EFFECT OF BACKGROUND COLOR ON SHRIMP PIGMENTATION*

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ABSTRACT

The aim of this work was to evaluate the effect of tank background color on color and total carotenoid content of *Litopenaeus vannamei* shrimp. Five hundred shrimp were grown in two white tanks. Thirty shrimp from each white tank were randomly taken and placed in two black tanks. Ten shrimp of each tank were collected after 30 days of commercial pellet feed. Color analyses showed that color of raw shrimp grown in black tanks was significantly darker (L* 26.02), green (a* -2.42) and blue (b* -2.85) than in white tanks (L* 33.45, a* -0.77, b* 3.88). After cooking, animals from black tanks presented more intense orange color (a* 21.68, *b 33.22) than in white tanks (a* 12.21, b* 22.37), but presenting similar total carotenoid content. Preliminary studies have reported that background color influences the color of shrimp, but does not interfere significantly in the carotenoid amount accumulated in shrimp.

Key words: Carotenoids; astaxanthin; visual appearance

EFEITO DA COR DO TANQUE DE CULTIVO SOBRE A PIGMENTAÇÃO DE CAMARÕES

RESUMO

O objetivo deste trabalho foi avaliar o efeito da cor do tanque de cultivo sobre a coloração e teor de carotenóides totais de camarões *Litopenaeus vannamei*. Foram cultivados 500 camarões em dois tanques brancos. Aleatoriamente, 30 camarões foram retirados de cada tanque branco e colocados em tanques pretos. Após 30 dias alimentados com ração comercial, foram coletados dez camarões de cada tratamento. As análises de cor mostraram que a cor dos camarões crus cultivados em tanques escuros foi mais escura (L* 26,02), mais esverdeada (a* -2,42) e mais azulada (b* -2,85) que os cultivados em tanques brancos (L* 33,45, a* -0,77, b* 3,88). Após o cozimento, os animais do tanque escuro apresentaram coloração laranja mais intensa (a* 21.68, *b 33.22) que os do tanque branco (a* 12.21, b* 22.37), mas similar teor de carotenóides totais. Estes resultados preliminares mostram que a coloração do meio de cultivo interfere na cor dos camarões, mas não interfere significantemente no acúmulo de carotenóides nos camarões.

Palavras chave: Carotenóides; astaxantina; aparência visual

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INTRODUCTION

Color is one of the most important attributes for shrimp acceptance and market value (LATSCHA, 1989; TUME *et al.*, 2009). The red/orange shrimp color is associated with freshness and product quality (LATSCHA, 1989), being astaxanthin, a carotenoid also found in salmon, the main pigment responsible for this color (BOONYARATPALIN *et al.*, 2001).

Carotenoids are acquired through diet once animals are not capable of carotenoid synthesis. Since the farm environment does not provide such nutrients, adding these compounds to the feed has enhanced the color of farmed shrimp, being astaxanthin the most effective pigment on shrimp color (YAMADA *et al.*, 1990; BOONYARATPALIN *et al.*, 2001; ARREDONDO-FIGUEROA *et al.*, 2003). According to the literature, carotenoid-supplemented feed enhances the color of shrimp, which becomes gray *in natura* and red/orange when cooked (SUPAMATTAYA *et al.*, 2005; PASSOS, 2006).

Carotenoids serve several functions as well as pigmentation: they are a source of pro-vitamin A (DALL, 1995; LIÑÁN-CABELLO *et al*., 2003), increase survival rate and weight gain (ARREDONDO-FIGUEROA *et al.*, 2003; SUPAMATTAYA *et al*., 2005), enhance resistance to the white spot syndrome virus (SUPAMATTAYA *et al*., 2005), maintain ammonia excess and resistance to stress (CHIEN *et al.*, 2003), improve hepatopancreatic function (PAN *et al*. 2003), protect cholesterol and polyunsaturated fatty acids from oxidation (PALOZZA *et al.*, 2008; MCNULTY *et al.*, 2008) and provide antioxidant protection (BRITTON, 1995). However, supplementation time and the amount of carotenoid needed to reach suitable pigmentation have not yet been determined and the cost of supplementation limits its use in commercial feed.

TUME *et al.* (2009) have observed that the color of growing tanks has interfered in shrimp color fed with astaxanthin-supplemented food. Concerning the high costs of astaxanthin supplementation, the objective of this paper is to observe if the tank color may modify shrimp color even without carotenoid supplementation.

MATERIALS AND METHODS

The experiments were carried out at the marine shrimp laboratory (Laboratório de Camarões Marinhos – LCM) at Universidade Federal de Santa Catarina (UFSC), in the city of Florianópolis, southern Brazil, in May 2008.

Litopenaeus vannamei (BOONE, 1931), also known as Pacific white shrimp, adult and weighing 8 ± 1.2g have been extracted from experimental reserves at LCM/UFSC and used in this experiment.

About 500 shrimp have been kept in two white tanks (black sides and white bottom). 30 shrimp were, randomly, taken from each white tank and placed in two black tanks (black sides and bottom). Water temperature was kept at 28 ± 2 °C and measured twice a day. Seawater was pumped through the aerated tanks. Shrimps have been kept in tanks under continuous simulated daylight system (12h of darkness).

Shrimp were fed with commercial feed three times a day. Carotenoid content in feed was lower than 3ppm as analyzed by UV-visible spectrophotometer as described below.

After 30 days, ten shrimp were removed from each tank and placed into a bucket containing water and ice for 5 minutes until death, being later removed and kept in ice for analyses. The following analyses were carried out using pooled samples of 10 shrimp per tank, being two samples from the dark tanks and two samples for the white tanks.

Shrimp color from each treatment was determined before and after cooking (1 min, 100 ml water at 100 ºC) and evaluated right after collection using a Minolta Chromo Meter CR 400 (Osaka, Japan, 2002) colorimeter calibrated with white reference. Measurements were taken from three parts along the shrimp body (close to head; middle; close to tail) and performed in the colorimetric space L* (lightness), a* (redness), b* (yellowness) (CIELab system), at 25 ± 1 ºC.

Color measurements in raw and cooked shrimps were carried out with exoskeleton and muscle without exoskeleton, respectively, in order to verify color of shrimps as commercialized in Brazil, whole (when *in natura*) and peeled (when cooked).

Extraction of carotenoids was determined according to TOLASA *et al.* (2005) with modifications. Cooked shrimp muscle (edible part) and cephalothoraxes were ground and blended for 1 min, separately. 8 g of the homogenized mixture have been extracted thrice with 30 ml acetone. Each acetone extract was collected in funnel separation. 25 ml hexane, 100 ml water, and 0.5 g of NaCl were added in order to separate water-soluble compounds. Funnels have been kept in dark for 20 min and shaken once. Hexane extract was filtered and dehydrated by anhydrous sodium sulfate and poured in a volumetric flask (25 ml). Total carotenoid concentration was determined spectrophotometrically by a Hewlett-Packard, HP 8452A (Cheadle Heath, Stockport Cheshire, UK) spectrophotometer at 470 nm and calculated according to a standard curve ($y = 0.1847x$; $r^2 = 0.9951$) of astaxanthin (Sigma, St. Louis, MO, USA, 98% purity). Analyses were performed in triplicate.

Data was summarized and presented as mean and standard deviation. One-tailed *t* test was used for mean comparisons with a significance level of 5% using BioEstat 5.0 as statistical treatment software.

RESULTS AND DISCUSSION

Shrimp kept in black tanks presented dark gray/blue color, whereas shrimp kept in white tanks showed a lighter gray color. When cooked, shrimp from black tanks presented more intense orange color than those grown in white tanks (Figure 1). Color differences noticed visually have been confirmed by instrumental color measurements, according to Table 1.

Figure 1. *In natura* and cooked shrimps grown in white (a) and black (b) tanks

Color of raw shrimp grown in black tanks was significantly darker (L* lower) and much greener (a* negative) and bluer (b* negative) than in white tanks. After cooking, animals from black tanks presented more intense orange color (a* representing red and *b representing yellow). Such colors have been observed for muscle as well as for cephalothoraxes samples (Table 1).

Table 1. L* (lightness), a* (redness), and b* (yellowness) values for samples of muscle and cephalothoraxes of raw and cooked shrimp grown in black and white tanks

	Raw		**Cooked**	
	Black tank	**White tank**	**Black tank**	**White tank**
Cephalotorax				
L*	26.02 ± 1.58 †	33.45 ± 4.47	55.42 ± 5.12 †	62.83 ± 7.34
a*	-2.42 ± 1.43 †	-0.77 ± 0.81	21.68 ± 2.55 †	12.21 ± 3.93
b*	-2.85 ± 2.33 †	3.88 ± 3.17	33.22 ± 4.70 †	22.37 ± 6.25
Body/muscle				
L*	33.42 ± 3.14 †	34.42 ± 1.79	65.71 ± 3.66	65.52 ± 6.52
a*	-3.43 ± 0.36 †	-1.62 ± 0.55	15.38 ± 3.91†	9.52 ± 3.85
b*	-7.55 ± 1.22 †	-0.71 ± 1.76	28.93 ± 7.57 †	15.51 ± 4.69

Mean ± standard deviation. One-tailed t test; † significant difference at confidence level of 5 % (n = 2)

The blue/gray color observed in *in natura* shrimp is due to the accumulation of crustacyanin, a protein-astaxanthin complex that becomes orange with complex dissociation. This dissociation may occur because of heat (cooking) or the addition of substances such as acetone (MURIANA *et al.,* 1993; VELU *et al.,* 2003).

This color change caused by formation/dissociation of the complex is due to the bathochromic effect (a change in the wavelength absorbed by compounds). Free astaxanthin absorbs light at 470-472 nm (yellow, orange and red) and with the formation of the astaxanthin-protein complex, absorption at 632 nm (green, blue or purple) is observed (WEESIE *et al*., 1995; CIANCI *et al.,* 2002).

This effect is biologically important once changing in shell color is a camouflage mechanism essential for defense strategies against predators (LATSCHA, 1989).

Regarding consumer preference, observed through subjective analysis, orange color after cooking is preferred for white shrimp (LATSCHA, 1989; BOONYARATPALIN *et al*., 2001; TUME *et al*., 2009). However, studies reporting color preferences for raw shrimp have not been found. These studies are important once the majority of shrimp is commercialized *in natura.*

Significant differences have not been observed as regards to total carotenoid content between shrimp grown in white and black tanks, for either cephalothoraxes (P = 0.3678) or muscle (P = 0.0930) (Table 2).

Table 2. Carotenoid content (mg 100 g^{-1}) in muscle and cephalothoraxes of shrimp kept in black and white tanks

Total carotenoids	Black tank	White tank
Cephalotorax	1.81 ± 0.13	1.62 ± 0.23
Muscle	0.67 ± 0.20	0.53 ± 0.10

Mean ± standard deviation. One-tailed t test; †significant difference at confidence level of 5 % (n = 2)

Such results indicate that the background tank color changes shrimp color but does not interfere significantly in the carotenoid amount accumulated in shrimp.

Results of the present study are in tune with TUME *et al.* (2009) who reported that shrimp fed with supplemented feed (40 ppm astaxanthin) during 28 days and grown in black or white tanks presented the same color differences as those presented in this study.

TUME *et al.* (2009) also observed a similarity in the amount of astaxanthin (major carotenoid) present in shrimp grown in black and white tanks, being the color differences observed using subjective assessment due to the location of pigmentation. For shrimp grown in black tanks, astaxanthin was spread in the epidermis while for shrimp grown in white tanks it was densely packed in chromatophores, i.e., the difference relies on the way these compounds, in particular astaxanthin, are deposited in these crustaceans and not on the accumulated amount.

According to the literature, feed supplemented with carotenoids such as β-carotene and astaxanthin has been recommended to grow more pigmented shrimp with higher carotenoid content (YAMADA *et al*., 1990; BOONYARATPALIN *et al*., 2001; ARREDONDO-FIGUEROA *et al*., 2003; SUPAMATTAYA *et al*., 2005; PASSOS, 2006). Other studies also demonstrate that the substrate light may cause the accumulation of astaxanthin in shrimp in order to protect against damage caused by luminosity excess, although it did not concerned shrimp color (YOU *et al*., *2006*). Nevertheless, studies reporting the relation between color tank, carotenoid content, and shrimp pigmentation are scarce. Therefore, these studies show that there are several ways to change color and/or astaxanthin content in shrimp, which assures this field as a very promising field of research.

The present work is the first to study the effect of background color tank on color of shrimp not fed with carotenoid-supplemented feed. The commercial feed used contains a small amount of carotenoid naturally present in the raw material formulation, which could have been converted in astaxanthin by shrimp, as demonstrated by other authors (BOONYARATPALIN *et al*., 2001; SUPAMATTAYA *et al*., 2005; PONCE-PALAFOX *et al*., 2006), and accumulated differently according to the color tank (TUME *et al*., 2009). It suggests that even without supplemented feed, it may be possible to change shrimp color through altering of the tank color.

It is important to say that, although it has been demonstrated that the color tank changes shrimp color, it does not change the amount of accumulated carotenoids. Therefore, the alterations in tank color may be a lower-cost alternative to intensify shrimp color that does not bring the benefits of shrimp development through carotenoid-supplemented diet (LIÑÁN-CABELLO *et al*., 2003; ARREDONDO-FIGUEROA *et al*., 2003; SUPAMATTAYA *et al*., 2005), as well as the nutritional qualities and benefits to consumer health that these nutrients ensure (GUERIN *et al*., 2003; HUSSEIN *et al*., 2006; PALOZZA *et al*., 2008).

Studies with larger number of samples and sensorial analyses are necessary in order to observe the acceptance of these shrimp as well as discussing the use of black tanks to obtain intensely-colored shrimp.

This preliminary study has reported that the background tank color influences the color of shrimp, but does not interfere significantly in the carotenoid amount accumulated in shrimp.

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