PROXIMATE COMPOSITION AND FATTY ACID PROFILE OF ANURANS MEAT

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ABSTRACT. The objective of this study is to analyze the proximate and fatty acid composition of anurans meat from local (*Limnonectes leporinus*) and exotic species (*Rana catesbeiana*). Comparison of both leg meat portions of the anurans were also made with chicken's leg muscle. Between the local versus exotic anuran species, there was significant difference ($p<0.05$) in the proximate composition. The result showed that *L.* leporinus had a lower content in ash, fat and protein (0.48%, 0.27% and 14.69% respectively). In distribution of fatty acids classes, *L. leporinus* had lower proportions of SFA (38.73%) and MUFA (22.57%) and conversely higher proportion of PUFA (38.24%) than those of *R. catesbeiana* which had 38.91% SFA, 33.52% MUFA and 27.57% PUFA. The main SFA were palmitic acid (C16:0) and stearic acid (C18:0) while oleic acid (C18:1) and linoleic acid (C18:2) were the dominant MUFA and PUFA. The PUFA:SFA ratio for *R. catesbeiana* and *L. leporinus* were 0.71 and 0.98 respectively. Compared to chicken meat, anuran meat is an excellent nutritional source of protein (14.69%-18.77%) and low in fat (0.27%-0.64%), whereas the chicken meat had lower protein content (14.48%) and significantly higher fat content 1.51% ($p<0.05$).

KEYWORDS. *Anurans, Limnonectes leporinus, Rana catesbeiana, proximate composition, fatty acids*

INTRODUCTION

Anurans are amphibians in the Order Anura which stands for tailless amphibians, or simply frogs and toads. The part mainly eaten is their meaty hind legs, which resemble a small chicken drumstick. Two species that are non-native to Malaysia and farmed commercially for consumption are the North American bullfrog (*Rana catesbeiana*) and the Taiwanese frog (*Holoplobatrachus rugulosus*) (John, 2005; Inger & Tan, 1996).

The consumption anuran meats are rising in many countries. The main producers are Taiwan, Indonesia, Brazil and Mexico while countries such as USA, France, Canada, Belgium, Italy and Spain are the major importers of anurans (Patel, 1993; FAO, 2007). Hence, farming for *Rana catesbeiana* are increasing in recent years. In Peninsular Malaysia, the population of domesticated anuran had increased 260.9% from 1 072 000 in 2003 to 3 869 000 colony of frogs in 2004 (Department Of Veterinary Services Malaysia, 2006).

In Sabah, there are about 100 species found in Borneo (Inger & Stuebing, 2005). Local anurans from the genera *Limnonectes* and *Fejervarya* are utmost favoured. The consumption and collection for trading have been done by local people living in West

Coast and Kudat Divisions of Sabah (Kueh, 2006). *Limnonectes leporinus* or Giant river frog lives along banks of medium to large-sized streams in the primary and disturbed forest (Inger & Stuebing, 2005). It is commonly sought due to its palatability and greater muscle mass. Adults *L. leporinus* can grow up to 90-125 mm in snout-vent length (SVL) compared to other edible anurans such as *L. khulii* (44-67 mm SVL), *R. erythreae* (32-75 mm SVL), and *F. cancrivora* (51-82 mm SVL) (Inger & Stuebing, 2005).

Though anuran meat has been consumed by the local people for a long time and with numerous claims made regarding its nutritional values, but there is no not evidence by scientific works. Although some analyses were performed on *Rana esculenta* and *Rana catesbeiana* (Nobrega *et al*., 2006; Mendez *et al.*, 1998; Yalcin *et al.*, 1995), but information on the nutritional composition of anuran meat from species found in Sabah is rarely found. Thus, the determination of nutrient composition of *Limnonectes leporinus* and *Rana catesbeiana* found in Sabah was carried out. Both of the anurans meat was also compared with nutrient content in chicken.

MATERIALS AND METHODS

Sample preparation

The anuran was identified by its feature properties i.e. webbing, skin color, shape, size, eardrum and mouth. Only the adult specimen were captured and slaughtered. The local species *Limnonectes leporinus* was captured from Sungai Togong at Kampung Kelingau and Sungai Dunkat at Kampung Pulatan. While, the exotic species, *Rana catesbeina* was collected from local frog's farm at Inanam. The local farmed chicken's thigh meat was purchased from the cold storage shop.

Meat from the leg portion was separated, skinned and debonned manually. The meat was then washed, packed in polypropylene container and then stored at -18ºC until analyzed. During analyses, the meat was placed overnight in refrigerator at 4ºC to thaw and homogenized using Waring blender.

Proximate analysis

Moisture was determined by air drying (ISO 1442, 1997) and ash from the incinerated residue (ISO 936, 1998). Crude protein content was calculated by converting the nitrogen content, determined by the Kjeldahl method (6.25 x N) (ISO 937:1978) and fat by Soxhlet extraction using diethyl ether (ISO 1443: 1973).

Determination of fatty acid composition

The total lipids were isolated from anuran meat according to the method by Kinsella *et. al* (1977), using a chloroform:methanol (2:1, v/v) solvent system. Fatty acid methyl esters (FAME) was prepared by transesterification according to standard ISO 5509 (2000). The FAMEs were analysed using Agilent 6890 series GC system instrument equipped with FID detector and DB-WAXTER column (J & W Scientific, 30 m X 0.25 mm X 0.25 µm). Operating conditions were: a helium flow rate of 17.2 mL/min, a FID detector at 250 ^{o}C, and a split-splitless injector at 250 ^{o}C with an injection volume of 1 µL. The temperature programme of the column was 50^{o}C for 1 min, to 200^{o}C at 18^{o}C/min to 240^{o}C at 1^{o}C/min and held for 18 min. The individual fatty acid peaks will be identified by comparison of

retention times and peak areas with those of known mixtures of FAMEs standard (Sigma, 18918) run under the same operating conditions.

Statistical analysis

Data were analyzed using one-way ANOVA and differences between samples means separated by Tukey's test.

RESULTS AND DISCUSSION

Proximate Analysis

The results for proximate analysis of *R. catesbeiana*, *L. leporinus* and chicken meat are presented in Table 1.

Table 1 Proximate analysis of anurans and chicken meat (wet weight basis)

Sample	Proximate value			
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
Chicken	$76.03^{a} \pm 0.15$	$0.92^{c} \pm 0.02$	$14.48^{a} \pm 0.18$	$1.51^{c} \pm 0.05$
Rana catesbeiana	$77.73^{b} \pm 0.30$	$0.87^{b} \pm 0.02$	$18.77^{b} \pm 0.20$	$0.64^{b} \pm 0.02$
Limnonectes leporinus	$82.87^{c} \pm 0.11$	$0.48^{a} \pm 0.02$	$14.69^{a} \pm 0.10$	$0.27^{a} \pm 0.01$

Data are expressed as means ± standard deviation. Means on the same column with different superscript are significantly different ($p<0.05$).

There is a significant different ($p<0.05$) in moisture contents between *R. catesbeiana* (77.73 ± 0.30%) and *L. leporinus* (82.87 ± 0.11%). The ash content in *R. catesbeiana* meat was 0.87% which is within the common range of ash present in meat and poultry (0.7-1.3%) (Nielsen, 1998). However, *Limnonectes leporinus* had significantly lower ash content with 0.48 ± 0.02%.

The protein content in anurans meats were 14.69% and 18.77% for *L. leporinus* and *R. catesbeiana* respectively. This present study showed a lower protein content in *R. catesbeiana* compared to a study conducted by Nóbrega *et al.* (2007) with 19.4%. However, in another study carried out by Olevera-Novoa *et al.* (2007), a lower protein range were obtained (13.87%-14.52%). In that study, *R. catesbeiana* were fed with the experimental diet containing dietary protein of 42-58%. It is apparent that the wild local anurans which did not receive supplementary feeds had a lower protein content than those farmed *R. catesbeiana*. The variations in protein content of anurans meat can be due to a number of factors such as types of species, diet, age, location, and portion of meat sampled (van Heerden *et al*., 2002; Sales & Hayes, 1996).

The anurans meat had a low fat content which was of less than 1.0% and is similar to that reported by Nobrega *et al.* (2006). *L. leporinus* (0.27 ± 0.01%) showed significant lower ($p<0.05$) fat content than *R. catesbeiana* (0.64 ± 0.02%).

In this study, edible anurans were analyzed and compared with chicken meat. Previous study has identified the three most potent aroma compounds found in *R. catesbeiana* that were similar to chicken which consist of unsaturated aliphatic aldehydes, in particularly alkadienals: (E-E)-2,4-decadienal, (E,Z)-2,4-decadienal and (E,Z)-2,6-nonadienal (Nóbrega *et al.*, 2006).

Moisture content of chicken meat (76.03 ± 0.15%) was significantly lower ($p<0.05$) than both the anuran meat (77.73%-82.87%) but higher ($p<0.05$) in fat and ash content when compare to the edible portion of anuran. Chicken meat had 14.48 ± 0.18% of protein which was lower compared to anurans.

Fatty Acid Composition

Fatty acid composition has a considerable effect on health. Meat fat basically comprises mostly monosaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). Table 2 showed the percentage of fatty acids composition of each meat. The major saturated fatty acids were palmitic acid (16:0) and stearic acid (18:0) while palmitoleic acid (16:1) and oleic acid (18:1) were the main monounsaturated fatty acids; and linoleic acid (18:2) and linolenic acid (18:3) were the dominant polyunsaturated fatty acid in these three types of meat sample. These data was similar to the result reported by Mendez *et al.* (1998) and van Heerden *et al*. (2001). However, the average value of each of the fatty acids composition in the meats sample with other published data vary because of the numerous factors which can affect fatty acids composition of each meat, i.e, geographical location, age, sex, diet, physiological, acclimatization, part of carcass used, etc (van Heerden *et al*., 2001; Mendez *et al*.,1998; Lauridsen *et al*.,1997; Scapin *et al*.,1990).

There were significant difference ($p<0.05$) observed between chicken meat and anurans meat except for myristic acid and linolenic acid. The content of palmitic acid and myristic acid in all meat samples were relatively low. Myristic and palmitic acids are main fatty acids that raise total and low-density lipoprotein (LDL) cholesterol (Valsta *et al*., 2004; Chizzolini *et al*, 1999; Enser *et al*., 1997).

The concentration of monosaturated fatty acids was higher in both chicken (55.95%) and *R. catesbeiana* (33.52%) which were farmed when compared to that in wild-captured *L. leporinus* (22.57%). The increased was caused by increased in oleic acid (C18:1) which may be attributed to fatty acid in feed diet and the increase is also possible to occur at the expense of stearic acid (C18:0) (Padre *et al.*, 2006; Valsta *et al*., 2005). In contrast, the concentration of PUFA in both muscles was lower in chicken and *R. catesbeiana*. The similar result was also shown in a study carry out by Srinivasan *et al.* (1998) between grain-supplemented cattle and grass-fed cattle.

Table 2. Fatty acid composition of total lipid in anurans and chicken meat

Fatty acids	Mean ± sd %		
	***Gallus gallus* (chicken)**	***Rana catesbeina***	***Limnonectes leporinus***
Lauric acid (C12:0)	0.05±0.01	n.d	n.d
Myristic acid (C14:0)	0.49±0.03[a]	0.85±0.08[b]	0.36±0.09[a]
Palmitic acid (C16:0)	14.69±0.16[a]	18.97±0.46[b]	21.52±1.45[c]
Palmitoleic acid (C16:1)	8.99±0.15[a]	4.77±0.48[b]	1.32±0.07[c]
Stearic acid (C18:0)	5.00±0.11[a]	7.65±0.34[b]	11.50±0.44[c]
Oleic acid (C18:1)	46.96±0.04[a]	28.75±1.16[b]	21.25±1.12[c]
Linoleic acid (C18:2)	23.15±0.18[a]	26.43±0.27[b]	34.77±0.85[c]
Linolenic acid (C18:3)	0.71±0.00[a]	1.14±0.48[a]	3.47±0.34[c]
Behenic acid (C22:0)	n.d	7.45±1.00[b]	2.22±0.45[c]
Lignoceric acid (C24:0)	n.d	3.99±0.53[b]	3.13±0.22[c]
SAT[1]	20.23	38.91	38.73
MUFA[1]	55.95	33.52	22.57
PUFA[1]	23.86	27.57	38.24
PUFA : SAT	1.17	0.71	0.98

[1] SAT, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid
[2] The means followed by different letters in the same row indicate a significant different ($P<0.05$)
[3] n.d.: not detected

Anurans are poikilothermic animals and are susceptible to environmental alterations. In this study, it was observed that the monounsaturated fatty acids in anurans meat was significantly lower than chicken meat. According to Scapin *et al*. (1990), the poikilothermic vertebrates would increase the content of shorter chain as well as monounsaturated fatty acids if environment was low in temperature to maintain its membrane fluidity. Since the anurans meat in this study was caught in warmer temperature it might resulted the anurans meat had more saturated fatty acids.

In the ratio of polyunsaturated fatty acids to saturated fatty acids (P: S value), *Gallus gallus, Rana catesbeiana* and *Limnonectes leporinus* are 1.17, 0.71 and 0.98 respectively. This ratio is important because the low ratio of P: S would indicate higher risk of cardiovascular disease (Hoffman & Wiklund, 2006). The present study showed that the chicken meat and anurans meat had a P: S value beyond the recommended ratio of not less than 0.4. The normal P:S ratio for meat is around 0.1 (Wood *et al*., 2003).

CONCLUSION

The anurans meat has good nutritional value especially high protein and low fat. Between anuran species, the local species i.e. *Limnonectes leporinus* had lower fat and higher essential fatty acids compare to exotic anurans, *R. catesbeiana*. The farmed *R. catesbeiana* however has higher protein content. Despite the higher saturated fatty acid content, the equal-sized portion of anuran meat compare to beef, lamb, or pork has lower levels of saturated fats due to the lower total fat content. Since the introduced *R. catesbeiana* caused the decline of native anurans populations, *L. leporinus* may possess fervent potential for farming and cottage industry in alternative food.

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