DOI: 10.2478/cjf-2024-0009
CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

CAROTENOIDS FROM SHRIMP WASTE AS A NATURAL DYE FOR THE FEATHER COLOURING OF CANARY BIRDS *Serinus canaria domestica*

Alexander Atanasoff[1*], Hristo Hristov[1], Dimitar Yorgov[2], Ferhat Cagiltay[3]

[1] *Trakia University, Faculty of Veterinary Medicine, 6014 Stara Zagora, Bulgaria*
[2] *Trakia University, Faculty of Agriculture, 6014 Stara Zagora, Bulgaria*
[3] *Akdeniz University, Faculty of Fisheries, 07058 Antalya, Türkiye*

*Corresponding Author: aleksandar.atanasov@trakia-uni.bg

ARTICLE INFO

Received: 13 September 2023
Accepted: 15 March 2024

ABSTRACT

Carotenoids are naturally occurring pigments in plants, algae, fungi, insects, and crustaceans. Krill and prawns contain high levels of some value-added nutrients for the aquaculture industry, such as astaxanthin which is used as a colouring agent. In birds with ornamental plumage, such as canaries, the carotenoid-pigmented plumage and red hues are exclusively due to the diet. In this regard, our aim was to study the possibilities of using shrimp waste for feather colouring in canary *Serinus canaria domestica*. Shrimp *Pandalus borealis* dried waste was included in the feed of six female red lipochrome mosaic canaries *Serinus canaria domestica* for three months during their third molt. The basic diet consisted of a seed mixture (canary seed, sunflower seed without shell, linseed, and rapeseed), rearing food (Quiko®Bianco), and conditioning food (Quiko®Rusk) with the supplementation of an oil suspension of dried shrimp waste (3%). The *ad libitum*-fed birds had additional free access to pasta (Legazin® Procria White Morbida). To evaluate the effect of shrimp waste on feather colouring, covert feathers were taken from the tail in the region of the uropygial gland. Diffuse reflectance spectroscopy of the most intensely coloured parts of the feathers was measured by a spectrophotometer. The chromaticity coordinates in a CIE xyY colour space were calculated from the measured spectroscopy. The results of the experiment showed that shrimp waste increased chromaticity and had no negative effect on the canaries. On this basis, the authors assumed that dried shrimp waste could be an alternative to synthetic dyes.

Keywords:
Astaxanthin
Canary birds
Pigmentation
Shrimp by-product

How to Cite

Atanasoff, A., Hristov, H., Yorgov, D., Cagiltay, F. (2024): Carotenoids from shrimp waste as a natural dye for the feather colouring of canary birds *Serinus canaria domestica*. Croatian Journal of Fisheries, 82, 73-77. DOI: 10.2478/cjf-2024-0009.

INTRODUCTION

The domestic canary, often simply known as canary bird *Serinus canaria domestica*, has been widely used in ornithological research as a model for carotenoid plumage colouration studies (Koch et al., 2016). The carotenoid-pigmented plumage and red hues of the birds are exclusively associated with the diet. There are over 800 types of carotenoids but only four major ones are used in colour feeding: beta-carotene (orange pigment), lutein and zeaxanthin (yellow pigment) and canthaxanthin (red pigment). The last one is the most powerful color-promoting substance for red factor canaries.
Astaxanthin (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) is a strong lipophilic red keto-carotenoid pigment and metabolite of zeaxanthin and/or canthaxanthin which naturally originates in rainwater microalgae *Haematococcus pluvialis*. They are consumed by crustaceans (e.g. shrimp, krill, crab, lobster, crayfish) (Martínez-Cámara et al., 2021). Astaxanthin extracted from *H. pluvialis* can be used as a nutritional supplement for humans and animals, as approved by the European Food Safety Authority (Turck et al., 2020). Therefore, recent studies tend to investigate the colouring role of astaxanthin in fish meat (Harith et al., 2022) and the feathers of some wading birds, e.g. flamingo and scarlet ibis (Johnson and Lewis, 1979). However, there is little information about the relationship between astaxanthin and ornamental bird pigmentation. In this regard, we studied the possibilities of using dried shrimp waste for feather colouring in canary bird *Serinus canaria domestica*.

MATERIALS AND METHODS

Animals and treatment

Shrimp meal is undecomposed, ground and oven-dried shrimp waste. It contains whole shrimp and/or shrimp parts of *Pandalus borealis*, such as heads or shells. In order to prevent possible contamination, dried shrimp waste should not contain any microorganisms (bacteria, viruses, parasites). For this reason, a microbiological quality assessment of raw shrimp was performed by applying Urku's method (2021). The proximate analysis of the samples was determined in laboratory conditions (Table 1). We used AOAC (2006; method 983.18) to prepare the samples and subjected them to moisture analyses using air drying AOAC (1997; method 950.46). Crude protein content was calculated by converting the nitrogen content by multiplying it by 6.25 because of the 16% nitrogen content in proteins (100/16 = 6.25), determined by the Kjeldahl method using a semi-automatic Kjeldahl system (UDK-139, Velp Scientifica, Italy). Lipid content was determined by Soxhlet extraction (AOAC 2006; method 960.39). Crude ash was obtained by incineration in a muffle furnace (MLW, Germany) at 550 °C for 8 h. Liquid chromatography with atmospheric pressure chemical ionization in mass spectrometry (LC-APCI-MS) was used for the identification of free astaxanthin (0.5%) in samples of dried shrimp meal.
Shrimp meal was added to the feed of six female (1) red lipochrome mosaic canaries for three months during their third molt. The colourfastness of carotenoids varies tremendously between wild and domestic birds, and therefore we selected only red lipochrome mosaic canary *Serinus canaria domestica* as the primary species for this study. One of the canaries suddenly died during the third month of the trial.
The basic diet consisted of a seed mixture (canary seed, sunflower seed without shell, linseed and rapeseed), rearing food (Quiko®Bianco) and conditioning food (Quiko®Rusk) with supplementation of an oil suspension of shrimp meal (3%). Canary birds can change the colour of their feathers only during molt, and thus we supplemented the feed during the respective season. *Ad libitum*-fed birds had additional free access to paste (Legazin® Procria White Morbida), according to breed requirement recommendations. We took covert feathers from the uropygial gland region to evaluate the effect of the shrimp meal on feather colouring. Water was provided *ad libitum*.

Table 1. Proximate analysis of shrimp meal (g/100 g sample)

Parameter	As fed	On DM
Dry matter, g	91.964 ± 4.11	100
Crude protein, g	49.582 ± 6.05	53.915
Crude fat, g	7.530 ± 0.85	8.188
Crude fibre, g	8.281 ± 1.48	9.005
Crude ash, g	19.891 ± 1.73	21.629

Spectrophotometry

Diffuse reflectance spectroscopy from the most intensely coloured parts of the feathers was measured by a spectrophotometer (Spekol 11, Carl Zeiss, Jena). The chromaticity coordinates in a CIE xyY colour space were calculated from the measured spectroscopy.
The diffuse reflectance coefficient $R(\lambda)$ at a given wavelength λ was calculated according to the formula:

$$R(\lambda)=\frac{I_{feathers}(\lambda)}{I_{WhiteStd}(\lambda)} \quad (1)$$

where $I_{feathers}(\lambda)$ is the measured intensity of the light at a wavelength λ, coming from the surface of the feathers, and $I_{WhiteStd}(\lambda)$ is the intensity at the same wavelength, coming from the white standard sample measured at the same conditions as the feathers.

The excitation purity *EP* which represents the degree of saturation of the colour was calculated according to the formula:

$$EP[\%] = \frac{WC^*}{WD^*} \times 100 \quad (2)$$

RESULTS

The colour measurements of the feathers were done by a spectrophotometer. Special measures were taken to measure only the most intensely coloured part because of the non-uniformity of the colouring of the canary feathers. This was done by placing a bundle of feathers between two pieces of black cardboard. One had a circular opening with a known diameter. This piece was placed in such a way that the most intensely coloured part of the feathers was seen through the opening (Fig. 1). As the field measured by the spectrophotometer was larger than the aperture in the cardboard, the raw signal was corrected by subtracting the signal coming from the black cardboard area.

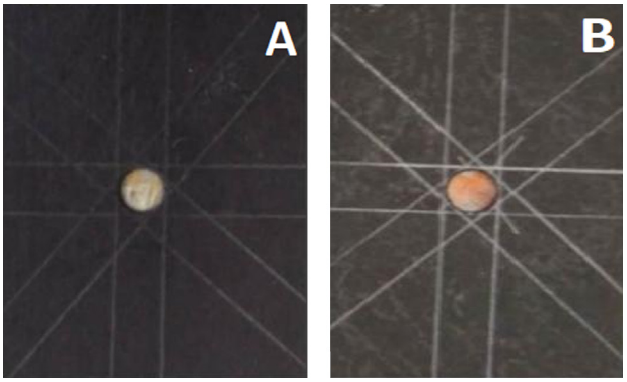

Fig 1. Samples of canary feathers (2-16) before feeding with 3% shrimp meal (A) and after three months of feeding (B)

Since our objective was to study the impact of shrimp meal on the intensity of feather colouring, it was enough to focus our attention on *x* and *y* chromaticity coordinates derived from *X, Y, Z* colour coordinates. The chromaticity coordinates x and y determine the colour on the CIE 1931 colour space chromaticity diagram.
The colour coordinates *X, Y, Z* were calculated from the diffuse reflectance spectroscopy of the samples measured at 40 wavelengths in the 380 - 770 nm spectrum interval (the entire visible part of the spectrum) with an increment of 10 nm. The measurement geometry was 45/0, i.e. illumination of the sample under the angle 45° and detection of the signal under 0°. Both angles were determined considering the direction perpendicular to the sample surface.
An example of diffuse reflectance spectroscopy of samples belonging to one of the experimental birds before and after alimentation with 3% shrimp meal in the feed is shown in Fig. 2. This diagram shows all the hues perceivable by the standard observer for various (*x, y*) pairs and also indicates the spectral wavelengths of the dominant single frequency colours.

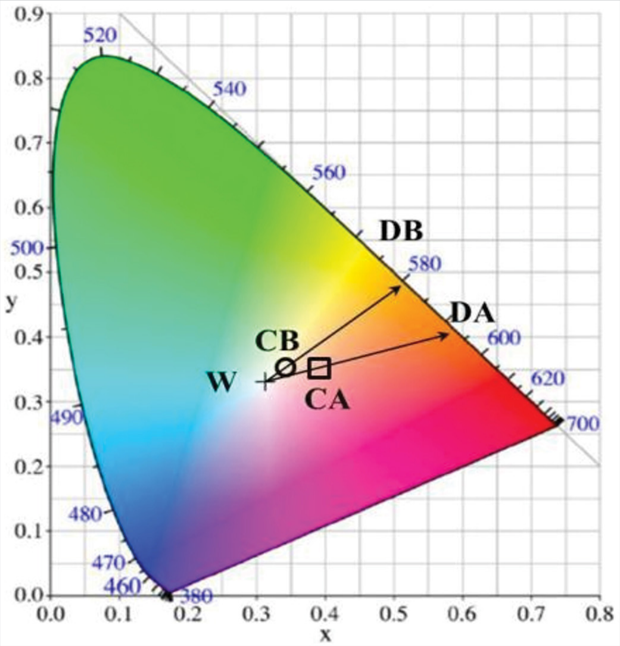

Fig 2. CIE 1931 colour space chromaticity diagram and location of the measured colours of the canary (2-16) before and after alimentation with 3% shrimp meal. Circle – before the diet; square – after the diet. W corresponds to the white colour; CB – the centre of the circle; CA – the centre of the square; points DB and DA indicate the corresponding dominant wavelengths.

The colour characteristics of the samples were calculated using a program in MS Excel supposing the illumination from a standardized *D65* light source (which corresponds to the stray sunlight).
The calculated chromaticity coordinates *x* and *y* for all measured samples are presented in Table 2.
The dominant wavelength λ_d and the excitation purity *EP* are shown as well. The dominant wavelength λ_d is determined by an intersection point *DB (DA)* between the line *W-CB (W-CA)* and the boundary line of the CIE 1931 colour space chromaticity diagram (Figure 3).

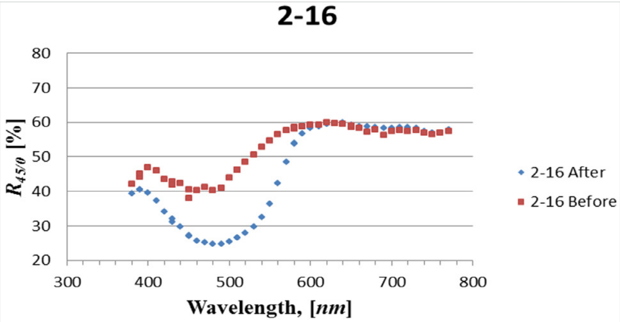

Fig 3. Diffuse reflectance spectroscopy before and after the diet with 3% shrimp meal. Red markers – before the diet; blue markers – after the diet.

Table 2. Values of the chromaticity coordinates x and y for all measured samples

Colour	Identification of birds									
	130-16		144-15		177-15		199-15		2-16	
	Before	After	Before	After	Before	After	Before	After	Before	After
X	0.39299	0.39493	0.30612	0.36870	0.35472	0.38688	0.38079	0.40904	0.34488	0.38910
Y	0.35192	0.36095	0.32514	0.37137	0.36061	0.35482	0.34173	0.37698	0.35684	0.35377
λ_d, nm	595	590	483*	582	582	592	602	585	580	593
EP, %	27.4	30.8	3.5*	29	20.4	26.8	18.7	39.3	14.6	28.2

λ_d determines the hue of the sample's colour. The dominant wavelengths before and after alimentation were different, but the results from these measurements were insufficient to find any correlations between λ_d and the feeding of the birds.

The point is located very close to the white point *W* but, because of the error of measurements, it appeared to the left from *W* and thus the dominant wavelength occurred in the blue region. Practically this sample has no dominant wavelength and its *EP* is 0. The excitation purity *(EP)* is higher for all samples after the treatment with shrimp meal additives. It means that after the alimentation, the feather colour had higher saturation.

DISCUSSION

Northern shrimp are usually peeled, cooked and frozen, with processing waste consisting of shells, heads and tails, which represent approximately 50-60% of the catch weight (Jiao et al., 2015). The results of this experiment were consistent with data from previous studies where the crude protein content of processing by-products was found to vary between 35-40% of the dry weight, and total fat content was about 0.7-0.8%, while the crude ash varied between 17-20% (Heu et al., 2003).

Astaxanthin has been established as the most prevalent carotenoid in shrimp, representing about 65-98% of the total carotenoids (Jiao et al., 2015). Astaxanthin is more polar than other carotenoids and usually combined with lipids. Since canary birds cannot synthesize carotenoids *de novo*, the right type and combination of carotenoids have to be delivered via the feed to obtain the desired feather colour. Thus, the colour intensity of feathers mostly depends on the rate of astaxanthin in the feed. Two sources of astaxanthin are used in birds: the first one is with *trans* (E) structure from biological acquisition and the second one is with *cis* (Z) structure from artificial chemical synthesis. The major natural sources are bacterium *Paracoccus carotinifaciens*, red yeast *Phaffia rhodozyma* and unicellular microalgae (*Heamatococcus pluvialis*, *Chlorococcum*, *Chlorella zofingiensis*), where astaxanthin can range from 0.15% to 5%. Generally, for chicken pigmentation, astaxanthin is used at rates of 10-30 mg/kg to give the desired feather colour (Honchar et al., 2022), but it is impossible to overdose on astaxanthin. Astaxanthins are fat-soluble compounds and the level of added fat in the feed directly affects the extent and rate of absorption and reddish colour. Walker et al. (2012) reveal that egg yolk colour can be improved by supplementing astaxanthin with palm toco. After being absorbed by the liver and circulated in the bloodstream, the lipoproteins with different densities are delivered and deposited in eggs, feathers and other target places. The results of the current study confirmed that supplementation of shrimp meal (3%) with an oil suspension in a canary diet is more effective for accumulating red colour in the bird feathers.

The current investigation provides practical and useful information on the change in feather colour through feed supplemented with shrimp meal for one of the most popular ornamental birds - canaries. These findings will be important for breeders of exhibition canaries and can foster discussions about using the natural aquaculture by-product. Since carotenoids are by nature susceptible to degradation by light, heat and other agents, further studies are required to understand the technology of preserving the integrity of molecules across the feed manufacturing process for ornamental birds.

KAROTENOIDI IZ OTPADAKA RAČIĆA KAO PRIRODNO BOJILO ZA BOJANJE PERJA KANARINACA *Serinus canaria domestica*

SAŽETAK

Karotenoidi su prirodni pigmenti u biljkama, algama, gljivama, kukcima i rakovima. Kril račići i kozice sadrže visoke razine određenih hranjivih tvari koje imaju dodatnu vrijednost za industriju akvakulture, poput astaksantina koji se koristi kao bojilo. Kod ptica s ukrasnim perjem, kao što su kanarinci, karotenoidno pigmentirano perje i crvene nijanse isključivo su posljedica prehrane. Cilj nam je bio istražiti mogućnosti korištenja otpadaka račića za bojanje perja kanarinca *Serinus canaria domestica*. Osušeni otpad račića *Pandalus borealis* uključen je u hranu šest ženki crvenih lipokromnih mozaičnih kanarinaca *Serinus canaria domestica* tri mjeseca tijekom njihovog trećeg linjanja. Osnovna prehrana sastojala se od mješavine sjemenki (kanarinčeve sjemenke, sjemenke suncokreta bez ljuske, lanene sjemenke i sjemenke uljane repice), hrane za uzgoj (Quiko®Bianco) i hrane za kondicioniranje (Quiko®Rusk) s dodatkom uljne suspenzije osušenih otpadaka račića (3%). *Ad libitum* hranjene ptice imale su dodatni slobodan pristup tjestenini (Legazin® Procria White Morbida). Kako bi se procijenio učinak otpadaka račića na boju perja, pokrovno perje uzeto je iz repa u području uropigijske žlijezde. Spektrofotometrom je mjerena spektroskopija difuzne refleksije najintenzivnije obojenih dijelova perja. Koordinate kromatičnosti u CIE xyY prostoru boja izračunate su iz izmjerene spektroskopije. Rezultati eksperimenta pokazali su da otpad od račića povećava kromatičnost i nema negativan učinak na kanarince. Na temelju toga, autori su pretpostavili da bi osušeni otpad od račića mogao biti alternativa sintetskim bojama.

Ključne riječi: astaksantin, kanarinci, pigmentacija, nusproizvod račića

REFERENCES

Harith, Z., Sukri, S.M., Remlee, N.F.S., Mohd Sabir, F.N., Azwanida Zakaria, N.N. (2022): Effects of dietary astaxanthin enrichment on enhancing the colour and growth of red tilapia, *Oreochromis* sp. Aquaculture and Fisheries, 9.1, 52-56.

Heu, M.S., Kim, J.S., Shahidi, F. (2003): Components and nutritional quality of shrimp processing by-products, Food chemistry, 82 (2); 235-242.

Honchar, V., Iakubchak, O., Shevchenko, L., Midyk, S., Korniyenko, V., Kondratiuk, V., Rozbytska, T., Melnik, V., Kryzhova, Y. (2022): The effect of astaxanthin and lycopene on the content of fatty acids in the yolks of chicken eggs under different storage regimes. Slovak Journal of Food Sciences, 16, 473-489.

Jiao, G., Hui, J.P.M., Burton, I., Thibault, M.H., Pelletier, C., Boudreau, J., Tchoukanova, N., Subramanian, B., Djaoued, Y., Ewart, S., Gagnon, J., Ewart, K.V., Zhang, J. (2015): Characterization of shrimp oil from *Pandalus borealis* by high performance liquid chromatography and high resolution mass spectrometry. Marine drugs, 13 (6): 3849-3876.

Johnson, E. A., Lewis, M. J. (1979): Astaxanthin formation by the yeast *Phaffia rhodozyma*. Microbiology, 115 (1), 173-183.

Koch, R., McGrew, K., Hill, G. (2016): Effects of diet on plumage coloration and carotenoid deposition in red and yellow domestic canaries (*Serinus canaria*). The Wilson Journal of Ornithology, 128 (2), 328-333.

Martínez-Cámara, S., Ibañez, A., Rubio, S., Barreiro, C., Barredo, J.L. (2021): Main carotenoids produced by microorganisms. Encyclopedia, (4), 1223-1245.

Turck, D., Castenmiller, J., de Henauw, S., Hirsch-Ernst, K.I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Engel, K., Frenzel, T., Heinonen, M., Marchelli, R., Neuhcauser-Berthold, M., Poulsen, M., Sanz, Y., Schlatter, J., van Loveren, H., Ackerl, R., Gelbmann, W., Steinkellner, H., Knutsen, H. (2020):. Safety of astaxanthin for its use as a novel food in food supplements. EFSA Journal, 18 (2), e05993

Urku, C. (2021): Isolation and characterization of *Pseudomonas putida* caused granulomas in cultured sea bass (*Dicentrarchus labrax*) in Turkey. Journal of the Hellenic Veterinary Medical Society, 72(1), 2661-2668.

Walker, T., Wang, H., Dolde, D. (2012): Supplementation of laying-hen feed with palm tocos and algae astaxanthin for egg yolk nutrient enrichment. Journal of Agricultural and Food Chemistry, 60, 1989-1999.