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Fishmeal-based diet decreases the redness of sunshine bass (*Morone chrysops* × *Morone saxatilis*) fillets☆

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ABSTRACT

The objectives of the present study were to determine the effects of feeding a fishmeal-based diet on color attributes and lipid oxidation in sunshine bass (*Morone chrysops* × *Morone saxatilis*) fillets during retail display. A balanced diet containing 30 percent fishmeal (FM) or a diet containing poultry byproduct meal as a complete replacement of fishmeal (PB) was fed to sunshine bass for fifteen months. Harvested fish were filleted, overwrapped with polyvinyl chloride film and stored at 2 °C (REF) or over ice (ICE), under an illuminated retail display. Samples ($n = 6$) were analyzed after 0, 3, 6, or 9 d storage for color attributes (CIE L^*, a^*, b^*, hue angle and chroma), thiobarbituric acid-reactive substances (TBARS), and pH. TBARS and pH increased ($P < 0.05$) during storage, indicating progress in lipid oxidation and protein changes. FM fillets demonstrated lower ($P < 0.05$) a^* (redness) value and greater ($P < 0.05$) hue angle than PB fillets. Since consumer acceptance of sunshine bass is dependant upon its white flesh, fishmeal supplementation could be used as a dietary strategy to improve fish marketability.

1. Introduction

Due to consumers' recognition of the health effects of fish consumption, the demand for fish has been steadily increasing in the United States. The realization that wild-caught fish is inadequate to meet the demand led to the rapid growth of commercial aquaculture (FAO, 2008; Fowler, Karahadian, Greenberg, & Harrell, 1994). Sunshine bass (*Morone chrysops* × *Morone saxatilis*) is a cost-effective commercial aquaculture species in the southeastern United States, with excellent adaptability to temperate regions (Boyd, Green, & LePors, 1992; USDA, 2004). In 2005, the total production of sunshine bass in the United States was 4.9 million kilograms, generating a revenue of $27.65 million (USDA-NASS, 2007). Sunshine bass is sought-after by the consumers because of its firm and white flesh, mild flavor, and low fat content (Boyd et al., 1992).

Carnivorous fish, such as sunshine bass, are predators preying on aquatic fauna for high-quality protein. Therefore, protein-rich diets (low in carbohydrate and high in fat) are required in sunshine bass farming. The cost of balanced diet, for proper fish growth and health, is the major expense in inland aquaculture and represents two-third of the recurring cost (Muzinic, Thompson, Metts, Dasgupta, & Webster, 2006; Webster, Tiu, Morgan, & Gannam, 1999). Different animal byproducts are utilized as excellent sources of dietary crude protein for sunshine bass, such as blood meal (Gallagher & LaDouceur, 1995), poultry byproduct meal (Webster, Thompson, Morgan, Grisby, & Gannam, 2000), meat and bone meal (Bharadwaj, Brignon, Gould, & Brown, 2002), turkey meal (Muzinic et al., 2006), and fishmeal (Webster et al., 1999; Webster, Tiu, Tidwell, Wyk, & Howerton, 1995; Xiong et al., 1996). Among animal byproducts, fishmeal is highly palatable and, therefore, is the most desirable animal protein ingredient. The balanced amino acid composition and high digestibility of fishmeal contribute to its superior nutritional value. Therefore, it is widely used in livestock, poultry, and fish feed (FAO, 2008; Regenstein, Goldhor, & Graves, 2003). Furthermore, fishmeal is a good source of polyunsaturated fatty acids (PUFAs; Howe, Downing, Grenyer, Grigonis-Deane, & Bryden, 2002).

PUFAs are critical nutrients for human health. They are essential for the proper development of the central nervous system in fetuses and are also reported to reduce the incidence of cardiovascular diseases (Ruxton, Reed, Simpson, & Millington, 2007). The awareness of the health benefits of PUFA is increasing in countries where consumption of meat is traditionally greater than fish, for instance the United States. In this perspective, fortification of muscle foods with PUFAs, via dietary manipulation, is an emerging area of interest. Inclusion of fishmeal in diet is a practical approach

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to increase the PUFA content in sunshine bass (Fowler et al., 1994; Harel & Place, 2003; Lane, Trushenski, & Kohler, 2006). However, PUFAs deposited in muscle tissues may rapidly undergo lipid oxidation (Wood et al., 1999), a process well-known to compromise the color attributes of muscle foods. In this perspective, information concerning the influence of fishmeal-based diet on color and lipid stability in sunshine bass fillets is limited. Hence, the objectives of the present study were to examine the effects of fishmeal-based diet on color attributes and lipid oxidation in aerobically packaged sunshine bass fillets during retail display.

2. Materials and methods

2.1. Fish production and processing

Sunshine bass were grown at the Aquaculture Research Centre, Kentucky State University from June 2006 to August 2007. Juvenile fingerlings with an average weight of 35 g were obtained from Keo Fish Farm (Keo, AR, USA) and stocked in six ponds (1.1 m deep) at the rate of 300 fingerlings per pond. The water temperature and dissolved oxygen in the ponds were monitored to ensure that water quality parameters were within the acceptable limits (Boyd, 1979). Fish were either fed a diet containing 30 percent fishmeal (FM) or a diet containing poultry byproduct meal (PB) as a complete replacement for fishmeal. Three ponds were allocated for each diet. The diets were formulated with commercially available ingredients to meet the nutrient and energy requirements of sunshine bass. The formulations of diets are presented in Table 1. Formulated diets contained 40 g/100 g protein and 11 g/100 g fat.

On September 4, 2007, fish were humanely harvested, chill-killed in ice-water bath, decapitated, gutted, and filleted immediately. Fillets from each diet were pooled, washed in a saline solution (3 percent sodium chloride) to remove excess fat, and shipped in ice to the Meat Laboratory, University of Kentucky within 2 h. Individual fillets were weighed, placed on styrofoam trays, and overwrapped with oxygen-permeable polyvinyl chloride film (15,500–16,275 $cm^3/m^2/24$ h oxygen transmission rate at 23 °C). Packaged fillets were randomly allotted to two retail display conditions typically used for seafood, viz. refrigerated storage at 2 °C (REF) or stored over ice in the refrigerator (ICE), under continuous warm-white, fluorescent lighting of approximately 1100 lux. The samples (six replicates per dietary treatment; $n = 6$) were analyzed on 0, 3, 6 and 9 d.

2.2. Moisture and fat content estimation

On day 0, the moisture and fat content of the fillets were determined using an HFT 2000 moisture and fat analyzer (Data Support Co., Inc., Encino, CA, USA).

Table 1
Formulations of poultry byproduct-based (PB) and fishmeal-based (FM) diets fed to sunshine bass.

Ingredient (g/100 g)	PB	FM
Soybean meal	37.00	40.00
Menhaden fishmeal	0.00	30.00
Poultry byproduct meal	33.00	0.00
Corn grain, ground flour	7.70	7.00
Wheat flour	13.20	13.20
Menhaden fish oil	7.00	5.00
Soybean oil	0.00	3.00
Monocalcium phosphate	1.00	1.00
Vitamin mix	0.40	0.40
Choline chloride	0.15	0.15
Stay C	0.15	0.15
Mineral mix	0.10	0.10
DL-Methionine	0.30	0.00

2.3. Analysis of pH

The pH of fish fillets was determined according to Strange, Benedict, Smith, and Swift (1977). Six grams of sample were dispersed in 30 ml deionized water using a hand-held tissue homogenizer (Fisher Scientific, Fair Lawn, NJ, USA) and the pH of the homogenate was determined with an Accumet pH meter (Fisher Scientific, Fair Lawn, NJ, USA).

2.4. Instrumental color evaluation

Instrumental color was evaluated using a HunterLab LabScan XE spectrophotometer (Hunter Associates Laboratory, Reston, VA, USA) on the light-exposed surface of the fillets, using a D65 illuminant, 10° standard observer and 2.54-cm diameter aperture (AMSA, 1991). The instrument was calibrated with standard black and white plates, every eight hours. CIE L^*, a^*, b^*, hue angle and chroma values were measured at four random locations on each fillet and the sub-samples were averaged for statistical analyses.

2.5. Lipid oxidation

Secondary products of lipid oxidation were measured using the thiobarbituric acid assay (Yin, Faustman, Riesen, & Williams, 1993). Briefly, 5 g samples were mixed with trichloroacetic acid, homogenized, and filtered. One ml of aqueous filtrate was mixed with 1 ml of aqueous thiobarbituric acid and incubated at 25 °C for 20 h. The absorbance of samples measured spectrophotometrically at 532 nm was reported as thiobarbituric acid-reactive substances (TBARS).

2.6. Statistical analysis

A completely randomized factorial ($2 \times 2 \times 4$) design was used in the study. The factors include two diets (FM and PB), two storage conditions (REF and ICE) and four storage time points (0, 3, 6, and 9 d). Six replicates ($n = 6$) per dietary treatment were analyzed on each storage day. Data were analyzed using analysis of variance and mean separation procedures of the general linear model (SAS, 2007). The differences among means were detected at the 5 percent level using the least significant difference (LSD) test.

3. Results and discussion

3.1. Moisture and fat

Proximate analyses revealed no difference ($P > 0.05$) in the moisture and lipid content of FM and PB fillets. The moisture (75.73 ± 0.95 percent) and lipid (3.35 ± 0.55 percent) contents of sunshine bass fillets observed in the present study were similar to those reported previously (Fowler et al., 1994; Lane et al., 2006). This observation indicated that fishmeal-based diet did not exert any influence on the body composition of sunshine bass. These results could be attributed to the same levels of protein (40 percent) and fat (11 percent) in both diets (Table 1). Our results are in agreement with previous findings (Fowler et al., 1994; Lane et al., 2006; Webster et al., 2000) that diet composition did not alter moisture, protein and lipid content of sunshine bass fillets. On the other hand, fishmeal supplementation at 7.5 percent level resulted in greater protein and moisture content in sunshine bass, compared to diets containing 0 or 15 percent fishmeal (Webster et al., 1999).

3.2. Evaluation of pH

There were significant two-way interactions (diet × storage time; retail display × storage time) for pH (Table 2). The initial pH

Table 2
Effect of diet, retail display and storage time on the pH of sunshine bass fillets.

	Storage time (days)			
	0	3	6	9
Diet				
PB	6.18 ± 0.02^{c}	6.29 ± 0.02^{b}	6.23 ± 0.02^{c}	6.42 ± 0.04^{a}
FM	6.19 ± 0.02^{c}	6.21 ± 0.02^{c}	6.24 ± 0.01^{bc}	6.30 ± 0.02^{b}
Retail display				
REF	6.18 ± 0.02^{de}	6.29 ± 0.02^{b}	6.21 ± 0.01^{cd}	6.43 ± 0.04^{a}
ICE	6.18 ± 0.02^{de}	6.21 ± 0.03^{cd}	6.26 ± 0.02^{bc}	6.29 ± 0.03^{b}

[a–e]Mean values without a common letter within an interaction differ significantly ($P < 0.05$).
PB = poultry byproduct-based diet; FM = fishmeal-based diet.
REF = refrigerated storage; ICE = storage over ice.

values of sunshine bass fillets were in good agreement with the previous report (Eifert, Hackney, Libey, & Flick, 1992). In both dietary treatments, pH increased during storage, albeit at different trends. Although pH increased in both display conditions, the magnitude of increase was lower ($P < 0.05$) in ICE than in REF display. At 9 d, the pH of fillets in ICE had lower ($P < 0.05$) pH than those stored in REF. A raise in pH indicated physico-chemical changes in proteins. Protein degradation leads to generation and accumulation of ammonia, which in turn increases the alkalinity of the food matrix (Uchiyama, Suzuki, Ehira, & Noguchi, 1966). Our observations agreed with those of Eifert et al. (1992) in which pH of sunshine bass fillets increased progressively over an 18-day refrigerated storage. Similarly, Choubert and Baccaunaud (2006) reported that the pH of rainbow trout fillets increased during a 4-week refrigerated storage under controlled atmosphere.

3.3. Instrumental color

Scientific literature on the color attributes of sunshine bass fillets is limited. In the present study, no 3-way interactions occurred for instrumental color parameters.

Lightness (L^* value) of fillets was influenced ($P < 0.05$) by diet. FM fillets were darker (lower L^*) than PB fillets (Table 3). Nevertheless, the numerical reduction in L^* value due to fishmeal supplementation was only 2 percent. Although not influenced ($P > 0.05$) by display conditions (Table 3), L^* values demonstrated inconsistent variation ($P < 0.05$) during storage (Table 4).

PB fillets demonstrated greater ($P < 0.05$) redness (a^* value) than those from fishmeal-fed bass (Table 3). Fishmeal-based diet resulted in a 33 percent reduction of a^* values. Low red color intensity in FM fillets could possibly be due to low levels of heme pigments, especially myoglobin. However, myoglobin content was not estimated to confirm this possibility. Consumers prefer sunshine bass due to its white flesh (Boyd et al., 1992). Therefore, fishmeal supplementation could be used to manipulate fillet appearance to improve the consumer acceptance of sunshine bass. Diet manipulation is widely employed to improve the color stability

Table 3
Diet and retail display main effect means for color traits of sunshine bass fillets.

Color trait	Diet		Retail display	
	PB	FM	REF	ICE
L^* value	49.10 ± 0.39^{a}	47.97 ± 0.32^{b}	48.39 ± 0.35^{a}	48.67 ± 0.38^{a}
a^* value	1.69 ± 0.19^{a}	1.11 ± 0.19^{b}	1.41 ± 0.21^{a}	1.39 ± 0.18^{a}
b^* value	6.15 ± 0.16^{a}	5.83 ± 0.14^{a}	5.88 ± 0.15^{a}	6.10 ± 0.15^{a}
Hue angle	75.40 ± 1.51^{b}	80.78 ± 1.78^{a}	78.14 ± 1.85^{a}	78.03 ± 1.50^{a}
Chroma	6.47 ± 0.18^{a}	6.09 ± 0.17^{a}	6.18 ± 0.17^{a}	6.35 ± 0.18^{a}

[a, b]Mean values for the same main effect in the same row without a common letter differ significantly ($P < 0.05$).
PB = poultry byproduct-based diet; FM = fishmeal-based diet.
REF = refrigerated storage; ICE = storage over ice.

Table 4
Storage time main effect means for color traits of sunshine bass fillets.

Color trait	Storage time (days)			
	0	3	6	9
L^* value	49.58 ± 0.42^{a}	47.06 ± 0.48^{b}	48.28 ± 0.54^{ab}	49.20 ± 0.36^{a}
a^* value	0.67 ± 0.20^{c}	1.67 ± 0.23^{ab}	2.25 ± 0.24^{a}	1.01 ± 0.31^{bc}
b^* value	5.40 ± 0.15^{b}	5.93 ± 0.19^{ab}	6.42 ± 0.22^{a}	6.23 ± 0.25^{a}
Hue angle	84.05 ± 2.18^{a}	75.10 ± 2.04^{b}	71.68 ± 1.74^{b}	81.51 ± 2.80^{a}
Chroma	5.53 ± 0.16^{c}	6.23 ± 0.22^{b}	6.87 ± 0.25^{a}	6.45 ± 0.28^{ab}

[a–c]Mean values in the same row without a common letter differ significantly ($P < 0.05$).

of red meats. While display conditions did not influence redness, a^* values increased ($P < 0.05$) until 6 d and decreased ($P < 0.05$) thereafter (Table 4). One possible reason for this increase in redness is oxygenation of deoxymyoglobin to cherry-red colored oxymyoglobin, which could have started immediately after fish harvest and proceeded until 6 d. After 6 d, oxymyoglobin (cherry-red pigment) might have oxidized to metmyoglobin (brown pigment) leading to a reduction in the red color intensity. Formation of brown methyoglobin is the major cause of color deterioration in muscle foods (Faustman & Cassens, 1990). In partial agreement with our observation, D'Souza, Skonberg, Stone, and Brown (2006) reported that a^* values in fresh refrigerated rainbow trout fillets deteriorated over the storage time.

There was significant diet × display interaction for b^* value (yellowness). PB fillets were more ($P < 0.05$) yellow than FM ones in ICE storage, whereas during REF storage there was no difference in b^* value (data not shown). However, overall, PB fillets demonstrated greater numerical b^* value (yellowness) than FM ones (Table 3). Nevertheless, as storage progressed, b^* values of fillets from both diets increased ($P < 0.05$; Table 4), indicating an increase in yellow discoloration. In agreement with our results, Handumrongkul and Silva (1994) also reported that the b^* values increased in sunshine bass during 10-day storage at 2 °C.

Hue angle ($\tan^{-1} b^*/a^*$) was greater ($P < 0.05$) for FM samples than PB samples (Table 3). Although not influenced by display conditions (Table 3), hue angle decreased ($P < 0.05$) from 0 d to 6 d, and then increased on 9 d in both dietary treatments (Table 4). Similarly, Choubert and Baccaunaud (2006) observed that hue angle in rainbow trout increased during refrigerated storage. Brewer, Zhu, and McKeith (2001) argued that since the coefficient of variation for hue angle is the lowest among instrumental color parameters, it is the most useful color parameter for monitoring color changes. Hue angle represents the degree of change from redness, and greater hue angle values in FM than PB indicated greater deviation from true red color axis (AMSA, 1991). This observation clearly pointed to the fact that consumer-desirable pale color (white flesh) of sunshine bass could be improved by feeding fishmeal-based diet.

On the other hand, there was a significant diet × display interaction on chroma. PB samples in ICE demonstrated greater ($P < 0.05$) chroma than the FM ones, whereas during REF storage diet did not exert any effect (data not shown). However, chroma was numerically greater in PB fillets than in FM fillets (Table 3). Furthermore, chroma increased ($P < 0.05$) in both dietary treatments during storage, indicating an increase in color intensity (Table 4). Chroma or saturation index indicates the vividness of color. Since chroma is calculated as $([a^{*2} + b^{*2}]^{1/2})$, the progressive increase in chroma during the first six days of storage could be logically attributed to the concomitant increase in a^* and b^* values during the same period.

3.4. Lipid oxidation

Lipid peroxidation is a major concern in fish quality because of higher levels of readily oxidizable PUFAs in fish muscles relative to

Table 5
Effect of diet, retail display and storage time on TBARS of sunshine bass fillets.

	Storage time (days)			
	0	3	6	9
Diet				
PB	0.016 ± 0.002^{cd}	0.012 ± 0.001^{de}	0.017 ± 0.002^{cd}	0.051 ± 0.005^{a}
FM	0.015 ± 0.002^{de}	0.018 ± 0.001^{cd}	0.022 ± 0.003^{c}	0.042 ± 0.004^{b}
Retail display				
REF	0.016 ± 0.002^{d}	0.013 ± 0.001^{d}	0.014 ± 0.001^{d}	0.056 ± 0.004^{a}
ICE	0.016 ± 0.002^{d}	0.017 ± 0.002^{d}	0.025 ± 0.003^{c}	0.037 ± 0.003^{b}

[a–e]Mean values without a common letter within an interaction differ significantly ($P < 0.05$).
PB = poultry byproduct-based diet; FM = fishmeal-based diet.
REF = refrigerated storage; ICE = storage over ice.

flesh from terrestrial livestock. Various pro-oxidants routinely encountered in fish processing, such as iron from filleting knives and heme iron in muscle pigments, catalyze lipid oxidation and accelerate quality deterioration. TBARS value is widely considered as a reliable index of lipid oxidative rancidity (Sun, Faustman, Senecal, Wilkinson, & Furr, 2001) and therefore is extensively used to analyze the oxidative stability of muscle foods (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). It is well documented that the flavor attributes of fish are highly correlated to the levels of TBARS (Fair & Williams, 1995).

Our results indicated significant two-way interactions (diet × storage time, retail display × storage time) in lipid oxidation (Table 5). TBARS values were similar in PB and FM fillets on 0 d, and increased ($P < 0.05$) during storage, albeit at a different rate. Earlier investigations by Boyd et al. (1992) reported that lipid oxidation increased progressively in sunshine bass over a 21-day storage period. In the present study, interestingly on 9 d, TBARS was greater ($P < 0.05$) in PB than in FM fillets. This observation could not be due to the differences in the amount of menhaden fish oil added to each diet because the same amount of fish oil (added fish oil plus oil from fishmeal) was present in each diet. Our observations are contrary to those of D'Souza et al. (2006), who reported that fishmeal supplementation increased the propensity of lipid oxidation in rainbow trout fillets. Species difference and diet formulation could have contributed to the observed variation in the lipid oxidation pattern. While lipid oxidation increased during storage, the 9 d samples in ICE demonstrated lower ($P < 0.05$) TBARS values than those in REF. In the present study, fresh ice was replaced every 24 h and this could have maintained the temperature of fillets very close to 0 °C in ICE (vs. 2 °C in REF). Lower temperature of samples stored in ICE display, compared to REF, could have slowed down the chain reactions of lipid peroxidation.

4. Conclusions

While proximate composition was not influenced by the fishmeal-based diet, pH was greater in PB fillets than those from fishmeal-fed sunshine bass. One major concern in fishmeal supplementation in animal diets is the rapid development of rancidity in muscle foods. However, our results indicated that lipid oxidation was not adversely influenced by the fishmeal-based diet. Fillets from fishmeal-fed sunshine bass were less red and demonstrated greater hue angle than PB fillets. Findings of our investigation suggested that the fishmeal-based diet could be fed to sunshine bass to decrease red color and impart a pale appearance to the flesh. Since consumer purchase decisions, at the point-of-sale, are widely based on the white flesh/pale appearance of sunshine bass, fishmeal supplementation could be employed as a potential dietary strategy to improve the marketability.

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