

## Pigmentation of Pacific white shrimp (*Penaeus vannamei*) using Aztec marigold (*Tagetes erecta*) extracts as the carotenoid source

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**SUMMARY.** Pacific white shrimp were fed diets containing natural pigments from Aztec marigold (*Tagetes erecta*) petals extracts (esterified and saponified). The pigmentation effect of these carotenoids was compared with a carotenoid-free control diet and a synthetic astaxanthin (Roche Carophyll Pink) supplemented diet. It was found that after 14 days of feeding, the shrimp showed that the saponified marigold extract and the high concentration esterified marigold extract supplemented diets produced a better pigmentation effect (between 50-70 %) in the exoskeleton than the astaxanthin, the low concentration esterified marigold extract and the basal diets, the latter diets being non-significantly different among themselves. However, during the same 14 day period, non-significantly different results were observed in the carotenoid content of the shrimp abdominal muscle when fed with all diets except the low concentration esterified marigold extract diet that showed a significantly lower value than the saponified marigold extract diet. These results indicate that the main carotenoid sources in *Tagetes erecta*, namely lutein and zeaxanthin, are possibly metabolized into astaxanthin and deposited by the Pacific white shrimp.

**RESUMEN.** Pigmentación del camarón blanco del Pacífico (*Penaeus vannamei*) utilizando extractos de flor de cempasúchil (*Tagetes erecta*) como fuente de carotenoides. El camarón blanco del Pacífico fue alimentado con dietas que contenían pigmentos naturales de extractos de pétalo de flor de cempasúchil (*Tagetes erecta*) esterificados y saponificados. El efecto pigmentante de los carotenoides fue comparado con una dieta control libre de carotenoides y una dieta de referencia con astaxantina sintética (Carofil rosa de Roche). Se encontró que después de 14 días de alimentación, los camarones mostraron que los extractos de cempasúchil saponificado y el esterificado de alta concentración produjeron un mejor efecto de pigmentación (50-70 %) que la astaxantina en el exoesqueleto. El extracto esterificado de baja concentración y la dieta basal no difieren significativamente entre ellos. Sin embargo, durante el mismo período de 14 días, no hubo diferencias significativas en el contenido de carotenoides del músculo abdominal del camarón, excepto con el extracto esterificado de baja concentración, que mostró un valor menor que el del extracto saponificado. Estos resultados indican que la fuente de carotenoides principal en *Tagetes erecta*, luteína y zeaxantina, son posiblemente metabolizados en astaxantina y depositados por el camarón.

### INTRODUCTION

One of the main quality parameters in some shrimp is their degree of coloration. The color in shrimp can be obtained from several pigmentation precursors in the diet, among which the carotenoids are the most important (1).

The main pigment that colors shrimp muscle is astaxanthin (3,3 dihydroxy- $\beta$ -carotene 4,4-dione), and when present in the diet it can be deposited directly in the tissue as an ester of astaxanthin (2).

There is evidence that some carotenoids, e.g.  $\beta$ -carotene and zeaxanthin, can be transformed into astaxanthin, by *P. japonicus*, through different metabolic pathways (2). The use of carotenoids from Aztec marigold (*Tagetes erecta*) petals extracts has been widely studied in the poultry industry (3), but very little is known of their pigmentation effect on fish and crustaceans. Other flowers extracts, e.g., *Adonis aestivalis* extracts, at a level of 10 mg total pigment/100g diet produced a noticeable pink coloration in trout (*Onchorhynchus mykiss*) skin after a 3 month feeding period (4).

Thus the main purpose of this work was to determine if the esterified and saponified carotenoids obtained from *Tagetes erecta* are bioavailable and deposited in the tissues of the Pacific white shrimp (*Penaeus vannamei*).

### MATERIAL AND METHODS

**Test diets:** A basal diet (BD) was formulated in accordance with the nutritional requirements of shrimp of 6 to 8 cm in length (5,6). To this diet the esterified and saponified pigments of Aztec marigold petals were incorporated at the following total carotenoids (TC) concentrations: diet EYL1, 278.45 g/kg of esterified xanthophylls; diet EYL2, 348 g/kg of esterified xanthophylls; diet SYL, 259.59 g/kg of saponified xanthophylls and diet RD, 200 g/kg astaxanthin. The concentration of astaxanthin used in this study falls within the range studied by Yamada et al. (7) with *P. japonicus*. The pigmentation extracts were supplied by Laboratorios Bioquímex S.A. C.V., and their carotenoid composition was determined following the method developed by Gregory et al (8) and reported in Table 1.

TABLE 1  
Carotenoid composition and content (g/kg) of the marigold petal oleoresin extracts, from which the microcapsules or colloid protected mesquite gum pigments were formulated.

| Pigment                  | Esterified marigold extract (g/kg) | Saponified marigold extract (g/kg) |
|--------------------------|------------------------------------|------------------------------------|
| $\beta$ -carotene        | 0.70                               | 0.41                               |
| cryptoxanthin            | 0.59                               | 0.40                               |
| cis-lutein               | 1.38                               | 0.72                               |
| trans-lutein             | 55.31                              | 19.12                              |
| trans-zeaxanthin         | 1.85                               | 1.24                               |
| epoxy-lutein             | 17.17                              | 2.91                               |
| Total carotenoid content | 77.0                               | 24.8                               |

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These extracts were then mixed with soybean oil and/or mesquite (*Prosopis juliflora*) gum in order to obtain stable, non-degrading microcapsules or colloid protect pigment formulations.

The formulated pigments were then spray-dried (mini-Buchii 190 spray drier). The dried ingredients indicated in Table 2 for each diet were mixed in a Hobart mixer for 10 min, after which the wet ingredients which include water and soybean lecithin were added with a further 10 min mixing.

The mixtures were extruded through a 2 mm die in a meat grinder. The extrudates were cut into 0.2 to 0.6 cm long pellets, slightly dried at 60 °C for 12 h, sealed in plastic bags and stored at  $-10.0 \pm 1.0$  °C until used.

The total carotenoid concentration (TC) and composition of the diets was determined after manufacture and during the two fortnights of the experiment. The data reported in Table 2 correspond to the diets fed to the shrimps during the 28 day trial.

TABLE 2  
Composition (% w/w) of the experimental diets for shrimp pigmentation

| Ingredient                     | DIET  |        |        |        |       |
|--------------------------------|-------|--------|--------|--------|-------|
|                                | BD    | EYL1   | EYL2   | SYL    | RD    |
| Fish meal                      | 27.00 | 27.00  | 27.00  | 27.00  | 27.00 |
| Soybean meal dehulled          | 20.00 | 19.25  | 19.06  | 18.35  | 19.75 |
| Yeast ( <i>S. cerevise</i> )   | 8.00  | 8.00   | 8.00   | 8.00   | 8.00  |
| Corn gluten                    | 12.00 | 12.00  | 12.00  | 12.00  | 12.00 |
| Wheat starch                   | 23.00 | 23.00  | 23.00  | 23.00  | 23.00 |
| Fish oil                       | 1.75  | 1.75   | 1.75   | 1.75   | 1.75  |
| Soybean lecithin               | 1.73  | 1.73   | 1.73   | 1.73   | 1.73  |
| Monosodium phosphate           | 0.50  | 0.50   | 0.50   | 0.50   | 0.50  |
| Sodium bicarbonate             | 2.50  | 2.50   | 2.50   | 2.50   | 2.50  |
| Bassfind (binder)              | 0.50  | 0.50   | 0.50   | 0.50   | 0.50  |
| Vitamin mixture <sup>a</sup>   | 3.00  | 3.00   | 3.00   | 3.00   | 3.00  |
| Pigmenting source <sup>b</sup> | 0.00  | 0.75   | 0.94   | 1.65   | 0.25  |
| Ethoxiquin (antioxidant)       | 0.02  | 0.02   | 0.02   | 0.02   | 0.02  |
| Total carotenoid (g/kg)        | <1    | 278.45 | 348.03 | 259.59 | 200.0 |
| <b>Nutrient</b>                |       |        |        |        |       |
| Crude protein (%)              | 40.84 | 39.71  | 39.47  | 40.70  | 42.65 |
| Crude fat (%)                  | 6.83  | 6.19   | 6.79   | 5.52   | 7.02  |
| Ash (%)                        | 8.25  | 8.13   | 7.35   | 8.24   | 8.50  |
| Gross energy (kJ/kg)           | 16.30 | 16.41  | 16.30  | 16.20  | 16.40 |

<sup>a</sup>Provide in mg per 100 g mixture: thiamine-HCl 150, pyridoxine-HCl 150, riboflavin 500, nicotinic acid 2000, Ca-pantothenate 750, inositol 10000, biotin 15, folic acid 37.5, amino benzoic acid 25000,  $\alpha$ -tocopherol 1000, vitamin D<sub>3</sub> 0.15, cyanocobalamin 1, vitamin A 500000 I.U./g. <sup>b</sup>Provide in accordance to pigment source concentration and composition for achieving the desired concentration in diet. Where BD=Basal diet; EYL1= Low concentration esterified marigold extract; EYL2= High concentration esterified marigold extract; SYL=Saponified marigold extract diet; RD=Reference diet.

**Feeding trials:** The shrimp used in this assay were of the species *Penaeus vannamei*, commonly known as the Pacific white shrimp. Three hundred organisms with a length of 6-8 and weighing 5.6-6.1

g each were allotted randomly to 15 flow-through aquaria (40 cm wide, 50 cm long and 30 cm deep), and fed in triplicate groups. Filtered sea water with a salinity of 33 ppt and a mean average temperature of  $28.0 \pm 1.5$  °C was circulated through the aquaria at a rate of 1 l/min. The aquaria were cleaned after the first week.

The shrimp were fed twice daily at a rate of 8% of biomass during 28 days. The amount of feed consumed, the number of moults and the dead shrimp were determined on a daily basis, but are reported as the value observed over the 28 day trial. At the beginning of the experimentation the shrimp in each aquaria were individually weighed. At day 14 and 28 eight shrimp were sampled each time at random from each aquarium, and sacrificed. The viscera (proventriculus, hepatopancreas and intestine) were removed from the shrimp, whilst the exoskeleton and abdominal muscle were immediately stored in liquid nitrogen, and subsequently analysed for total carotenoid content.

The exoskeleton and abdominal muscle of the eight sacrificed shrimp were pooled as one sample, respectively, and the extraction and determination of the carotenoid content was done using the technique reported in the A.O.A.C. (9).

Finally, in order to ascertain if the inclusion of the pigments in the basal diet (BD) had an effect on the shrimp, the specific growth rate (SGR), the food conversion rate (FCR) and the food intake (FI), were determined for the overall experimental period, using the procedures outlined by Olvera et al (10).

The data were subjected to analysis of variance (ANOVA) using Duncan's multiple range test to determine whether differences among means were statistically significant at the 5% level (11,12).

## RESULTS AND DISCUSSION

The total carotenoid concentration analysis in the muscle and the exoskeleton (Table 3) shows: Firstly, that at any time period the content of pigments was approximately 10 times higher in the exoskeleton than in the abdominal muscle. Secondly, that the total carotenoid content unexpectedly showed only slightly higher values in the exoskeleton with the reference diet (RD), the low concentration esterified marigold extract diet (EYL1) and the basal diet (BD), and in the abdominal muscle with diet EYL1 at day 28 with regard to day 14. The rest of the diets showed a remarkable decrease in pigmentation in the exoskeleton and abdominal muscle between days 14 and 28. Yamada et al (7) have reported similar results in the pigmentation of *P. japonicus* fed with 200 and 400 g/kg of astaxanthin esters during 8 weeks, possibly due to tissue saturation at these concentrations, followed by a depletion of the pigments from the tissues at both concentrations. Also, as the diets were analysed for TC concentration during the experimental trial, and no diminution in pigment concentration was found, the explanation by Yamada et al (7) seems plausible. These results, point out the feeding trials should not exceed 14 days as no perceptible gain in pigmentation is obtained by extending the feeding trial to 28 days. The effect of tissue saturation has been noted in other species as well. Choubert and Storebakken (13) reported that rainbow trout fed various dietary carotenoid concentrations showed a decreasing rate of accumulation of pigments, which became more pronounced, with longer feeding time and increasing carotenoid concentrations.

**TABLE 3**  
Carotenoid concentration (g/kg) in the shrimp fed the experimental diets

| DIET                    | BD                | EYL1              | EYL2               | SYL                 | RD                 |
|-------------------------|-------------------|-------------------|--------------------|---------------------|--------------------|
| Dietary carotenoids     | <1                | 278.4             | 348.0              | 259.5               | 200.0              |
| <b>ABDOMINAL MUSCLE</b> |                   |                   |                    |                     |                    |
| 0 day                   | 3.0               | 3.0               | 3.0                | 3.0                 | 3.0                |
| 14 days                 | 9.0 <sup>bc</sup> | 7.9 <sup>c</sup>  | 9.1 <sup>b,c</sup> | 11.0 <sup>a,b</sup> | 9.1 <sup>b,c</sup> |
| 28 days                 | 8.0 <sup>a</sup>  | 8.5 <sup>a</sup>  | 8.5 <sup>a</sup>   | 8.5 <sup>a</sup>    | 8.5 <sup>a</sup>   |
| <b>EXOSKELETON</b>      |                   |                   |                    |                     |                    |
| 0 day                   | 40.0              | 40.0              | 40.0               | 40.0                | 40.0               |
| 14 days                 | 70.7 <sup>b</sup> | 75.0 <sup>b</sup> | 113.0 <sup>a</sup> | 120.0 <sup>a</sup>  | 80.0 <sup>b</sup>  |
| 28 days                 | 70.8 <sup>b</sup> | 76.0 <sup>b</sup> | 92.0 <sup>a</sup>  | 87.0 <sup>a</sup>   | 89.0 <sup>a</sup>  |

a,b,c,d Figures in a common row with varying superscripts are significantly different (p<0.05); BD= Basal diet; EYL1= Low concentration esterified marigold extract; EYL2= High concentration esterified marigold extract; SYL= Saponified marigold extract diet; RD= Reference diet.

**Abdomen pigmentation.** Table 3 shows the deposit of pigments in the abdominal muscle of the shrimp for the different treatments at days 14 and 28. At day 14, the data show that the higher pigment assimilation value is from the saponified marigold extract (SYL) diet, followed by the high concentration (328.03 g/kg) esterified marigold extract (EYL2) diet, the 200 g/kg astaxanthin (RD) diet and the basal diet (BD). Although, between these four diets there exists a non-significant different pigmentation effect, there exists a significant different pigmentation effect between the SYL diet and the low concentration (278.45 g/kg) marigold extract (EYL 1).

These results suggest that the saponified marigold extracts are more readily assimilated by the shrimp than the esterified marigold extracts.

As mentioned earlier, at day 28 the pigment concentration in the abdominal muscle dropped from the values exhibited at day 14. Furthermore, there exist no significant difference (p<0.05) in the level of pigmentation among all the treatments. These results indicate that the inclusion of esterified or saponified xanthophylls or of synthetic astaxanthin in the basal diet does not improve the pigmentation of the abdominal muscle of this particular species of shrimp. It also confirms the importance of determining the length of time and the pigment concentrations used in feeding trials.

**Exoskeleton pigmentation.** As shown in Table 3, the best treatments at day 14 are the saponified marigold extract (SYL) diet and the high concentration esterified marigold extract (EYL2) which are not significantly different (p<0.05) between them but are significantly different from diets EYL 1, RD and BD.

As the feeding trial continues to day 28, the total carotenoid concentration in the exoskeleton diminishes for diets SYL and EYL2, while it increases for diets RD, EYL 1 and BD. Now the RD diet does not show a significant difference with regard to diets SYL and EYL2. Although, caution must be exercised with these results as the total carotenoid content of the SYL diet is around 30 % higher than the RD diet.

However, it is confirmed that the saponified marigold extracts are better utilized by the shrimp than the esterified marigold pigments,

and that the pigment concentrations and feeding trials periods are of the utmost importance for obtaining a satisfactory pigmentation level.

Finally, the addition of saponified and esterified marigold extracts to the basal diet of the Pacific white shrimp promotes its exoskeleton pigmentation.

**Shrimp growth indicators.** The growth results are shown in Table 4. All the parameters evaluated were significantly influenced by the experimental diets. Notwithstanding, the data is very difficult to interpret as it tends to indicate contradictory results. For example, we have mentioned that diets SYL and EYL2 gave the best pigmentation effects in exoskeleton of shrimp after the 28 day trial, both being significantly different from the BD diet. However, in Table 4 it can be observed that EYL 2 diet had a survival rate and a weight gain higher than that of the BD and the SYL diets. Meanwhile the SYL diet showed a lower survival rate and a lower food intake than the BD diet, having a significantly lower FCR than both the EYL2 and BD diets.

Surprisingly the RD diet showed very poor figures of weight gain, food intake and food conversion rate, although it reported a high survival rate.

Whatever the results of the growth indicators, none of the parameters has a significant influence on the degree of pigmentation of the shrimp.

**TABLE 4**  
Influence of the 28 days feeding trials on different shrimp growth indicators

| Diet                          | BD                | EYL1                | EYL2                | SYL                | RD                |
|-------------------------------|-------------------|---------------------|---------------------|--------------------|-------------------|
| Initial body weight (g)       | 5.9               | 5.8                 | 6.0                 | 5.7                | 5.8               |
| Final body weight (g)         | 7.8               | 8.0                 | 8.0                 | 8.3                | 7.4               |
| Survival (%)                  | 90.0 <sup>a</sup> | 73.3 <sup>d</sup>   | 100.0 <sup>a</sup>  | 83.3 <sup>c</sup>  | 90.0 <sup>b</sup> |
| Weight gain (mg/day)          | 67.9 <sup>d</sup> | 78.5 <sup>b,c</sup> | 71.8 <sup>c,d</sup> | 92.92 <sup>a</sup> | 57.1 <sup>e</sup> |
| Specific growth rate (% /day) | 4.3 <sup>c</sup>  | 4.9 <sup>b</sup>    | 4.5 <sup>c</sup>    | 5.8 <sup>a</sup>   | 3.8 <sup>d</sup>  |
| Food intake (% /day)          | 0.18 <sup>b</sup> | 0.13 <sup>d</sup>   | 0.19 <sup>a</sup>   | 0.15 <sup>c</sup>  | 0.15 <sup>c</sup> |
| Food conversion rate          | 2.6 <sup>a</sup>  | 1.6 <sup>b</sup>    | 2.6 <sup>a</sup>    | 1.5 <sup>b</sup>   | 2.6 <sup>a</sup>  |

a,b,c,d Figures in a common row with varying superscripts are significantly different (p<0.05). BD=Basal diet; EYL1= Low concentration esterified marigold extract; EYL2= High concentration esterified marigold extract; SYL=Saponified marigold extract diet; RD=Reference diet.

### CONCLUSIONS

The diets containing saponified marigold extracts and a high concentration of esterified marigold extracts have a good pigmenting effect on Pacific white shrimp exoskeleton, being superior to that of synthetic astaxanthin after 28 days.

However, it may be considered that the pigmenting effect of the saponified marigold extracts is more effective than that of the esterified pigments, as these were added to the diets in higher concentrations. Both, the pigment concentration and the feeding trial duration play an important role in the final level of pigmentation

found in shrimp tissues.

The results indicate that this particular species of shrimp (*P. vannamei*) seems not to have the capacity of enhancing its abdominal muscle pigmentation by the addition of pigmentation sources to the diet. On the other hand, this species can increase its exoskeleton pigmentation by incorporating pigments in its diet, which undoubtedly constitutes an interesting fact.

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#### REFERENCES

- Latcha T. 1990. Carotenoid pigments in crustaceans. La Roche Ltd; Dept. of Animal Nutrition and Health. Basel, Switzerland. 1990.
- Tanaka Y., H. Matsuguchi, T. Katayama, K.L. Simon & C.O. Chichester. The biosynthesis of astaxanthin XVI. The carotenoids in crustacea. *Comp. Biochem. Physiol.* 54B: 391-393. 1976.
- Food and Drug Administration (FDA). Guidelines for evaluation of effectiveness of new animal drugs for use in poultry feeds for pigmentation. Bureau of Veterinary Medicine. 1982.
- Kamata T., G. Neamtu, Y. Tanaka, M. Sameshina & K.L. Simpson. Utilization of *Adonis aestivalis* as a dietary pigment source for rainbow trout *Salmo gairdneri*. *Bull. Jap. Soc. Sci. Fish* 56(5): 783-788. 1990.
- Foster J.R. & T.W. Beard. Growth experiments with the prawn *Palaemon serratus* fed with fresh and compounded foods. *Fish Invest. Ser.* 1127(7): 145-149. 1973.
- Kanazawa A., S. Teshima & S. Tokiwa. Nutritional requirements of prawn. VII Effect of dietary lipids on growth. *Bull. Japan Soc. Sci. Fish.* 43(7): 849-856. 1977.
- Yamada S., Y. Tamaka, M. Sameshima & Y. Ito. Effect of dietary astaxanthin,  $\beta$ -carotene and canthaxanthin on pigmentation of the prawn. *Aquaculture* 87: 323-330. 1990.
- Gregory G.K., T. Chen & T. Philip. Quantitative analysis of lutein esters in marigold flowers (*Tagetes erecta*) by high performance liquid chromatography. *J. Food Sci.* 51(4): 1093. 1986.
- Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 15th edition. Washington, D.C. pp 1108. 1990.
- Olvera M.A.N., C.A.P. Martínez, C. Reyna Galván & C.S. Chávez. The use of the seed of the leguminous plant *Sesbania glandiflora* as a partial replacement for fish meal in diets for tilapia (*Oreochromis mossambicus*). *Aquaculture* 71:51-60. 1988.
- Duncan D.B. Multiple range and multiple F tests. *Biometrics* 11: 1-42. 1955.
- Amerine M.A., R.M. Pangborn & E.B. Roessler. Principles of Sensory Evaluation of Food. Food Science and Technology Monographs. Academic Press, New York. 1965.
- Choubert G. & T. Storebakken. Dose response to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoid concentrations. *Aquaculture* 81(1): 69-77. 1989.

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