

Research Notes

Dietary Marine Algae Maintains Egg Consumer Acceptability While Enhancing Yolk Color

S. M. HERBER-MCNEILL and M. E. VAN ELSWYK¹

Department of Poultry Science, Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843-2472

ABSTRACT A drum-dried docosahexaenoic acid (DHA; C_{22:6n-3}) enriched marine microalgal product (MA) was investigated as a n-3 fatty acid (n-3 FA) source in laying hen diets. Hen diets were supplemented with 2.4 or 4.8% MA. Eggs were analyzed for yolk color following 4 wk of feeding as well as weekly for 4 wk. Egg flavor was evaluated by consumer panelists.

Feeding MA significantly ($P < 0.01$) increased yolk a* values in a dose response manner as early as 1 wk post-MA feeding. Consumer panelists found n-3 FA enriched eggs as acceptable as typical eggs. These data suggest that dietary MA is useful for enhancing yolk n-3 FA and color while maintaining consumer acceptability of the resulting egg product.

(Key words: egg yolk, n-3 fatty acids, algae, yolk color, egg flavor)

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INTRODUCTION

Despite recommendations to consume fish as a source of n-3 fatty acids (n-3 FA) for the reduction of coronary heart disease risk, fish consumption in the U.S. averages less than one serving per week (USDA, 1994, 1995). To promote n-3 FA consumption, production of a n-3 FA enriched food source that is affordable and more common in the U.S. diet has been recommended (Nettleton, 1991; O'Brien, 1995). Modification of laying hen diets to include a source of n-3 FA readily results in the production of a shell egg enriched in n-3 FA (Hargis and Van Elswyk, 1993). A recent advance in the heterotrophic production of docosahexaenoic acid (DHA; C_{22:6n-3}) enriched marine microalgae (MA) may provide an attractive option for n-3 FA supplementation of poultry diets (Barclay *et al.*, 1994). Marine microalgae are the original source of n-3 FA in the diets of fish, and therefore represent a more direct dietary source of these healthful fatty acids. In fact, feeding a minimum of 2.4% MA to laying hens results in the production of an egg with as much n-3 FA as a serving of lean fish (Herber and Van Elswyk, 1996). However, the sensory quality of n-3 FA enriched eggs produced by DHA enriched MA fed hens remains to be reported.

Previous work in the area of n-3 FA enriched egg flavor quality has focused on products resulting from a mixture of dietary n-3 FA, specifically eicosapentaenoic acid (EPA; C_{20:5n-3}) and DHA from sources such as

menhaden oil (MO; Adams *et al.*, 1989; Van Elswyk *et al.*, 1992; Van Elswyk *et al.*, 1995). As MA utilized in the current study is mainly enriched in DHA, supplementation of this unique MA allows an opportunity to evaluate the impact of supplementing an individual n-3 FA on egg sensory quality.

In addition to serving as an enriched source of the n-3 FA, DHA, these MA are also a source of naturally occurring carotenoids. Carotenoids have been promoted for their antioxidant potential (Bendich, 1989) as well as their usefulness for pigmenting food products. Of the carotenoids present in the MA, β -carotene and canthaxanthin have been quantitated and are reported to be present in concentrations of 13 to 25 mg/kg and 0.6 mg/kg of MA, respectively (W. R. Barclay, Omega Tech, Inc., 5766 Central Avenue, Boulder, CO 80301, personal communication). Although it is not in the scope of the current study, a source of dietary n-3 FA containing naturally occurring antioxidants may offer advantages over n-3 FA sources such as menhaden oil whose lipids are stabilized using the synthetic antioxidant ethoxyquin.

Less than 1% of dietary β -carotene is deposited in yolk and thus it is relatively ineffective for use in yolk pigmentation (NRC, 1994). Additional carotenoids present in the MA are likely contributing to its characteristic red-orange color and may be more effective in pigmenting egg yolk. For example, Haq and Bailey (1996) reported 19 times greater yolk carotenoid

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¹To whom correspondence should be addressed: mvanelsw@poultry.tamu.edu

Abbreviation Key: EPA = eicosapentaenoic acid; FA = fatty acid; DHA = docosahexaenoic acid; MA = microalgae; SCWL = Single Comb White Leghorn.

deposition from supplemental canthaxanthin and lutein, than from the same level of supplemental β -carotene. Many carotenoids have been recognized for their usefulness in enhancing the color of poultry products (Hencken, 1992; Sunde, 1992). Consumers in South America, Mexico, and many of the Mediterranean peoples prefer egg yolk and poultry skin with deep orange-red hues (Sunde, 1992). Naturally enhancing yolk pigmentation of n-3 FA enriched table eggs through the use of dietary MA could also serve as a creative marketing niche, helping consumers to identify enriched eggs as different from typical eggs. Therefore, the specific objectives of the current study were 1) to evaluate egg consumer acceptability of n-3 FA enriched eggs produced from graded levels of dietary MA and 2) to determine the influence of dietary MA on enriched yolk color.

MATERIALS AND METHODS

Feeding Regimens

In Experiment 1, 72 56-wk-old Single Comb White Leghorn (SCWL) hens were divided among three diets ($n = 24$ per treatment; 8 per replication). Diets were described previously (Herber and Van Elswyk, 1996) and included a typical corn-soybean control with no added n-3 FA, 2.4% MA,² or 4.8% (4.8% MA). Diets containing 2.4% MA provided 200 mg DHA and 69 μg β -carotene/d. The 4.8% MA diet supplied 400 mg DHA and 138 μg β -carotene/d (Herber and Van Elswyk, 1996). The concentrations of the remaining carotenoids provided by dietary MA are unknown. Macroingredients met the National Research Council (1994) requirements and micronutrients exceeded laying hen requirements as a turkey breeder vitamin mineral premix³ was utilized (Herber and Van Elswyk, 1996).

In Experiment 2, the same diets were fed to 48 24-wk-old SCWL hens ($n = 16$ per treatment; 8 per replication). In both experiments, diets were fed for a minimum of 4 wk prior to egg flavor analysis to allow yolk n-3 FA incorporation to reach a plateau (Hargis *et al.*, 1991). The MA were aliquoted and vacuum packaged upon receipt and no supplemental antioxidants were added. Marine algae were stored at 4 C until use in feed preparation. Hens consumed feed *ad libitum* daily. Diets were mixed biweekly in each experiment and stored at room temperature (25 C). Residual feed was vacuumed from troughs weekly to remove potentially oxidized feed.

Flavor Analysis

For each experiment, untrained consumer panels ($n = 2$ panels per experiment; 30 to 35 panelists per panel) were

completed on consecutive days. Panelists were random volunteers from the student and faculty population at Texas A&M University. Egg samples were prepared from scrambled whole eggs ($n = 3$ eggs per pool). Scrambled eggs were chosen as this egg product has been found by untrained and trained panelists to be the easiest to detect off flavors associated with n-3 FA enriched eggs (Van Elswyk *et al.*, 1992). Blended egg samples were cooked, without mixing to an internal temperature of 78 C in a nonstick skillet. No seasonings or oil were added to the eggs during preparation. Egg samples were cut into $2 \times 2 \times 1$ cm³ pieces for presentation. Throughout the panel, samples were stored in covered glass Pyrex dishes in a heated waterbath maintained at 68 C. Fresh samples were prepared every 15 min. Samples were presented to consumer panelists in random order. Samples were served under red lighting to prevent any bias associated with yolk color. To cleanse their palate between samples, panelists were provided crackers with unsalted tops and purified water at room temperature (25 C). Panelists were instructed to evaluate egg flavor using a nine point anchored hedonic scale (Meilgaard *et al.*, 1989). "Dislike extremely" was represented by a score of 1, 5 represented "neither like nor dislike", and 9 represented "like extremely".

Color Analysis

In Experiment 1, eggs were collected for color analysis at Week 4. In Experiment 2, eggs were collected weekly for the first 4 wk of feeding to monitor the increment of change in egg yolk color. Changes in egg yolk color were measured as an indirect indicator of yolk carotenoid deposition. For analysis of yolk color, egg yolks ($n = 3$ per pool) were separated and blended into pools ($n = 2$ per treatment per week). Each yolk pool was poured into a clean 60 \times 15 mm glass Petri dish. A glass lid was placed flush against the yolk surface to prevent air pockets. A Minolta CR-200 Chroma Meter⁴ was calibrated using a standard yellow calibration tile, model CRA471 for the CR-200 Chroma Meter. The tip of the Chroma Meter measuring head was placed flat against the surface of the Petri dish and yolk reflective color was determined from the average of three consecutive pulses from the optical chamber of the Chroma Meter. Data are reported in the L*a*b* color notation system with L* axis representing lightness, the a* axis representing the red-green color axis (redness) and the b* axis representing the blue-yellow (yellowness) color axis (Minolta, 1994).

Statistical Analysis

In each experiment, L*a*b* values and flavor scores were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS[®] Institute (1994) to compare means of replication, experiment, week, and treatment. The interactions of replication by treatment, experiment by treatment (flavor acceptability), and treat-

²Schizochytrium, OmegaTech Inc., Boulder, CO 80301.

³Hoffman-LaRoche, Inc., Nutley, NJ 07110.

⁴Minolta, Ramsey, NJ 07446.

ment by week (yolk color) were tested. Significantly different means were further separated using Duncan's multiple range test. Upon evaluation of flavor acceptability data, there was no statistical interaction between experiment and treatment, therefore treatment means from both experiments were combined.

RESULTS AND DISCUSSION

A minimum score of 5, representing "neither like nor dislike", was considered to represent an acceptable egg product, below 5 was considered unacceptable as this marked a shift from indifference to dislike. Eggs from hens fed both levels of MA received acceptable flavor scores (2.4% MA = 5.6 ± 0.3 ; 4.8% MA = 5.2 ± 0.3) that were not significantly different than CON (5.7 ± 0.2). The acceptable flavor scores of eggs from hens fed 2.4% MA are not surprising as they contain a similar total n-3 FA content (160 to 170 mg per egg) as eggs from hens fed 1.5% MO (155 to 165 mg per egg) (Herber and Van Elswyk, 1996) that have previously been identified as acceptable by both trained and untrained panelists (Marshall *et al.*, 1994; Van Elswyk *et al.*, 1995). Interestingly, however, were the acceptable flavor scores of eggs from hens fed 4.8% MA that contained similar total yolk n-3 FA (205 to 215 mg per egg) (Herber and Van Elswyk, 1996) as that resulting from supplementation of 3.0% MO in previous studies (Adams *et al.*, 1989; Hargis and Van Elswyk, 1991; Van Elswyk *et al.*, 1992). Eggs containing 200 mg or more of total n-3 FA per yolk from dietary menhaden oil supplementation have been found to be of poor sensory quality (Holdas and May, 1966; Van Elswyk *et al.*, 1992; Van Elswyk *et al.*, 1995). This difference in results is especially interesting when one considers that although MO provides both EPA and DHA, only DHA is significantly deposited in eggs from hens fed MO (Herber and Van Elswyk, 1996). Therefore, the FA profiles of eggs from hens fed MO is quite similar to that of eggs from hens fed MA (Herber and Van Elswyk, 1996). However, one of the major differences between MO and MA that may contribute to flavor acceptance is the oxidative stability advantage that MA may provide through the carotenoids it contains and because the lipids of MA are naturally encapsulated within the plant cell wall.

In Experiment 1, yolk color was analyzed in Week 4 only. Eggs from hens fed MA had significantly increased yolk a^* values as compared to controls (Table 1). The negative a^* values of egg yolks from control hens indicate chromaticity in the direction of green, whereas the positive values of yolks from hens fed MA indicate a shift toward red color. Enhancement of yolk a^* values most likely reflects the deposition of the MA carotenoids. In contrast to yolk n-3 FA incorporation, where doubling the amount of MA from 2.4% MA to 4.8% MA did not double n-3 FA deposited (Herber and Van Elswyk, 1996), yolk a^* values did increase by a factor slightly greater than two (Tables 1 and 2) suggesting a

TABLE 1. Influence of dietary marine algae on yolk color, Experiment 1

Color	Treatment ¹		
	Control	2.4% MA	4.8% MA
L* values ²	53.0 ± 0.9 ^a	48.5 ± 0.1 ^{bc}	47.8 ± 0.4 ^c
a* values	-0.10 ± 0.1 ^c	0.99 ± 0.9 ^b	2.67 ± 0.4 ^a
b* values	46.3 ± 2.9	47.2 ± 0.4	46.3 ± 0.8

^{a-c}Means ± SEM within rows with no common superscript differ significantly ($P < 0.05$).

¹2.4% MA = 2.4% marine algae; 4.8% MA = 4.8% marine algae.

²Measured in L*a*b* color notation system.

more efficient uptake of dietary pigments compared to dietary FA. Previous work by Haq and co-workers (1996) confirms that carotenoids such as canthaxanthin are efficiently deposited in egg yolk when added to poultry feeding regimens. Decreased L* values in the MA treatments are not surprising given the significant deepening of yolk red/orange hues (Table 1). Diet did not influence yolk b* values (Table 1).

In Experiment 2, yolk color was monitored weekly to determine changes over time. L* and b* values were not affected in this experiment (data not shown). However, after only 1 wk of feeding, yolk a^* values in all MA treatments were significantly enhanced as compared to controls (Table 2). The effects of MA on egg yolk color had reached a plateau after 14 d, which was sustained throughout the experiment. This observation is consistent with previous work showing dietary pigments are maximally incorporated into yolk within 2 wk of diet initiation (Haq and Bailey, 1996). Interestingly, dietary n-3 FA incorporation has also been reported to plateau following 2 wk on MA supplemented diets (Herber and Van Elswyk, 1996). The potential for enhanced yolk lipid stabilization by the deposition of antioxidant pigments remains to be elucidated. Yolk deposition of these pigments may also translate into additional health benefits to consumers as the role of antioxidants in the prevention of cardiovascular disease and cancer are supported.

TABLE 2. Influence of dietary marine algae (MA) on yolk a^* values (redness), Experiment 2

Week	Treatment ¹		
	Control	2.4% MA	4.8% MA
1	-0.4 ± 0 ^{b,x}	1.4 ± 0.1 ^{a,x}	1.9 ± 0.6 ^{a,y}
2	-1.5 ± 0.1 ^{c,x}	1.5 ± 0.3 ^{b,x}	3.5 ± 0.2 ^{a,x}
3	-0.5 ± 0.3 ^{c,x}	1.4 ± 0.2 ^{b,x}	4.0 ± 0.1 ^{a,x}
4	-0.5 ± 0.6 ^{c,x}	1.6 ± 0.3 ^{b,x}	3.8 ± 0.7 ^{a,x}

^{a-c}Means ± SEM with no common superscript differ significantly ($P < 0.05$).

^{x,y}Means ± SEM within columns with no common superscript differ significantly ($P < 0.05$).

¹4.8% marine algae.

Conclusion

Dietary MA as a source of DHA is useful for enhancing the n-3 FA content, and thus the nutritional quality of shell eggs, without significantly impacting consumer acceptance. Dietary MA may also be useful in certain geographical regions for enhancing yolk color. Additional health benefits to the consumer from yolk carotenoid consumption remain to be investigated.

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