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Full Length Research Paper

Effects of different additives on colorimetry and melanosis prevention of Atlantic seabob shrimp (*Xyphopenaeus kroyeri)* stored under refrigeration

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The aim of this work was to evaluate the effect of the food additives, sodium chloride, sodium metabisulfite, sodium nitrite and citric acid on the anti-melanotic activity in shrimp (*Xyphopenaeus kroyeri*) kept under refrigeration for 13 days post-capture. A sensory panel and color measurements (L*: luminosity, a*: red-green axis saturation and b*: yellow-blue axis saturation) was conducted during storage to evaluate the development stages of melanosis. Statistical differences were found in the colorimetric indexes (L*, a* and b*) and melanosis levels in all the treatments. The best results were found in 2.5% sodium metabisulfite. However, 2% sodium chloride had similar results and presented advantages such as low cost, maintaining firmness, general appearance, flavor, microbiological control, besides not causing allergic reactions. The sodium chloride is an excellent alternative to sodium metabisulfite.

Key words: sodium chloride, shrimp storage, sodium metabisulfite, shelf-life.

INTRODUCTION

The Brazilian fish market is in active development, with a continental extractive fishing yield of 249,600.2 t in 2011, according to the Fishing and Aquaculture Ministry (MPA) (Brasil, 2011). Shrimp is among the seafood with an increase of 4.49% from 2009 (5519.7 t) to 2011 (5779.5 t).

The *Xyphopenaeus kroyeri*, popularly known as Atlantic seabob is among the species of shrimp produced in Brazil. This is an endemic species in the western Atlantic Ocean (between the state of North Carolina [USA] and the state of Rio Grande do Sul [Brazil]). The *X. kroyeri* is the main crustacean caught in the Brazilian marine fisheries since 2009, because it is a coastal species and affordable to small and medium-scale fisheries along Brazilian states, from the Amapa to the Rio Grande do

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Sul (Ibama, 2011).

The high consumption of *X. kroyeri* shrimp is related to its excellent nutritional quality, freshness, variety and flavor. However, despite the sector's growth in recent decades, it still needs a lot of progress with regards to it shelf life. In general, fresh shelf life shrimp is short, since after capturing, numerous biochemical and enzymatic mechanisms are activated, starting the deterioration and consequently the loss of natural shrimp characteristics, which may cause unacceptability of the product (Martinez-Alvarez et al., 2005; Nirmal and Benjakul, 2009a; Pardio et al., 2011).

One of the biggest problems found in this crustacean is melanosis (black spots) development, a dark pigment that arises by polyphenol (PFO) biochemical action, which are able to oxidize phenolic compounds into quinones (Nirmal and Benjakul, 2011) even under cold storage. Shrimp usually have limited shelf life due to melanosis formation; although its presence is harmless to consumers, it causes drastic reduction of the value in market products and the acceptance in consumer sensory (Nirmal and Benjakul, 2009b).

The fishing industry has been using food additives that have preservation ability to maintain and/or improve the quality of the final product (Okpala et al., 2014). According to the Identity and Quality Technical Regulation (RTIQ) for fresh shrimp, Ordinance n. 456 of the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA), sodium metabisulphite is the only preservative allowed for conservation purposes (Brasil, 2010).
Among these preservatives, the citric acid prevent oxidation and reducing dehydration of crustaceans, while the sodium metabisulfite is widely used to maintain the product organoleptic properties and shelf life (Mol and Turkmen, 2010). However, the overuse of sulfite is associated with adverse reactions in people with asthma, thus its residual concentration in food is legally controlled (Gómez-Guillén et al., 2005).

Another preservative commonly used in dried shrimps is sodium chloride, which intensifies flavor attributes, hardness and reduces microorganism levels (Niamnuy et al., 2007). Nitrite is also used in curing salts in order to increase conservation of marine products by controlling microbial growth during storage, inhibiting pathogenic and deteriorating microorganisms (Lyhs et al., 1998).

In this context, the aim of this work was to evaluate the colorimetric changes and anti-melanotic action of the additives sodium chloride, sodium metabisulfite, sodium nitrite and citric acid in fresh shrimp (*Xyphopenaeus kroyeri*) stored under refrigeration for 13 days.

MATERIALS AND METHODS

Samples collection and storage

The shrimps (*X. kroyeri)* used were from the *Lagoa dos Patos*, in Tramandai, RS, Brazil (29°59'05''S and 58°08'01''W). The temperature at the time of capture ranged from 20 to 23°C, and their individual weight was 5.00±0.50 g. Whole shrimps were collected immediately after taken to the boats and kept on ice at 1:2 (shrimp : ice) and sent to the Teaching, Research and Meat Technology Center (Centro de Ensino, Pesquisa e Tecnologia de Carne - CEPETEC), Faculty of Veterinary Medicine, Rio Grande do Sul Federal University (UFRGS).

Treatments and collection days

Shrimps were washed in running water, drained, randomly distributed into groups and subjected to five immersion treatments: T1: distilled water (no preservative - control group), T2: sodium chloride solution (2% w/v), T3: sodium metabisulfite solution (2.5% w/v) T4: Sodium nitrite solution (1% w/v) T5: citric acid solution (2% w/v). Each treatment consisted of 2.0 kg of shrimp, which were submerged in the treatments for 15 min. Afterwards, the shrimps were packaged in polyethylene bags and stored at 5°C for 13 days. During this period, aliquots were sampled in order to perform analyzes.

Analytical measurement of colors

Determinations of the shrimp color were performed after the samples remained in an acclimatized room at 15°C for 30 min to oxygenate their surface, using a portable colorimeter Konica Minolta® (Chroma Meter CR-410), with a D65 light source, at an observation angle of 10° and 30 mm of measuring cell opening. The device was systematically calibrated with a white (L*=93.80, a*=-0.89, b*=0.95) and a black (L=1.19*, a*=1.27, b*=1.92) standard.

Each sample, consisting of 10 shrimp, were subjected to readings using the CIELab system parameters (L* indicating the luminosity, a* the color and saturation at the red-green axis, and b* the color and saturation at the yellow-blue axis), established in 1967 by the International Commission on Illumination (Parisenti et al., 2011). The measurements were performed in three locations of the sample surface, and the daily averages were calculated for the first to the thirteenth days of storage.

Sensory evaluation of melanosis

A semi-trained sensory panel of 11 judges evaluated the melanosis. Visual evaluations were performed, classifying the melanosis development stages in a four-point scale: 1- without melanosis presence or absence of discoloration; 2- low color change, from mild to moderate (more than 30% of the shrimps' surface affected); 3- severe melanosis (30-70% of shrimps' surface affected in less than 50% of subjects); and 4- extremely severe darkening (70-100% of the shrimps' surface affected in most individuals), following the pictures model of Montero et al. (2004). Each judge evaluated 10 different samples from each treatment each evaluation day, performed on days 1, 2, 3, 4, 6, 7, 9 and 11.

Statistical analysis

Analysis of variance (ANOVA) was used for melanosis levels, L* (luminosity), a* (red level) and b* (yellow level). In the case of 5% statistical variation, the test for multiple comparisons of Bonferroni was performed. The correlation analysis were performed using the linear correlation Pearson (p <0.05). The statistical programs used were the Statistical Analysis System 9.0 (SAS) and the Statistical Package for the Social Sciences 18 (SPSS/PASWSTAT).

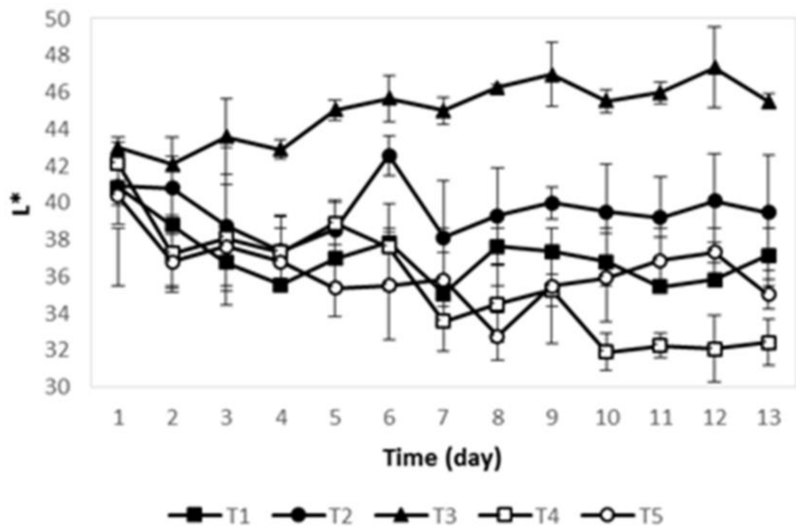

Figure 1. Values of L* (luminosity) of shrimps (*X. kroyeri*) treated with water (T1), 2% sodium chloride (T2), 2.5% sodium metabisulphite (T3), 1% sodium nitrite (T4) and 2% citric acid (T5), stored under conventional refrigeration for 13 days.

RESULTS AND DISCUSSION

The L* parameter began to show changes and differences among treatments from the fourth day of storage (Figure 1). The shrimp treated with sodium metabisulfite (T3) positively differed from the other treatments ($P < 0.05$) on days 4, 5, 8, 9 and 11, showing higher L* values, hence presenting greater control of melanosis. However, it was statistically similar to the sodium chloride treatment (T2) on days 6, 7, 10, 12 and 13, and to the citric acid treatment on day 7 ($p < 0.05$).

The lower the L* value, the higher the melanosis development will be (Yokoyama, 2007). A significantly lower value ($p < 0.05$) of the L* parameter was related to the appearance of "black spots" in shrimps *Parapenaeus longirstris* (Lopez-Caballero et al., 2007). A decrease in L* value may be considered as an indicator of darkening (Martinez-Alvarez et al., 2007). Thus, the high efficiency of treatments 2 and 3 was noted with higher scores for the L* parameter over the shelf life.

Rotllant et al. (2002) found significantly higher luminosity levels in shrimps *Aristeus antennatus* treated with sulfites-based additives compared to samples without preservatives, demonstrating that sulfites are strong reducing agents, able to whiten crustaceans. Similar results were found in this study with the treatment based on sodium metabisulphite (T3) obtaining the best luminosity results over the 13 days of evaluation.

The sodium chloride in shrimps leads to a denaturation temperature decrease of the protein. The luminosity difference (ΔL*) usually increases as the salt concentration increases, providing a fresh appearance to the product. The denaturation and myofibrillar coagulation associated with the collagen shrinkage and the low water retention capacity also causes an increase in shrimp hardness, another factor that contributes to the maintenance of the fresh product characteristics (Niamnuy et al., 2007). These characteristics also explain the results of treatment 2 (sodium chloride) in this study.

In refrigerated shrimp without additives, a gradual increase of a* values is expected to occur, due to melanosis development which causes a shrimp darkening. In a study with Norway lobsters (*Nephrops norvegicus*), the red intensity (a*) tended to decrease during storage, indicating that the color of this product tends to change gradually to greenish hues (Martinez-Alvarez et al., 2007). Mol and Turkemen (2010) showed that decreases in a* values suggest a darkening of the meat, becoming less red and more greenish. However, the present study showed increase of a* values (red intensity) from day 2 one (Figure 2). Treatment 3 showed higher values ($p < 0.05$), except at day 3, when the results were similar ($p > 0.05$) to treatments 1, 2 and 5, and at day 11 to treatment 2 ($p > 0.05$). Similar results were reported by Arancibia et al. (2015) which found increased values of a* in *Litopenaeus vannamei* during 14 days storage at 5°C. Different species have different values of a*, suggesting a difference in carotenoid contents between species (Benjakul et al., 2008). This would explain the differences found in the parameter a* in different studies and species.

The values of b* (yellow intensity) presented differences among treatments only on days 5, 11 and 12 (Figure 3). A gradual increase of the values in all treatments was observed confirming the occurrence of degradation and product quality loss from day 5. In a

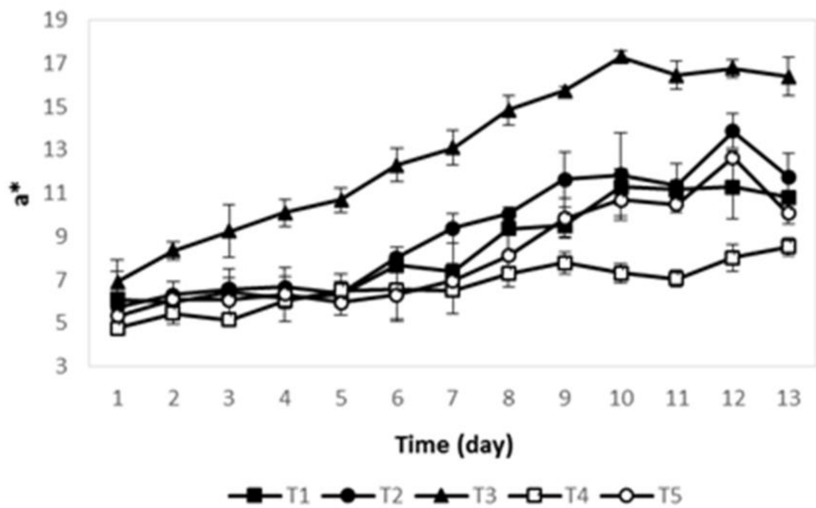

Figure 2. Values of a* (red intensity) of shrimps (*X. kroyeri*) treated with water (T1), 2% sodium chloride (T2), 2.5% sodium metabisulphite (T3), 1% sodium nitrite (T4) and 2% citric acid (T5), stored under conventional refrigeration for 13 days.

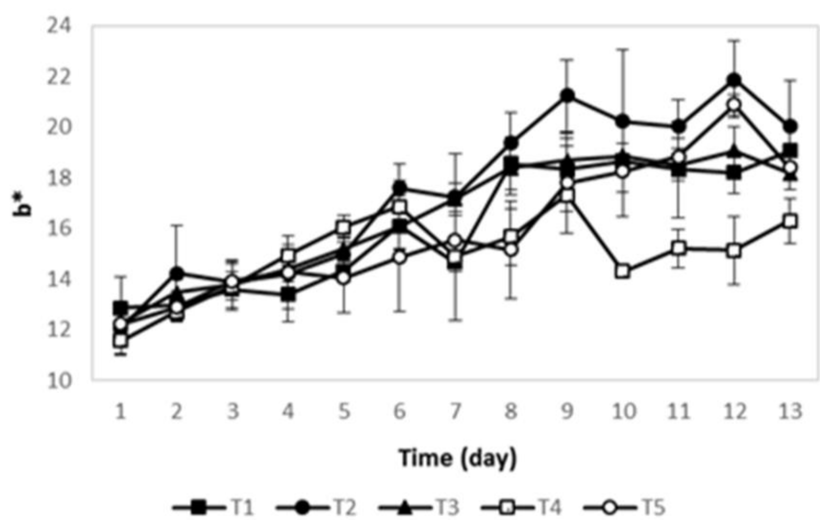

Figure 3. Values of b * (yellow intensity) of shrimps (*X. kroyeri*) treated with water (T1), 2% sodium chloride (T2), 2.5% sodium metabisulphite (T3), 1% sodium nitrite (T4) and 2% citric acid (T5), stored under conventional refrigeration for 13 days.

study with Norway lobsters (*N. norvegicus*), the yellow intensity (b*) presented no defined course during storage period (Martinez-Alvarez et al., 2007). However, Mu et al. (2012) verified maintenance of yellow intensity in Pacific white shrimps (*Litopenaeus vannamei*) preserved with cinnamaldehyde, showing that the additive can prevent the shrimp melanosis and redness along storage. Authors found increasing values of b* during the storage period, assuming that it occurred through antioxidant activity of the concentrated proteins and lipids (Arancibia et al., 2015; Wang, 2014). The shrimp general appearance and the melanosis development levels in the different treatments during the storage period is shown in Figure 4.

The use of melanosis inhibitor additives is essential for the fishing industry to ensure the quality of crustaceans for greater shelf life, since the low temperatures alone cannot prevent the occurrence of these black spots (Figure 4). The enzymes responsible for melanosis development remain active during refrigeration, storage

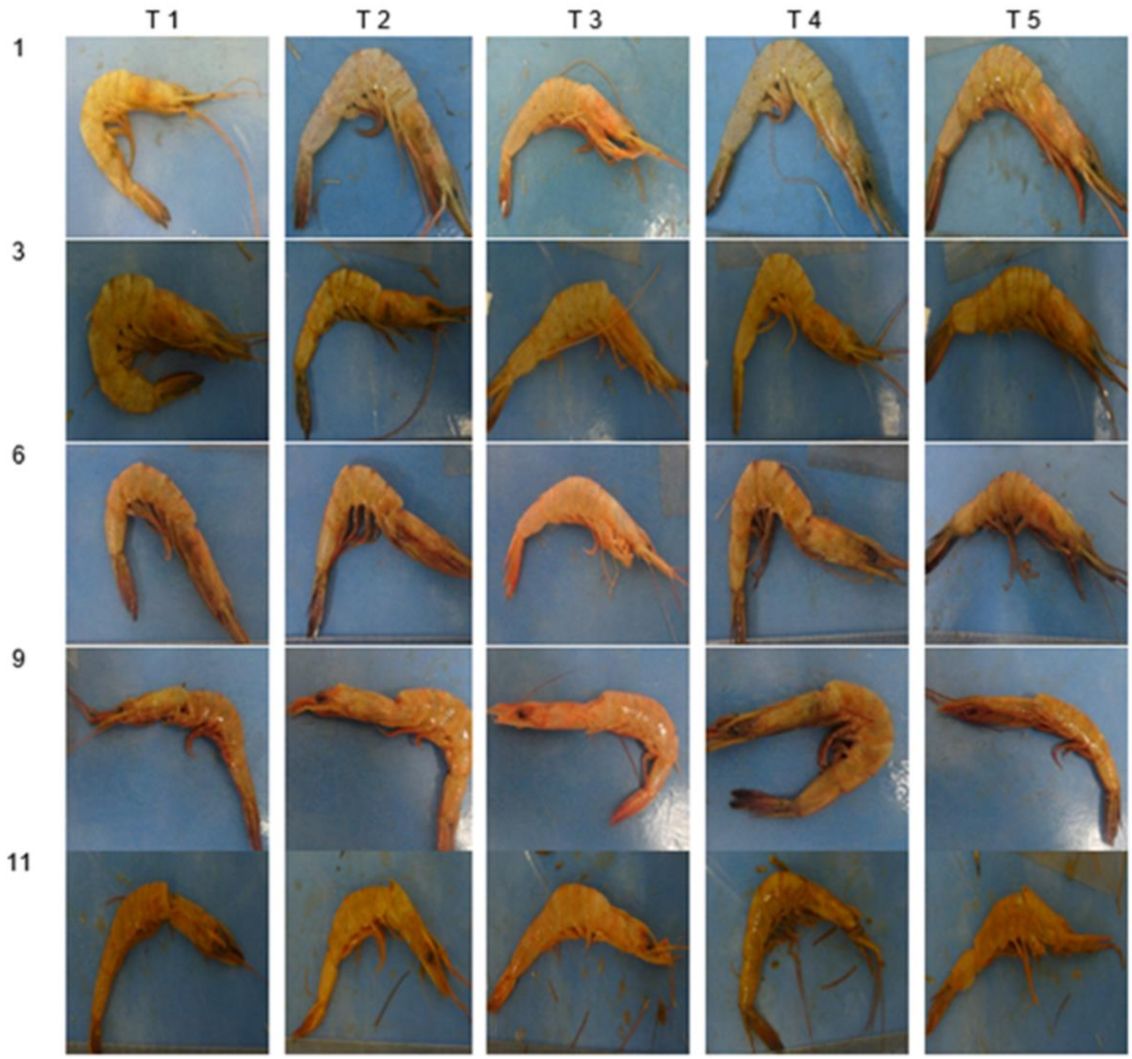

Figure 4. General appearance and melanosis levels in shrimps *X. kroyeri* kept under refrigeration (5°C) and treated with water (T1), 2% sodium chloride (T2), 2.5% sodium metabisulphite (T3), 1% sodium nitrite (T4) and 2% citric acid (T5), up to 11 days.

on ice or after the freezing process (Pardio et al., 2011). Melanosis development levels (Figure 5) showed significant differences ($p < 0.05$) in all evaluated days and treatments. Shrimps treated with sodium metabisulfite (T3) had lower melanosis levels (1.27 ± 0.47; $p < 0.05$) while the other treatments had average of 2.17±0.75 (T1), 1.73±0.63 (T2), 2.52±0.86 (T4) and 2.16±0.72 (T5).

The sodium metabisulphite (T3) treatment maintained melanosis levels (1.40±0.54, 1.32±0.54, 1.17±0.42, 1.16±0.37, 1.38±0.50, 1.20±0.43, 1.27±0.44; $p > 0.05$) lower than the other treatments on days 1, 3, 4, 6, 7, 9 and 11 and consequently had effective control over this phenomenon. On the other hand, the melanosis levels on the sodium chloride (T2) treatment on day 9 (1.76±0.60) and 11 (1.78±0.56), was statistically superior to the others treatments. Therefore, sodium chloride (T2) may be an inexpensive and low toxicity alternative for industry as compared to sodium metabisulphite (Figure 2). A previous study suggests that sodium chloride could be used as excipient to delay the melanosis appearance when used as a spray (Montero et al., 2006). The results of this study are similar to the treatments which used low doses of the 4-hexylresorcinol to delay melanosis development. Sodium chloride seems to be efficient as some anti-melanotics, because besides preventing melanosis development, it also improves others quality parameters, such as maintaining firmness and bacterial control.

Despite the widespread use of sulfite in the food industry, some adverse health effects are related to its consumption, such as: nausea, location gastric irritation, hives and bronchospasm in sensitive asthmatic subjects, hence the importance of knowing alternative components to sulphite-derivative compounds (Machado et al., 2006;

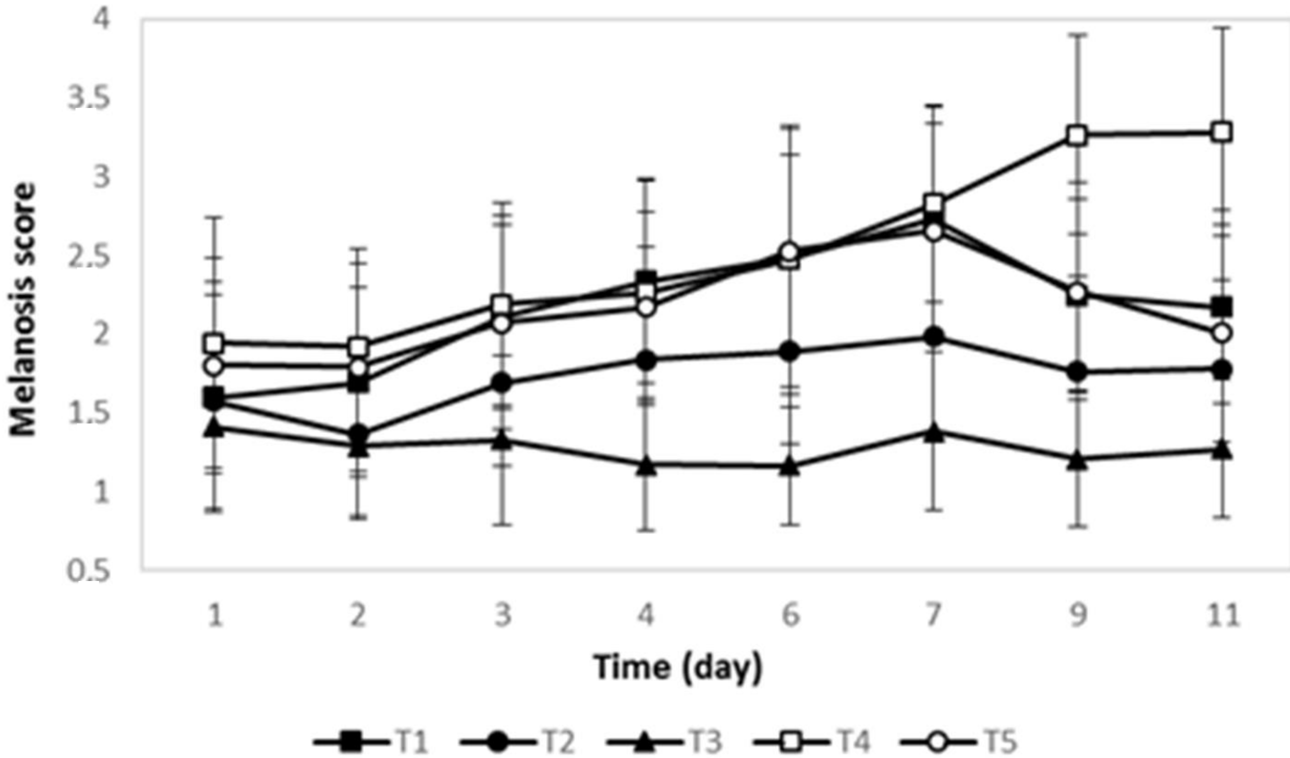

Figure 5. Melanosis levels of shrimps (*X. kroyeri*) treated with water (T1), 2% sodium chloride (T2), 2.5% sodium metabisulphite (T3), 1% sodium nitrite (T4) and 2% citric acid (T5) stored under refrigeration for 11 days.

Table 1. Correlation coefficient (r) between the parameters L*, a*, b* and shrimp (*X. kroyeri)* melanosis levels.

	L*	a*	b*	Melanosis
L*	1.0000	-	-	-
a*	0.5279*	1.0000	-	-
b*	0.0436	0.7525*	1.0000	-
Melanosis	-0.8636*	-0.3972	0.0873	1.0000

* P <0.01.

Martinez-Alvarez et al., 2007). Different results found in melanosis studies is because of the different shrimp species, the shrimps initial condition and the difference among the types and concentrations of additives used (Gokoglu and Yerlikaya, 2008).

Through the Pearson's correlation analysis (Table 1), significant correlations were found between L* values and melanosis levels (r =-0.8636, p <0.00001), between the L* and a* values (r=0.5279, p = 0.0005) and between the a* and b* values (r=0.7525, p <0.00001). Gokoglu and Yerlikaya (2008) also found correlation (r) between the L* values and melanosis levels in shrimps, *P. longirostris* immersed in water and in concentrations of grape seed extract, between a* values and melanosis levels in and between b* values and melanosis levels in the treatments.

Conclusion

The literature points out that the melanosis presence is not a public health problem, however it is responsible for significant losses in the fishing industry, reducing the shelf life of crustaceans. Therefore, the use of anti-melanotics is needed for control and maintenance of the organoleptic quality of the final product. This study showed positive results regarding the variables: melanosis levels, L* (luminosity), a* (red intensity) and b* (yellow intensity) in shrimps (*X. kroyeri)* treated with 2.5% of sodium metabisulfite. However, 2% sodium chloride obtained satisfactory results as compared to 2.5% sodium metabisulfite. Sodium chloride, different from sodium metabisulphite, has the advantage of not causing allergic reactions. Furthermore, sodium chloride has other advantages, such as low cost, maintaining firmness, general appearance, flavor and microbiological control. Therefore, sodium chloride is an excellent alternative to sodium metabisulfite. The authors suggest that studies should be conducted with higher concentrations of sodium chloride, different immersion times and increased storage in order to verify its anti-melanotic action and shelf-life.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth performance of three tilapia fish species raised at varied pond sizes and water depths

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An on-farm study was conducted in Chingale area in Zomba, Southern Malawi to assess the growth of three tilapia fish species in earthen ponds of different sizes and water depths. The experiment was laid out in a factorial design of 200 and 400 m^2 pond sizes, 0.8 and 1.2 m pond water depths, and fish species: *Oreochromis karongae*, *Oreochromis shiranus* and *Tilapia rendalli*, replicated thrice among randomly selected farmers. Ponds were fertilized monthly with fresh chicken manure at an application rate of 1 ton/ha and fish were fed on maize bran as a supplement at 5% body weight. Fish were sampled and weighed every four weeks over 6 months. *O. karongae* attained the highest weight gain (65.75 g) in smaller ponds (200 m^2) translating into an overall higher gross yield of 2.91 tons/ha/year ($P<0.05$). Overall mean weight gain for *O. shiranus* (49.70 g) and *O. karongae* (43.87 g) was not significantly different in 400 m^2 ($P>0.05$). Fish in deeper ponds had a significantly higher overall mean final weight (52.26 g) ($P<0.05$). *T. rendalli* exhibited the lowest average daily weight gain (0.27) especially in 200 m^2 ponds, but had the highest specific growth rate (1.65%) while overall, *O. karongae* was the most advanced of the three tilapia species in terms of growth. Findings from this study suggest that for small-commercial fish farmers, smaller but deeper ponds produce better fish production results, hence should be adopted.

Key words: Tilapia, pond water depth, pond size, weight gain.

INTRODUCTION

One of the areas where culture of tilapia has flourished in recent years is Chingale in Zomba district of Southern Malawi. The area has the potential for developing fish farming due to its perennial water supply from Zomba

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Mountain and availability of locally produced fish feed ingredients. This has motivated the Malawi Department of Fisheries (DoF) and organizations such as World Vision International, Worldfish and the Japanese International Cooperation Agency (JICA) in supporting fish farming in the area. These initiatives have resulted into mushrooming of fishponds with more than 1000 farmers organized into vibrant working groups known as Fish Farmers' Clubs. However, despite all the efforts and resources invested, production of fish has remained low due to, among many factors, poor performing indigenous species.

Tilapia is the commonest cultured fish species in Malawi and there are mainly three species that are commonly cultured which include; *Oreochromis karongae* (Lake Malawi Tilapia), *Tilapia rendalli* (Red breasted Tilapia) and *Oreochromis shiranus* (Shire River Tilapia strain). These species are also raised by small scale farmers in Chingale. *O. karongae* is the most sought after species and exhibits better growth characteristics, and it is preferred by consumers for taste and flavor. The main challenge to raising this species by farmers in ponds has been its difficulty to reproduce in captivity (Sakala and Musuka, 2014). *T. rendalli* is also preferred for being more herbivorous and hence less reliant on supplemented diet (Koekemoer and Steyn, 2014). *O. shiranus* on the other hand, is the most prolific breeder of the three tilapias but the slowest grower (Sakala and Musuka, 2014).

Earlier pilot studies by JICA (ADiM, 2005), demonstrated that some challenges with production of tilapia such as breeding and growth can be eliminated through manipulation of the pond environment. This study was therefore designed and carried out to determine tilapia species that grows best (out of the three), and pond design (size and water depth) combination that would produce better fish production with locally available inputs amongst small-commercial fish farmers in Chingale area.

MATERIALS AND METHODS

Area of study

The on-farm research was conducted in Chingale area in Zomba district of Southern Malawi involving 48 farmers over a period of 180 days (6 months).

Experimental design and activities

Three species of tilapia namely: *O. karongae*, *O. shiranus* and *T. rendalli* were raised in earthen ponds of two different sizes (200 and 400 m^2) and pond water depths (0.8 and 1.2 m). The experiment was laid out in a 2×2×3 factorial design involving the two pond sizes, pond water depths and three tilapia fish species. The earthen ponds were fertilized monthly with chicken manure which was locally sourced at an application rate of 1 ton/ha. Fish were fed on maize bran as a supplement at 5% body weight.

Fish sampling and data collection

Fish were sampled and weighed monthly for over 6 months throughout the experimental period. This was a small scale farmer managed study, with local, easy and non-costly water quality control methods, such as using the hand to assess turbidity and water fertility, rather than the conventional laboratory methods. Pegs with predetermined water level marks were put into every pond to help farmers check and maintain water depths.

Cost (buying price) of the fingerlings, feed (maize bran) and chicken manure including the prevailing selling price of the fish per kg as well as the total harvest (kg) at the end of the trial were computed to determine gross margins for the three tilapia species.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPPS) statistical software for Windows version 16. Univariate analysis showed that data were normally distributed. Therefore, the data were subjected to analysis of variance (ANOVA) using the general linear model. The Tukey's-b was used to carry out multiple comparisons of means for interactions. Weight gain was determined by subtracting initial (stocking) weight (W_o) of the fingerlings from the final weight (W_t). Specific growth rate (SGR) was determined using the formula: $SGR=100\times(\ln W_t-\ln Wt_0)/t$; and average daily weight gain as; ADG (gd^{-1})=Weight gain(g)/t; where W_0 is initial weight, W_t is final weight at time t, t is the number of days (180 days) over which the fish were raised. Gross margins were calculated by subtracting total variable costs (TC) from the total revenue (TR). Variable costs included fingerlings (US$0.07/piece), feed (US$0.13 kg^{-1}) and manure (US$0.02 kg^{-1}). Total revenue was the product of total harvest (kg) and prevailing tilapia wholesale price in the country (US$4 kg^{-1}).

RESULTS

Weight gain of fish species versus pond size and depth

Results (Figure 1) showed that *O. karongae* attained the highest mean weight gain of 65.75 g in 200 m^2 ponds while weight gain for *T. rendalli* and *O. shiranus* were not significantly different (p=0.037). Means of weight gain for *O. shiranus* (49.70 g) and *O. karongae* (43.87 g) in 400 m^2 ponds were significantly higher (p=0.037) than these of *T. rendalli*. Results furthermore showed that pond depth had an effect on the final mean weight of the fish, regardless of species. Higher pond water depth (1.2 m) resulted in higher final fish weight gain (52.26 g) than shallow (0.8 m) deep ponds (p=0.043).

Specific growth rate (SGR) of fish species by pond size and pond depth

There were no significant differences in SGR between pond size, pond depth and fish species (p=0.601) (Table 1). Differences in SGR were however, observed between pond size and fish species, where *T. rendalli* had the highest SGR (1.653%) than *O. karongae* (1.367%) and *O. shiranus* (0.900%), respectively (p=0.001). The effect

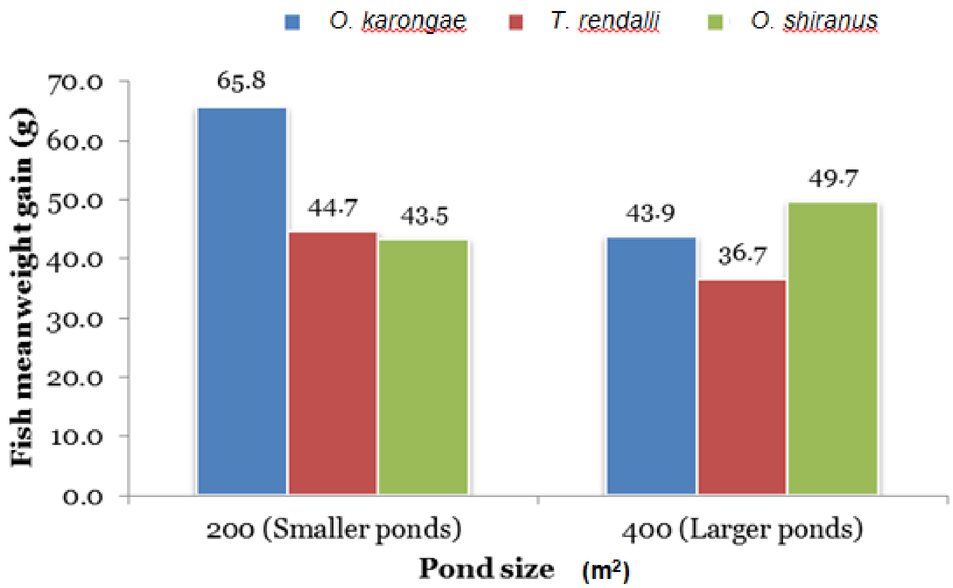

Figure 1. Mean weight gain (g) of fish species raised in 200m^2 and 400m^2 ponds for 6 months.

Table 1. Specific growth rate (SGR) of fish species by pond size and pond depth for 6 months.

Parameter	Fish Species	Pond size (m^2)(p=0.001)		Pond depth (m) (p=0.043)	
		200	400	0.8	1.2
SGR (%)	*O. karongae*	1.367±0.077^b	1.050±0.077^c	1.183±0.077^a	1.233±0.077^a
	T. rendalli	1.653±0.092^a	1.227±0.080^d	1.210±0.063^b	1.671±0.111^b
	O. shiranus	0.900±0.077^b	1.167±0.077^c	0.983±0.077^a	1.083±0.077^a

Values with different superscripts in the same column under the same factor are significantly different (P<0.05).

of pond depth on SGR of fish species had a similar pattern as that of pond size on fish species but was lower (p=0.043).

Average daily weight gain (ADG) of fish species by pond sizes and pond depths

Average daily weight gain (ADG) was significantly influenced by pond size and fish species raised (Table 2). Average daily weight gain for *T. rendalli* was 0.274 g dy-1 being lower (p=0.028) than for *O. karongae* (0.383 g dy-1) and *O. shiranus* (0.267 g dy-1), respectively, whose ADG were not significantly different in 200 m^2. The trend was similar for the 400 m^2 pond except for *O. shiranus* whose ADG (0.267 g dy-1) was higher (p=0.028) than that of *O. karongae* (0.233 g dy-1) and *T. rendalli* (0.221 g dy-1), and the latter two were not significantly different since weight gain had a similar trend as observed in 200 m^2 pond. There were no significant differences in ADG between pond depth and fish species (p=0.067), and neither did pond depth have independent effect on ADG of the fish.

Gross yields and extrapolated gross margins analysis

Mean gross yield of the fish (Table 3) was only affected by pond size (p=0.021) but not pond water depth (p=0.867) and fish species (p=0.101) with highest mean fish yields (2.91 tons/ha/year) reported in smaller ponds (200m^2) which agrees with the weight gain results. Results also showed that these factors did not significantly affect the extrapolated gross margins (Table 4).

DISCUSSION

Higher mean weight gain for *O. shiranus* and *O. karongae* than *T. rendalli* in 400 m^2 could be due to the fact that farmers found it easier to manage the 200 m^2 ponds (smaller ponds), despite attempts to standardize

Table 2. Average daily weight gain (ADG) of fish species by pond size and pond depth for 6 months.

Parameter	Fish Species	Pond size (m^2) (p=0.001)		Pond depth (m) (p=0.043)	
		200	400	0.8	1.2
	O. karongae	0.383±0.027[a]	0.233±0.027[c]	0.317±0.027[a]	0.300±0.027[a]
ADG	*T. rendalli*	0.274±0.030[b]	0.221±0.028[c]	0.186±0.022[a]	0.309±0.038[a]
	O. shiranus	0.267±0.027[b]	0.267±0.027[b]	0.233±0.027[a]	0.300±0.027[a]

Table 3. Gross margins of fish in 200 and 400 m^2 ponds at 0.8 and 1.2 m water depth raised for 6 months.

Pond size (m^2)	Pond water depth (m)	Fish species	Mean gross margin (US$)
200	0.8	*O. karongae*	73.997±34.738[a]
		T. rendalli	89.830±30.084*
		O. shiranus	70.663±34.738[a]
	1.2	*O. karongae*	33.330±42.545[a]
		T. rendalli	41.330±60.167*
		O. shiranus	103.997±34.738[a]
400	0.8	*O. karongae*	74.670±42.545[a]
		T. rendalli	56.670±42.545 *
		O. shiranus	88.670±34.738[a]
	1.2	*O. karongae*	82.670±60.167[a]
		T. rendalli	90.670±42.545*
		O. shiranus	130.003±34.738[a]

Values with different superscripts in the same column under the same factor are significantly different (P<0.05).

key management parameters during the on-farm study. Pond management is usually a challenge among small scale fish farmers in integrated farming systems and it is often variable (Nsonga and Mwiya, 2014). For *O. shiranus*, the effect of overcrowding due to prolific breeding was higher hence poor performance in the smaller pond. Lower mean weight gain for *T. rendalli* in bigger ponds can also be attributed to the generally poor and irregular management practices that affect pond fertility (primary production) was compromised also observed during farmer pond assessment tours. Fish therefore depended more on the supplementary feed (maize bran) hence a nutrition challenge. *T. rendalli* is predominantly herbivorous feeding on macrophytes, algae and also insects, crustaceans, aquatic invertebrates and small fish (Skelton, 2001). Better performance of *O. karongae* could be due to its ability to respond better to supplementary feed than *O. shiranus* (Malcom and Brendan, 2000).

Better growth of all fish species in deeper ponds suggests that pond water depth is a management parameter which farmers can adopt to improve fish production irrespective of species. Unpublished results by the JICA Project in the area of study showed that *O. karongae* and *T. rendalli* (species that have breeding problems in ponds) exhibited higher breeding performance in ponds that were more than a metre deep. Deeper ponds entail more water volume, that is, lower volumetric stocking density which provides more space and relatively more natural food for the fish. Implicitly, there was more available oxygen and low water deterioration rate from fish wastes. When surface water becomes warmer due to high temperatures, fish seek refuge at the deeper end to avoid stress (Walberg, 2011).

Overall lowest average daily weight gain (ADG) for *T. rendalli* may be due to the fact that the average daily gain was general throughout the culture periods. As mentioned earlier, adequate fertilization necessary for higher aquatic plants in the ponds which is a major requirement for *T. rendalli* (Hlophe and Moyo, 2013), was not achieved due to irregular manure application by the farmers. The highest specific growth rate (SGR) nevertheless, could be due to the cumulative growth advantage which the species had owing to the smallest initial stocking weight. Experience at the Malawi National Aquaculture Center shows that Tilapia fingerlings stocked at smaller sizes exhibit high SGR as they easily adapt to the new environment and have relatively high metabolic

Table 4. Mean fish gross yield by experimental factor.

Factor	Level/type	Mean fish yield (tons/ha/year)
Pond size (p=0.021)	200 m^2	2.91±1.375[a]
	400 m^2	2.05±0.912[b]
Pond water depth (p=0.867)	0.8 m	2.54±1.279[a]
	1.2 m	2.46±1.227[a]
Fish species (p=0.101)	*O. karongae*	2.03±1.007[a]
	T. rendalli	2.40±1.489[*]
	O. shiranus	3.09±1.016[a]

Values with different superscripts in the same column under the same factor are significantly different (P<0.05).

rate. This finding agrees with earlier research work of M'balaka et al. (2012).

SGR and ADG values attained in this on-farm research are comparable with some earlier researched works but with lower values than other researchers reported previously some but lower than others earlier reports. Khosa (2008), who conducted an on-station study and used a mixture of organic and in-organic fertilizer, supplemented with feed, reported SGR for *O. shiranus* ranging from 1.08 to 1.24%, as well as ADG between 0.35 and 0.49 g dy^{-1}. Kang'ombe et al. (2006) reported between 0.13 and 0.77 g day^{-1}) for *T. rendalli* raised in fish ponds fertilized with cattle, pig and chicken manure.

Although results seem to suggest that *O. shiranus* gave higher yields and gross margins than *O. karongae* which could be attributed to prolific breeding (Valeta et al., 2012) and not necessarily good growth performance (weight gain) the data are highly dispersed and skewed, this may likely be due to unreported harvesting practices, which distorted the final harvest biomass data.

CONCLUSIONS AND RECOMMENDATIONS

The study has demonstrated the possibility of attaining high mean fish weight gain in small ponds (200 m^2) which are manageable at small scale on-farm level using basic rates of affordable and locally available maize bran as a supplementary feed and chicken manure as pond fertilizer. The study has also demonstrated to farmers that maintaining high pond water depth (more than a metre) results in higher fish growth performance and yields. The potential for commercial fish farming at small scale fish farming level exists especially for *O. karongae* in deeper ponds but be carefully supported with supplementary feed and organic fertilizers in small ponds.

It is recommended, that small scale fish farmers should adopt raising of *O. karongae* in deeper (more than a metre water depth) and small ponds and adopt using manure and supplementary feed as the first step towards commercial fish farming. With improved management practices, bigger ponds should be used to obtain higher fish biomass at harvest. This on-farm researches and results should be replicated for validation, and out-scaled to other areas where fish farming is failing to develop owing to poor technology and management.

Conflict of interests

The authors have not declared any conflict of interests

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