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Evaluation of Coloring Potential of *Dietzia natronolimnaea* Biomass as Source of Canthaxanthin for Egg Yolk Pigmentation

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ABSTRACT : An experiment was conducted to evaluate the effect of different levels of extracted pigment from *Dietzia natronolimnaea* biomass as a source of canthaxanthin in comparison with synthetic canthaxanthin on egg yolk pigmentation. The experiment used a completely randomized design (CRD). A total of 63 laying hens, 68 weeks old, were used and the birds were allotted to 7 dietary treatments with each treatment replicated three times with three hens per replicate. Treatments consisted of 3 levels of synthetic canthaxanthin (4, 8 and 16 ppm), 3 levels of extracted pigment from *D. natronolimnaea* biomass (4, 8 and 16 ppm) and control. Changes in yolk color were determined in 2 eggs taken at random, during the four week experimental period from each replicate. Supplementation of extracted pigment from *D. natronolimnaea* biomass had a significant effect on the color of egg yolks ($p<0.05$). Yolk color score of the control group was 6.83 in BASF color fan and the yolk color score of different extracted pigment levels was 11.00, 12.50 and 14.50, respectively. The yolk colors of different levels of synthetic canthaxanthin were 12.00, 14.00 and 15.00, respectively. The effect of pigment supplementation on egg yolk color was better explained by polynomial response curves. The R^2 indicated that for 3 supplementation levels of each pigment studied, over 90% of the color variation could be explained by the pigment concentration. The egg yolk color after 15 and 30 days of storage was not significantly different, but boiling reduced egg yolk color significantly ($p<0.05$). (**Key Words :** Microbial Pigment, Dietzia Natronolimnaea, Canthaxanthin, Laying Hen, Egg Yolk Color)

INTRODUCTION

Egg yolk color is an important criterion for consumer's choice of eggs. The color is used as a tool to assess the quality of eggs. Yolk color is indeed named at third position under egg quality traits (Hernandez and Blanch, 2000a, b). With this regard, oxy-carotenoids, called xanthophylls, are used to color food products of animal origin. To achieve a golden yellow yolk color diets have to be supplemented with both yellow and red pigments (Kang et al., 2003).

Canthaxanthin (4, 4'-diketo-β-carotene) is a ketocarotenoid found in certain animals, plants, and microorganisms (Bhosale and Bernstein, 2005), plus it is responsible for the orange-red color of egg yolk and the flesh of many marine animals (Nelis and De Leenheer, 1989) (Figure 1).

Sources of xanthophylls can be natural or synthetic. Consumer's preferences for natural organic products in their food are increasing (Williams, 1992; Sikder et al., 1998).

At present, the large market for carotenoids is satisfied through chemical synthesis, although this has various disadvantages, as the chemical synthesis of carotenoids requires a very high level of control and can produce compounds that have undesired side effects and may be allergens in certain consumers. Therefore, in view of the global economic value of carotenoids and increasing awareness of consumers, the production of these materials from natural sources has become an area of intensive investigation (Ausich, 1997). For the industrial production of carotenoids, microorganisms are preferred over other natural sources, such as vegetables and fruits, owing to problems of seasonal and geographic variability in production. In addition, there are economic advantages to microbial processes that use agricultural waste and industrial wastewater as substrates (Buzzini, 2000). Nonetheless and despite the importance of the microbial production of these compounds, a few microorganisms have been identified that suits commercial applications of ketocarotenoids, which include the yeast *Phaffia rhodozyma* (Johnson and An, 1991; Kim et al., 2006), fresh-water green alga *Haematococcus pluvialis* (Margalith, 1999), and green

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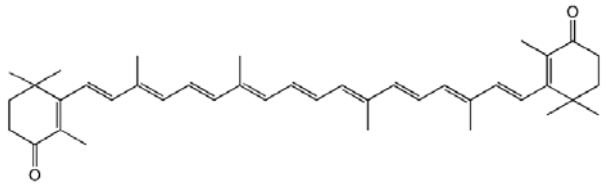

Figure 1. Chemical structure of canthaxanthin.

alga *Chlorococcum sp.* strain MA-1 (Zhang and Lee, 2001), all of which accumulate astaxanthin. However, very little data are available on the commercial production of canthaxanthin by microorganisms (Bhosale and Bernstein, 2005). Thus, the search continues for microbial sources for canthaxanthin production and the optimum conditions for the production of these compounds.

The bacterium *Dietzia natronolimnaea* is Gram positive, catalase positive, and oxidase negative with orange colonies (Duckworth et al., 1998), and *D. natronolimnaea* HS-1 was isolated during a routine screening of pigmented microorganisms. In preliminary experiments, the main pigment of this strain was identified to be a canthaxanthin (Razavi, 2004).

The aim of this study was to evaluate the ability of different levels of extracted pigment from *D. natronolimnaea* HS-1 biomass in compare with synthetic canthaxanthin in enhancing egg yolk pigmentation.

MATERIALS AND METHODS

Materials

The glucose, peptone, yeast extract, sugars and agar were all obtained from the Sigma-Aldrich Chemical Company (USA). The pure ethanol (99.9%) was purchased from the Bidestan Company (Iran). Lucantin®red (Synthetic Canthaxanthin) from the BASF Company (Germany).

Microorganism

The strain *D. natronolimnaea* HS-1 (DSM 44860) was isolated in the Laboratoire des Science du Génie Chimique by Razavi (Razavi, 2004), and maintained on yeast/malt (YM) agar plates containing (per liter): 10 g glucose, 5 g peptone, 5 g yeast extract, and 15 g agar. Single colonies were transferred to a fresh plate every month, incubated for 4 days, and thereafter kept under refrigeration at 4°C.

Culture conditions

A pure culture of *D. natronolimnaea* HS-1 from the YM agar was transferred into 500-ml Erlenmeyer flasks containing 200 ml of a GPY medium (per liter: 10 g glucose, 10 g peptone, 6 g yeast extract), incubated in a rotary shaker (180 rpm) at 28±1°C for 150 h.

Table 1. Ingredient and calculated analysis of basal diet

Ingredient	%
Corn grain	64.53
Soybean meal	21.19
Oyster shell	9.94
soybean oil	2.11
Dicalcium phosphate	1.19
Common salt (NaCl)	0.33
Vitamin-mineral premix[1]	0.5
DL-methionine	0.21
Calculated analysis	
ME (Kcal/kg)	2,820
CP (%)	15
Calcium (%)	4.1
Available phosphorus (%)	0.34
Na. (%)	0.18
Lysine (%)	0.76
Met+cys	0.72
Methionine (%)	0.47

[1] Contained followings per kg of premix: vitamin A, 1,600,000 IU; vitamin D_3, 300,000 IU; vitamin E, 800 IU; vitamin K_3, 132 mg; vitamin B_2, 1,000 mg; vitamin B_{12}, 1,200 mg; niacin, 2,000 mg; pantothenate Ca, 800 mg; folic acid, 60 mg; choline chloride, 35,000 mg; dl-methionine, 6,000 mg; iron, 4,000 mg; copper, 500 mg; manganese, 12,000 mg; zinc, 9,000 mg; cobalt, 100 mg; BHT, 6,000 mg; iodide, 250 mg.

Extraction and measurement of carotenoids

After 150 h incubation, Ten-ml aliquots were centrifuged at 5,000×g for 10 min at 4°C. Next, the supernatant (biomass) was resuspended in 3 ml of pure ethanol and put in water bath (45°C) for 2 min and then vortexed for 5 min, and the pellets centrifuged for extraction pigment. For full extraction pigment, this process repeated three times. Thereafter, the carotenoid extracts subsequently filtered through a 0.2-μm hydrophobic fluorophore membrane (Sigma-Aldrich Co., USA) and analyzed by scanning the absorbance of the wavelength spectra of 300-600 nm using a spectrophotometer (UV-Visible, Cary 300, Varians Co., Germany). The maximum absorbance was determined at a wavelength of 474 nm, which conformed to standard canthaxanthin λmax. The total carotenoid concentration was calculated following the formula provided by An et al. (1989).

Animals and diets

A total of 63 Hy-Line laying hens, 68 weeks old, were used in this study. The birds were allotted to 7 dietary treatments with each treatment replicated 3 times with 3 hens per replicate. A typical corn-soybean basal diet was formulated as the control (Table 1). UFFDA Software was used for diet formulation (Pesti et al., 1992). The birds received feed and water *ad libitum*. During a pre-experimental period of 15 days the birds were fed a basal diet without pigment supplementation. The experimental

Table 2. Experimental treatments

Treatment No.	Treatment groups
1	Control
2	Synthetic canthaxanthin (4 ppm)
3	Synthetic canthaxanthin (8 ppm)
4	Synthetic canthaxanthin (16 ppm)
5	*D. natronolimnaea* biomass extracted pigment (4 ppm)
6	*D. natronolimnaea* biomass extracted pigment (8 ppm)
7	*D. natronolimnaea* biomass extracted pigment (16 ppm)

period lasted for 30 days. The lighting regimen was 16 h of light per day. Treatments consisted of 3 levels of extracted pigment from *D. natronolimnaea* biomass (4, 8 and 16 ppm), 3 levels of synthetic canthaxanthin (4, 8 and 16 ppm) and control (Table 2). Eggs were collected daily and egg production, egg weight, feed intake, and feed conversion (g feed:g egg) were recorded. Egg yolk color was measured by BASF color fan. Changes in yolk color were determined in 2 eggs taken at random, during the four weeks of experimental period from each replicate. Per treatment, the yolk color was determined in 20 eggs after boiling in a water bath (93°C for 15 min).

Statistical analysis

Statistical analysis was performed with SAS 6.1 (1992); data were analyzed by General Linear Model in SAS program. The experiment used a completely randomized design, with 7 treatments and 3 replicates. Yolk color scores results were subjected to polynomial regression. Egg production, egg weight, feed intake, feed conversion, and yolk color data were analyzed by analysis of variance. Differences among treatments were tested using Duncan's multiple range test at the significance level of 0.05.

RESULTS AND DISCUSSION

The effects of the treatments on hen performance and yolk color scores are presented in Table 3. Egg production, egg weight, feed intake and feed conversion were not affected by any of the treatments, but the egg yolk color changed significantly ($p<0.05$) according to the pigments and concentrations used. In general, Pigment supplementation had not been associated with changes in production (Angeles and Scheideler, 1998; Garcia et al., 2002; Soto-Salanova, 2003). Supplementation of extracted pigment from *D. natronolimnaea* biomass had a significant effect on the color of egg yolks ($p<0.05$). Color scores of egg yolks from treatments with extracted pigment from *D. natronolimnaea* biomass, at all levels, tested in this experiment, were higher compared with the ones observed in the control group. Yolk color score of control group was 6.83 in BASF color fan and the yolk color score of different extracted pigment levels were 11.00, 12.50 and 14.50, respectively and the yolk colors of different levels of synthetic canthaxanthin were 12.00, 14.00 and 15.00, respectively (Table 3). Each increase in the supplementation level of extracted pigment caused an additional effect on color score. As oxycarotenoids, they are well absorbed by the birds' gastrointestinal tract and accumulate at various sites, including the egg yolk, in which they are deposited mainly as free carotenoids (Latscha, 1990). The oxycarotenoids deposition began quickly, after approximately 24 h of feeding and reached the highest scores around 11 d of feeding (Figures 2 and 3). At this time, a plateau was observed, which indicated the maximum color score to be reached for each extracted pigment supplementation level. This result was agreed with the previous studies (Grashorn et al., 2000; Sidibè, 2001). Recent trials performed by Sidibè (2001) give additional data on canthaxanthin deposition in the egg yolk after feeding canthaxanthin to laying hens at levels of 2-6 mg/kg feed. The color intensity of the egg yolk reached a plateau after 10 days and the canthaxanthin levels in the egg yolk measured between day 19 and 25 reflect a stable relationship between canthaxanthin in feed and egg yolk (Sidibè, 2001).

The egg yolk color after 15 and 30 days of storage did not show significant difference (data not shown). Boiling

Table 3. Effect of treatments on egg production, egg weight, feed intake, feed conversion, and yolk color[A]

Treatment No.	Egg production (%)	Egg weight (g)	Feed intake	Feed conversion	Yolk color (BASF color fan)
1	81.35±0.37[a]	63.02±0.44[a]	112.52±0.36[a]	1.79±0.020[a]	6.83±0.11[e]
2	81.48±0.98[a]	63.09±0.21[a]	112.97±0.38[a]	1.78±0.007[a]	12.00±0.17[c]
3	82.22±0.64[a]	63.21±0.41[a]	112.78±0.35[a]	1.78±0.012[a]	14.00±0.12[b]
4	82.22±1.11[a]	62.95±0.34[a]	112.66±0.31[a]	1.79±0.008[a]	15.00±0.12[a]
5	82.59±0.98[a]	63.10±0.50[a]	112.61±0.47[a]	1.78±0.019[a]	11.00±0.17[d]
6	81.85±1.33[a]	63.14±0.17[a]	113.06±0.40[a]	1.79±0.010[a]	12.50±0.23[c]
7	81.11±1.11[a]	62.65±0.75[a]	112.59±0.51[a]	1.80±0.022[a]	14.50±0.15[ab]
p-value	0.9474	0.9795	0.6779	0.981	<0.0001
CV	2.07	1.21	0.62	1.47	4.52

[a-e] Means in a column with different superscripts differ significantly ($p<0.05$). [A] Means±SE.

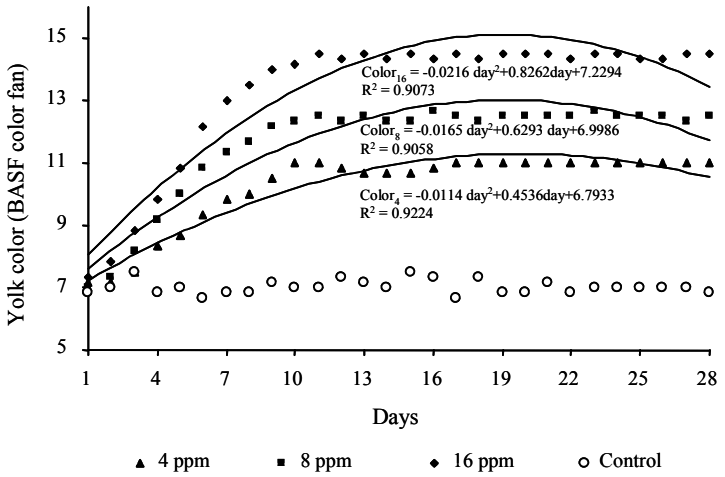

Figure 2. The effect of *D. natronolimnaea* oxycarotenoids deposition curves on egg yolks and their polynomial equations.

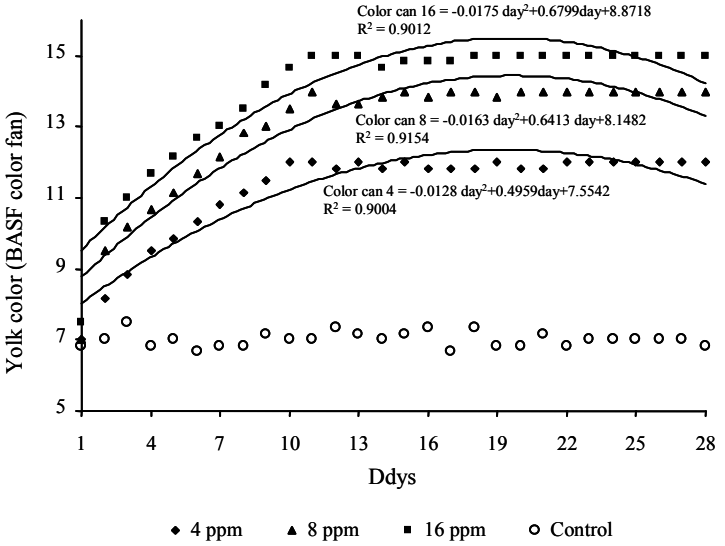

Figure 3. The effect of synthetic canthaxanthin deposition curves on egg yolks and their polynomial equations.

affected on egg yolk color ($p<0.05$) and reduced the yolk color score (Table 4). The visual color score is reduced by at least one unit in yolks of eggs that not supplemented by pigment (control). This reduction was higher in other experimental groups with pigment supplementation. This result was agreed with result that reported by Grashorn and Steinberg (Grashorn and Steinberg, 2002). The coloring potential of cis-carotenoids is lower than trans-carotenoids. Heating during egg boiling, transforms the trans-carotenoids of egg yolk to cis isomer and for this reason, the yolk color score will be reduced.

The effect of pigment supplementation on egg yolk color was better explained by polynomial effect response curves. The R^2 indicate that for 3 supplementation levels of

Table 4. Yolk color of fresh egg in compare with boiled egg[A]

Treatment No.	Yolk color (BASF color fan)		p-value	CV
	Fresh egg	Boiled egg		
1	6.83±0.29[a]	5.50±0.23[b]	<0.0001	10.67
2	12.00±0.26[a]	10.00±0.26[b]	<0.0001	5.75
3	14.00±0.17[a]	11.67±0.33[b]	<0.0001	5.06
4	15.00±0.26[a]	12.17±0.31[b]	<0.0001	5.12
5	11.00±0.26[a]	9.33±0.21[b]	<0.0001	5.68
6	12.50±0.34[a]	10.17±0.31[b]	<0.0001	7.02
7	14.50±0.34[a]	12.00±0.52[b]	<0.0001	8.09

[a, b] Means in a row with different superscripts differ significantly ($p<0.05$). [A] Means±SE.

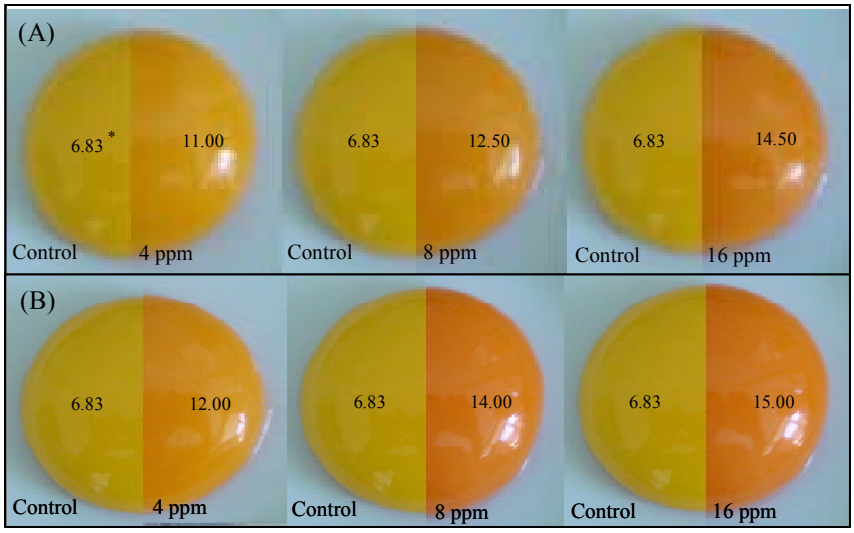

Figure 4. Comparison between egg yolk color of control group and different levels of extracted pigment from *D. natronolimnaea* biomass (A), and egg yolk color of control group and different levels of synthetic canthaxanthin (B). * Yolk color scale (BASf color fan).

each pigment studied, over 90% of the color variation could be explained by the pigment concentration. This result was in agreement with the report of Ponsano et al. (2004).

Comparison between egg yolk color of control group, different levels of synthetic canthaxanthin and different levels of extracted pigment from *D. natronolimnaea* biomass are presented in Figure 4.

In finally this study indicated that extracted pigment from *D. natronolimnaea* biomass could create suitable egg yolk color and it is proper natural pigment.

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