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Potential Use of Proximate and Fatty Acid Composition to Distinguish Between Cultured and Wild Largemouth Bass (*Micropterus salmoides*)

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Abstract: Cultured largemouth bass (LMB) cannot be sold as food in some lucrative markets due to regulatory restrictions that protect wild LMB. Distinguishing between cultured and wild fish could open food markets for cultured fish. Wild LMB eat freshwater fish and cultured LMB eat diets high in marine fish meal and oil, which should produce differences in flesh composition. We analyzed the proximate and fatty acid composition of wild and cultured LMB muscle to determine the potential for distinguishing fish origin analytically. Protein and moisture were higher in wild fish, while lipid was higher in cultured fish. The n-3 and n-6 fatty acids, long-chain polyunsaturated fatty acids (LC-PUFA), and the ratio of n-3 to n-6 fatty acids all differed between cultured and wild fish. The n-3 to n-6 ratio and n-3 LC-PUFA were higher in cultured fish, while elevated arachidonic acid (20:4n-6) in wild fish was a key distinguishing feature.

Keywords: Arachidonic acid, Fatty acids, Largemouth bass, Lipid, Proximate, Wild and cultured.

INTRODUCTION

The largemouth bass (LMB)(*Micropterus salmoides)* is native to the midwestern and southeastern United States and northeastern Mexico, and has been introduced throughout the USA and other countries. This species is economically important as one of the most popular sportfishes in the USA. It is also valuable as a foodfish sold live in fish markets patronized by Asian customers in major cities in the US and Canada. With the dual importance of maintaining wild populations of LMB and satisfying foodfish markets, state wildlife managers are concerned that wild LMB will be caught and sold as cultured foodfish. This would deplete wild stocks, so many states prohibit the sale of all LMB to protect the sportfishing industry [1]. The development of a test that would distinguish cultured from wild LMB unambiguously could provide the basis for regulations that would allow the sale of cultured LMB while protecting wild populations.

Diets of wild and cultured LMB are distinctive, which might produce differences in body composition. Fatty acid composition has been used successfully to distinguish cultured and wild members of other fish species [2-4]. The fatty acid composition of fish tissues correlates directly with dietary fatty acids [5, 6]. Currently, culture of LMB depends mostly on salmonid diets that contain high levels of marine fish meal and oil [7]. These diets contain high concentrations of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), including eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). Previous growth trials with LMB fed a commercial trout diet showed that LMB retain these LC-PUFA preferentially in the tissues [8]. Freshwater wild fish generally contain higher levels of C-18 fatty acids (such as linolenic acid, 18:3n-3), but also substantial concentrations of EPA and DHA [9-12]. In addition, freshwater fish are characterized by high proportions of n-6 fatty acids such as linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) [13]. Freshwater algae, crustaceans, and aquatic fish larvae are rich in 18:2n-6, 18:3n-3, and 20:5n-3, although there are considerable differences among species, seasons, and geographical locations [14-16]. Largemouth bass have a limited ability to modify dietary fatty acids, and the fatty acids in the diet are invariably reflected in the fatty acid profile of the tissues. Therefore, we analyzed the proximate and fatty acid composition of the muscle of cultured and wild LMB to determine the feasibility of this approach.

MATERIALS AND METHODOLOGY

Seven cultured LMB were obtained live from a producer who raises fish in outdoor ponds with a commercial diet similar to those used for trout. The labeled composition of the diet (Skretting USA, formerly Silvercup, Murray, Utah, USA) was 45% protein and 20% lipid. Most of the protein and lipid was from marine fish meal and oil, respectively, and the majority of largemouth bass producers in the region (midwestern and southeastern United States) used this diet.

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Table 1. Hepatosomatic Index (HSI) and Proximate Composition (g/100g) of the Muscle of Cultured and Wild LMB[a].

Analyzed composition	Cultured	Wild
HSI[b,c]	0.78 ± 0.06	0.81 ± 0.07
Moisture	76.7 ± 0.5**	79.6 ± 0.1*
Protein[c]	18.1 ± 0.1**	18.9 ± 0.1*
Lipid[c]	3.1 ± 0.2*	0.9 ± 0.0**
Ash[c]	1.1 ± 0.02	1.1 ± 0.03

[a]The values are means ± SEM of 6-7 fish per treatment. A student's *t*-test was performed to compare the difference in the means. Means within rows with different numbers of asterisks (*) are significantly different ($P \leq 0.05$).
[b]Hepatosomatic index (HSI) = Liver weight (g)/ body weight (g) x 100.
[c]Wet weight basis.

The diet contained key LC-PUFA including 20:4n-6 (2.0%), 20:5n-3 (13.8%), and 22:6n-3 (5.3%). Seven wild LMB were caught by hook and line in the Arkansas River near Pine Bluff, Arkansas. Both wild and cultured fish were collected during June and July, 2005. The cultured fish were in their second growing season (about 18 months old). All wild and cultured fish were marketable size (393-793 g). Mean (SEM) weights of whole fish were 502.6 (93.4) and 564.4 (122.3) g for farmed and wild fish, respectively, and were not statistically different (P>0.05).

Fish were handled according to the Guidelines for the Use of Fishes in Research [17]. Fish were euthanized with an overdose of MS-222 and kept completely covered with ice until dissection (up to 2 hours). Individual weights of whole fish were determined and the fillets were removed and stored frozen until proximate and fatty acid analysis. The livers were removed and weighed, and the hepatosomatic index (HSI) was calculated as liver weight (g) / body weight (g) x 100. The muscle from each fish was homogenized separately prior to analysis. Proximate analysis of muscle was conducted on at least six fish per treatment (cultured or wild), and fatty acid analysis was conducted on muscle from four fish per treatment. Muscle samples were analyzed for nitrogen to determine protein content using the Kjeldahl method, and dry matter and ash were analyzed using standard methods [18]. Total lipids from muscle were extracted and quantified with chloroform and methanol [19], then saponified and methylated using potassium hydroxide and boron trifluoride prior to fatty acid analysis [20, 21]. Fatty acid methyl esters were quantified using a flame ionization gas chromatograph (Varian, Model CP3800, Walnut Creek, California, USA) equipped with a capillary column (50 m x 0.25 mm) (Varian CP Select for Fame #CP7420) as described previously [20, 21]. Concentrations of the fatty acids were expressed as grams/ 100 g to perform the calculations. Data were subjected to an unpaired student's *t*-test to determine differences ($P \leq 0.05$) between treatment means.

RESULTS AND DISCUSSION

The percentage of total lipid in muscle of cultured LMB (3.1%) was significantly higher than that of wild LMB (0.9%) (Table **1**). Cultured fish usually receive a commercial diet on a regular basis, but wild fish forage in areas where prey availability varies. In general, wild fish are more susceptible to periods of inadequate energy intake. Therefore, cultured fish frequently contain a higher concentration of lipid than wild fish [22-24]. Muscle lipid in wild and cultured red porgy (*Pagrus pagrus*) was 0.65% and 3.03%, respectively [25], which is comparable to our results. Similarly, muscle lipid in wild and cultured sharpsnout sea bream (*Diplodus puntazzo*) was 1.73% and 3.87%, respectively [2]. Fillets of cultured salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), and channel catfish (*Ictalurus punctatus*) are all higher in lipid than their wild counterparts [26]. Nevertheless, based on our data and the classification cited by Ackman [27] both cultured and wild LMB can be considered 'lean'. Moisture and protein in muscle of wild LMB were higher than those of cultured LMB, but muscle ash and hepatosomatic index did not differ between treatments (Table **1**). The sizes of cultured and wild LMB in this study were similar, so differences in proximate composition were most likely due to diet and possibly other environmental factors, but not fish size [28, 29].

Cultured and wild LMB could be distinguished on the basis of muscle saturates, monounsaturates, total n-3 and n-6 fatty acids, total n-3 LC-PUFA, and the ratio of n-3 to n-6 fatty acids (Table **2**). Of the individual fatty acids, arachidonic acid (20:4n-6) was exceptionally high in the wild fish (12.6 g/ 100g) compared to the cultured fish (2.9 g/ 100g). Similar results were obtained in red porgy [25] and gilthead sea bream (*Sparus aurata*) [3]. Concentrations of 20:4n-6 are low in cultured fish because practical dietary lipid sources such as marine fish oils contain small amounts of this fatty acid [5]. In contrast, the 20:5n-3 was lower in wild bass (1.8 g/ 100g) than in the cultured ones (7.1 g/ 100g). This difference is also attributable to the use of marine fish oils in practical diets, which are rich in 20:5n-3. The natural diets of wild freshwater fish such as largemouth bass contain more 22:6n-3 than 20:5n-3, since 22:6n-3 is the predominant n-3 LC-PUFA in insects, other invertebrates, and the fish that consume them [28, 30]. The concentration of DHA did not differ between wild and cultured fish, which is consistent with the maintenance of DHA at a physiologically optimal level regardless of dietary concentrations [31, 32]. Total n-3 fatty acids and n-3 LC-PUFA were higher in cultured than in wild LMB, which reflects the marine fish meal and oil content of commercial diets typically fed to cultured LMB.

Table 2. Fatty Acid Composition of Muscle (g/100g of total fatty acids by weight) of Cultured and Wild Largemouth Bass[a,b].

Fatty Acids	Cultured	Wild
16:0	14.4 ±(0.2)	15.6 ± (0.8)
18:0	4.0 ± 0.3**	7.8 ± 0.2*
Saturates[c]	27.3 ± 0.5**	29.7 ± 0.8*
16:1	6.8 ± 0.4*	3.9 ± 0.7**
18:1[d]	26.9 ± 0.9*	20.1 ± 2.9**
MUFA[e]	36.7 ± 1.6*	27.5 ± 3.4**
18:2n-6	8.3 ± 0.1*	5.3 ± 0.3**
18:3n-3	1.1 ± 0.1**	3.1 ± 0.3*
20:4n-6	2.9 ± 0.3**	12.7 ± 1.3*
20:5n-3	7.1 ± 0.3*	1.8 ± 0.2**
22:6n-3	15.9 ± 0.6	16.5 ± 1.5
∑ n-6[f]	12.0 ± 0.4**	21.6 ± 2.6*
∑ n-3[g]	29.1 ± 0.7*	25.1 ± 2.1**
∑ n-3 LC-PUFA[h]	27.9 ± 0.7*	22.0 ± 2.1**
n-3/n-6 ratio	2.4 ± 0.1*	1.2 ± 0.1**

[a]Values are means ± SEM of 4 fish per treatment. A student's *t*-test was used to compare treatment means. Means within rows followed by different numbers of asterisks (*) were significantly different ($P \leq 0.05$).
[b]Fatty acids detected at ≤ 0.1 g/ 100g of total fatty acids by weight are not included.
[c]Saturates included 14:0, 16:0, 18:0 and 20:0.
[d]Total n-9 and n-7 isomers.
[e]Monounsaturates included 14:1, 16:1, 18:1 and 20:1.
[f] Total n- 6 fatty acids included 18:2n-6, 20:3n-6 and 20:4n-6.
[g] Total n-3 fatty acids included 18:3n-3, 20:5n-3 and 22:6n-3.
[h]Total n-3 LC-PUFA included 20:5n-3 and 22:6n-3. The only n-6 LC-PUFA detected was 20:4n-6.

CONCLUSION

There were numerous differences in both proximate and fatty acid composition of cultured and wild LMB. Differences in muscle lipid composition and fatty acid composition (especially arachidonic acid) could be used as key determinants of fish origin. Application of this assay to test fish samples from markets would ensure that wild bass populations are protected. These results are based on a small sample of LMB consisting of cultured fish from a single farm and wild fish from a known location in the same region. Additional studies with larger numbers of fish are needed to determine how consistent and robust these differences are with respect to geographical location, season, and diet.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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