat contents. The following species: Swamp barb C and Flying barb that were studied had high ash content; this may be due to the fact that their viscera, bones, and head were included in the analysis. Similarly, the high ash content of shellfish (River snail and *Margaritiferidae* members) was likely due to the inclusion of their viscera in the analysis.

Table 2. Proximate nutritional compositions in fish, snail and clam[1].

Kind	Sample name	Energy (kcal)***		Moisture (g)**		Protein (g)**		Fat (g)**		Carbohydrate (g)**		Ash (g)**	
		Village	VTE	Village	VTE	Village	VTE	Village	VTE	Village	VTE	Village	VTE
Fish	Walking catfish	101.5 ± 2.1	79.0	76.7 ± 0.6	80.0	20.6 ± 0.6	19.1	2.4 ± 0.1	0.3	0.0	0.0	1.1 ± 0.1	1.2
Fish	Broadhead catfish	—	94.0 ± 14.1	—	77.4 ± 1.7	—	19.9 ± 0.1	—	1.6 ± 1.5	—	0.2 ± 0.2	—	1.2 ± 0.1
Fish	Climbing perch	118.5 ± 0.7	125.0	74.5 ± 0.6	74.0	19.4 ± 0.2	19.2	4.3 ± 0.1	5.3	0.6 ± 0.8	0.0	1.6 ± 0.1	1.5
Fish	Swamp barb A*	—	97.0	—	77.7	—	17.8	—	2.5	—	0.7	—	1.3
Fish	Swamp barb B*	—	108.0	—	74.8	—	19.5	—	3.1	—	0.5	—	2.1
Fish	Swamp barb C*	—	168.0	—	65.5	—	19.1	—	10.1	—	0.2	—	5.1
Fish	Striped snakehead	88.0 ± 0.0	83.5 ± 3.5	77.3 ± 0.1	79.1 ± 0.8	20.7 ± 0.3	19.9 ± 0.6	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	1.4 ± 0.1	1.2 ± 0.0
Fish	Dwarf snakehead	87.0	—	77.6	—	20.9	—	0.4	—	0.0	—	1.4	—
Fish	Flying barb	93.5 ± 17.7	114.0 ± 11.3	76.8 ± 0.9	74.8 ± 2.0	14.7 ± 1.3	14.9 ± 1.7	3.7 ± 2.6	5.8 ± 0.4	0.4 ± 0.0	0.6 ± 0.3	4.5 ± 0.4	4.0 ± 0.4
Snail	River snail	59.0 ± 2.8	66.0	82.7 ± 0.6	80.4	8.7 ± 0.1	9.6	0.5 ± 0.0	0.6	4.9 ± 0.9	5.6	3.2 ± 0.1	3.8
Snail	Apple snail	55.5 ± 10.6	70.0 ± 7.1	85.0 ± 3.0	80.8 ± 1.2	10.3 ± 1.6	12.3 ± 1.1	0.5 ± 0.1	0.5 ± 0.1	2.6 ± 0.9	4.4 ± 0.6	1.8 ± 0.4	2.3 ± 0.5
Clam	Margaritiferidae	52.0	83.0	85.1	77.1	7.8	8.8	0.7	0.9	3.7	10.0	2.7	3.2

Values are the mean ± standard deviation, except some samples, which are shown as a value n = 1. The all value show g or kcal/100g (wet weight basis). * Swamp barb A: *Henicorhynchus siamensis*; B: *Puntius brevis*; C: *Rasbora aurotaenia*. ** The value shows mg/100g sample (wet weight basis). *** The value shows kcal/100g sample (wet weight basis).
1) Sources: Fujita et al. (2019)

Seasonal and size- differences of nutrition composition in four fish species

There were differences in proximate composition relative to size and season. Significant increases in protein and ash content were recorded as fish size changed from large to small. In contrast, fat content increased with increasing fish size (Fig. 1). These findings suggest a significant relationship between these proximate compositions and fish size.

The seasonal differences in the proximate compositions of the four fish species are shown in Fig. 1. The tendencies towards seasonal differences were weak compared to those of size-dependent differences. Filet protein content was lowest in March, while fat content of the filet was highest in March, except for Striped snakehead.

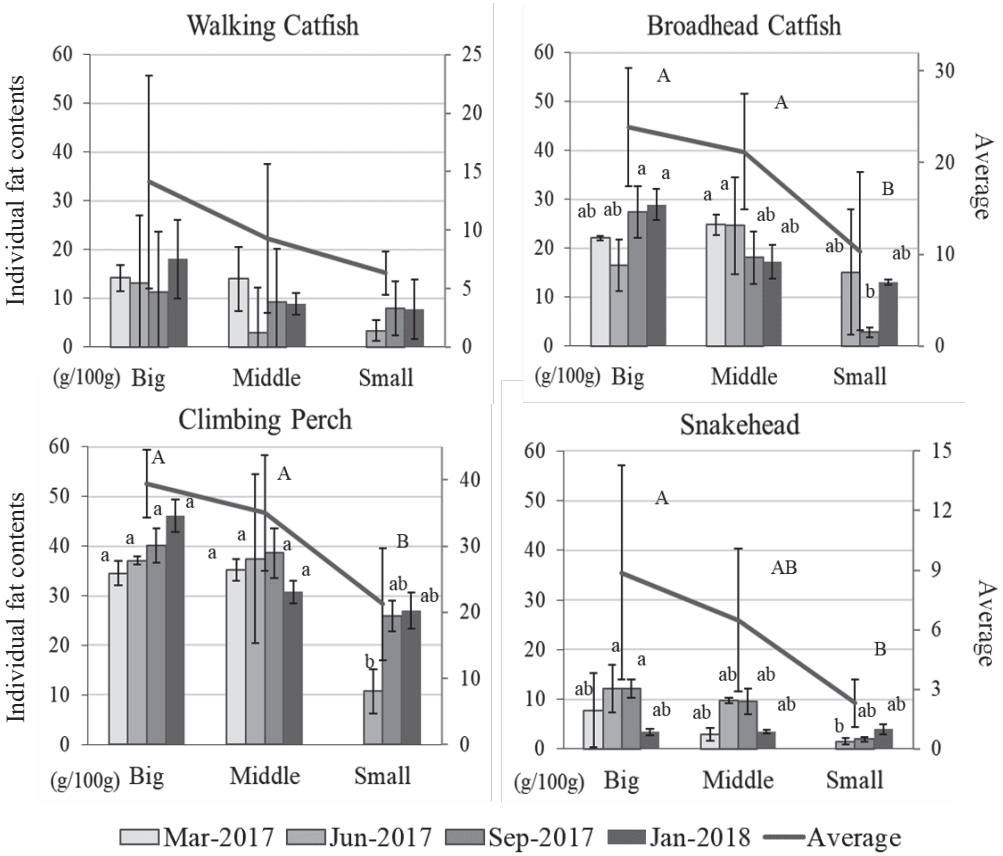

Fig. 1. Comparison of fat content (g/100 g) in the filets of four fish species (dry weight basis) (mean ± SD) by seasons and sizes[1].
The different characters show significant differences (P＜0.01); uppercase letters show significant differences in the averages, lowercase letters show significant differences in individual seasons. 1) Sources: Fujita et al. (2020)

Amino acids properties of fish and shellfish

The amino acid composition of each sample is shown in Table 3 (Fujita et al. 2019). Lysine is an essential amino acid for human, especially in rural areas of Asia where there is predominance of rice farming and consumption, which may cause dietary deficiency in this amino acid (Vasal, 2002). Notably, the lysine content of Walking catfish and Striped snakehead were higher than the previously reported values by Nurhasan et al. (2010). In this study, the lysine content of fish (1,330–1,870 mg/100 g sample, 82.6–97.9 mg/g protein) was higher than that of shellfish (580–680 mg/100 g sample, 59.6–76.1 mg/g protein). It is also clarified that the lysine content of fish is higher than in livestock animal proteins such as rib roast of lean and fat meat in beef (83–87 mg/g), loin of lean and fat meat in pork (88 mg/g) and chicken portions (78–99 mg/g) (STFCJ 2015). As regarding to the other amino acids, these sample had also rich essential amino acids,

such as leucine, valine and threonine, and they were also richly non-essential amino acids such as testy components, in particular high amount of glutamic acid and aspartic acid.

Protein quality can be predicted based on the indispensable amino acid score from a comparison of the pattern of its amino acid composition to the pattern of human amino acid requirement (FAO 2013). The scores were calculated for each age category based on the new amino acid scoring pattern (WHO/FAO/UNU 2007). The analysed fish and shellfish samples in the present study were rich in essential amino acids and showed a good balance of protein in all categories for each age with appropriate amino acid scores (>100, data not shown), particularly for children from the age of 1 to 18 years. As previously mentioned, lysine is a critical component of the typical diet in rural Asia where milled rice—which is low in lysine, an essential amino acid in protein synthesis—is the staple food (STFCJ 2015). Thus, aquatic animals consumed by the people of Nameuang village can potentially prevent malnutrition.

Table 3. Amino acid compositions in fish, snail and clam[1].

Sample name	Walking catfish		Climbing perch		Striped snakehead		Flying barb		River snail		Apple snail		Margaritiferidae	
	mg/g*	mg/100g**	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g	mg/g	mg/100g
Essential amino acids														
Histidine	22.5	430	23.4	450	24.6	480	28.6	460	22.9	220	18.4	210	20.5	180
Isoleucine	46.1	880	42.2	810	44.1	860	41.0	660	38.5	370	38.6	440	43.2	380
Leucine	83.2	1590	78.6	1510	80.5	1570	74.5	1200	76.0	730	80.7	920	76.1	670
Lysine	97.9	1870	91.1	1750	93.8	1830	82.6	1330	60.4	580	59.6	680	76.1	670
Methionine	29.8	570	28.6	550	30.3	590	26.7	430	19.8	190	17.5	200	25.0	220
Cysteine	9.9	190	9.9	190	10.3	200	9.3	150	12.5	120	10.5	120	12.5	110
Phenylalanine	44.0	840	43.2	830	43.1	840	41.0	660	40.6	390	37.7	430	37.5	330
Tyrosine	34.0	650	31.8	610	33.8	660	31.7	510	34.4	330	32.5	370	34.1	300
Threonine	49.2	940	45.3	870	45.1	880	41.6	670	46.9	450	44.7	510	45.5	400
Tryptophan	9.9	190	9.9	190	10.3	200	9.3	150	12.5	120	10.5	120	11.4	100
Valine	50.3	960	47.4	910	47.2	920	45.3	730	46.9	450	45.6	520	46.6	410
Non-essential amino acids														
Arginine	67.5	1290	65.6	1260	64.1	1250	61.5	990	76.0	730	85.1	970	72.7	640
Alanine	66.0	1260	69.3	1330	64.6	1260	65.8	1060	55.2	530	61.4	700	51.1	450
Glycine	73.3	1400	74.0	1420	60.5	1180	70.8	1140	60.4	580	70.2	800	46.6	410
Proline	44.0	840	44.8	860	38.5	750	44.7	720	45.8	440	49.1	560	35.2	310
Glutamic acid	161.8	3090	152.1	2920	155.9	3040	144.1	2320	144.8	1390	160.5	1830	145.5	1280
Aspartic acid	109.4	2090	102.6	1970	104.1	2030	96.3	1550	105.2	1010	102.6	1170	95.5	840
Serine	43.5	830	41.7	800	42.1	820	39.8	640	45.8	440	47.4	540	47.7	420

*** mg/g protein, ** mg/100g sample**

1) Sources: Fujita et al. (2019)

Conclusions

The purpose of this study was to evaluate the nutrient composition of fish and shellfish species consumed in Laos with a specific focus on rural areas. The results demonstrate that the aquatic animals analysed here, in particular fish such as the Broad catfish, Climbing perch, and Striped snakehead, had high protein contents and quality with appropriate amino acid scores. These types of fish also had high lysine contents. Although their supply in rural areas is currently considered insufficient, increase in supply through promotion of fishery/aquaculture may contribute to reduction in malnutrition caused by protein deficiency.

Moreover, because these freshwater fish and shellfish species are widely consumed inland globally, these data may also be useful from a nutritional public health standpoint for improving the nutritional status in rural areas in other developing countries. In addition, the Climbing perch had the highest fat content compared to the other three species. This information is useful from a public health perspective for residents in the Indochinese regions where these species are frequently consumed. Besides the four species above, there are other small-sized trash fishes, e.g., *Esomus metallicus*, *Rasbora spp*. and *Puntius brevis* of *Cyprinidae* (K. Fujita, unpubl. data). Others include *Parambassis siamensis* of *Ambassidae* and *Trichopsis spp*. of *Osphronemidae* (S. Morioka, unpubl. data). They are all important daily food sources particularly in mountainous areas of rural Laos, but their nutritional value is yet to be analysed. Since insufficient animal protein intake for residents of rural Laos is a major concern, nutritional evaluations of these trash fishes are also important for improving nutritional status in these areas.

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