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Supplemental microalgal astaxanthin produced coordinated changes in intrinsic antioxidant systems of layer hens exposed to heat stress

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ABSTRACT

Astaxanthin is a well-known antioxidant phytochemical that has limited bioavailability to humans. The present study was conducted to determine if microalgal astaxanthin was bioavailable to layer hens and affected antioxidant status of their tissues and eggs. A total of 50 White Leghorn Shavers (21-wk old) were divided into 5 groups (n = 10/group), caged individually in an environmentally-controlled room, and fed a corn-soybean meal basal diet supplemented with microalgal (*Haematococcus pluvialis*) astaxanthin (Heliae, Gibert, AZ) at 0, 10, 20, 40, and 80 mg/kg for 6 wk. Heat stress was induced during weeks 4–6 when the ambient temperature was raised 3 °C above the optimal temperature. Supplemental microalgal astaxanthin resulted in dose-dependent enrichments ($P < 0.05$) of astaxanthin and total carotenoids in the plasma and egg yolk of hens. The maximal concentrations of astaxanthin reached 4.1 μg/mL, 5.8 mg/kg, and 36 mg/kg, whereas those of total carotenoids reached 7.0 μg/mL, 75 mg/kg, and 114 mg/kg (on fresh tissue basis), respectively, in the plasma, liver, and egg yolk of hens. The oxygen radical absorbance capacity was also enhanced ($P < 0.05$) in a dose-dependent fashion in the liver and egg yolk of hens. Meanwhile, total glutathione concentration and activities of glutathione peroxidase and glutathione-S transferase in the liver of hens were decreased ($P < 0.05$) by the supplemental astaxanthin, compared with the control. Egg yolk color was changed ($P < 0.05$, more red) by the supplementation, and total PUFA concentration increased ($P < 0.05$) in the highest astaxanthin treatment group. In conclusion, supplemental dietary microalgal astaxanthin seemed to be highly bioavailable to be deposited in the plasma, liver, and eggs of hens, independent of heat stress, and resulted in coordinated changes in the intrinsic antioxidant systems.

1. Introduction

Over 8 billion chickens are slaughtered for meat and 99 billion eggs are produced within the United States alone every year (USDA) [1]. Microalgal biomass has been shown to serve as an excellent substitute for conventional ingredients corn and soybean in diets for both broiler chicks and laying hens, and is capable of enriching their meat and eggs with omega-3 fatty acids [2–4]. Certain strains of microalgae contain high concentrations of phytochemicals including phenols, flavonoids, and other bioactive compounds that benefit both chicken and human health [5]. One carotenoid, in particular, astaxanthin, is abundant in many microalgal species and has strong anti-oxidant potency [6]. It is an excellent electron donor and has the ability to protect cells from free radicals and subsequent oxidative stress under both in vitro and in vivo conditions [7,8]. Supplementation of astaxanthin at 7 to 100 mg/kg of diets can be readily absorbed by chickens, including laying hens, and subsequently incorporated into their tissues [9–13]. Another benefit of astaxanthin is to improve meat quality, by increasing shelf life through decreasing oxidative radicals and lipid peroxidation [14].

However, it was unclear if astaxanthin could be digested by hens and improve their performance under heat stress conditions. The majority of poultry production in the U.S. occurs within southern States where temperature is high in the summer, resulting in heat stress in laying hens [15]. Oxidative stress is the key factor as to how heat stress impacts the health and performance of the birds [16,17]. Dietary supplementation of anti-oxidants ameliorated the negative impacts of heat

Abbreviations: AKP, Alkaline phosphatase; ALT, Alanine transaminase; ANOVA, Analysis of variance; GSH, Glutathione; GPX, Glutathione peroxidase; GR, Glutathione reductase; GST, Glutathione-S transferase; HPLC, High performance liquid chromatography; MDA, Malondialdehyde; MUFA, Monounsaturated fatty acid; NEFA, Non-esterified fatty acid; GSSG, Oxidized glutathione; ORAC, Oxygen radical absorbance capacity; PIP, Plasma inorganic phosphorus; PUFA, Polyunsaturated fatty acid; SFA, Saturated fatty acid; SOD, Super oxide dismutase; TRAP, Tartrate resistant acid phosphatase; TC, Total cholesterol; TG, Triglyceride

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stress [18–20]. Likewise, dietary supplementation of microalgae rich in phytochemicals improved the antioxidant status of animals. However, it remained to be determined if this elevated antioxidant potential by supplemental microalgae could be translated into a protection against oxidation and lipid peroxidation induced by heat stress in hens [21,22].

Therefore, this study was conducted to feed laying hens under heat stress with supplemental microalgal astaxanthin at 10, 20, 40, and 80 mg/kg of a corn-soybean meal diet for 6 weeks. Our objectives were to determine: 1) if the supplemental astaxanthin was bioavailable to laying hens to be deposited into their eggs and tissues and how this metabolism was affected by heat stress; 2) if the enriched astaxanthin improved the antioxidant status of the eggs and tissues; 3) if the supplemental astaxanthin affected the egg production performance, egg quality, and health status of the hens.

2. Materials and methods

2.1. Animals, diets, and management

All protocols of this experiment were approved by the Cornell University Institutional Animal Care and Use Committee. Supplemental microalgae *Haematococcus pluvialis* were provided by Heliae Development, LLC (Gilbert, Arizona, USA). Proximate analyses and astaxanthin concentration of these ingredients are presented in Table 1. A total of 50 shaver leghorn laying hens (19-week old, donated by Kreher Farmers, Clarence, NY, USA) were used. They were housed in individual cages in an environmentally controlled room maintained at 25 °C, 55% relative humidity with 8:16 dark:light cycles and with free access to water and fed for 6 weeks. Starting at week 4 temperatures were gradually elevated over several days to a set point of 28 °C to induce heat stress which was maintained until the end of the experiment.

Table 1
Composition (g/kg) of experimental diets for laying hens.

Treatment	Control	1	2	3	4
Calculated astaxanthin (mg/kg)	0	10	20	40	80
Corn, grain	653	651	653	643	651
Soybean meal 48%	215	211	214	201	213
Defatted microalgae	0.00	5.00	0.00	20.0	0.00
Full fatted microalgae	0.00	0.00	0.80	0.00	3.20
Corn oil	20.0	20.0	20.0	20.0	20.0
Dicalcium phosphate	9.50	9.50	9.50	9.50	9.50
Limestone	78.9	78.9	78.9	78.9	78.9
Choline	2.60	2.60	2.60	2.60	2.60
DL-Methionine	1.60	1.60	1.60	1.60	1.60
Isoleucine	1.40	1.40	1.40	1.40	1.40
Tyrosine	4.20	4.20	4.20	4.20	4.20
Valine	1.20	1.20	1.20	1.20	1.20
Tryptophan	0.20	0.20	0.20	0.20	0.20
Sodium chloride	4.00	4.00	4.00	4.00	4.00
Vit/Min mixture[a]	0.90	0.90	0.90	0.90	0.90
Cellite	7.80	8.80	7.90	11.7	8.20
Nutritive value					
ME, MJ/kg	12.13	12.13	12.13	12.13	12.13
Crude protein, g/kg	150	150	150	150	150
Astaxanthin, mg/kg	0.00	7.96	23.9	54.0	109
Methionine, g/kg	3.88	3.89	3.88	3.93	3.89
Methionine + cysteine, g/kg	6.32	6.32	6.33	6.29	6.33
Lysine, g/kg	7.16	7.18	7.17	7.26	7.19
Phosphorus, g/kg	2.52	2.51	2.52	2.49	2.52
Calcium, g/kg	32.5	32.5	32.5	32.5	32.5

[a] Vitamin and mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 4500 IU; vitamin D, 450 IU; vitamin E, 7.5 IU; menadione, 0.75 mg; riboflavin, 3.75 mg; D-Ca pantothenate, 3 mg; niacin, 15 mg; vitamin B-12, 0.006 mg; biotin, 0.15 mg; folic acid, 0.375 mg; thiamine-HCl, 1.05 mg; pyridoxine-HCl, 3.75 mg; $CuSO_4 \cdot 5H_2O$, 12 mg; KI, 0.054 mg; $MnSO_4 \cdot H_2O$, 30 mg; Na_2SeO_3, 0.09 mg; ZnO, 53 mg; $FeSO_4$, 68 mg.

Hens were divided into five treatment groups (n = 10/treatment) based on average egg production and body weight (Table 1).The control group was fed a corn-soy basal diet without microalgae. Treatment groups 1 and 3 were fed the basal diet supplemented with 0.5% and 2.0% of defatted *Haematococcus pluvialis* to provide 10 and 40 mg of astaxanthin/kg of diets, respectively. Treatment groups 2 and 4 were fed the basal diet supplemented with 0.08% and 0.32% of full fatted *Haematococcus pluvialis* to provide 20 and 80 mg of astaxanthin/kg of diets, respectively. All experimental diets were formulated based on NRC requirements (1994) [23].

2.2. Performance, egg quality, and biochemical analyses

Body weights were measured weekly, feed intake biweekly, and egg production daily. Eggs from each treatment were collected at the beginning and at the ends of weeks 3 and 6 to be evaluated for egg component weights and biochemical parameters. Blood was collected from the wing veins at the three same time-points to prepare plasma samples that were stored at −20 °C until analyses.

Plasma alkaline phosphatase (AKP) activity was determined using the method established by Bowers and McComb [24]. Plasma tartrate-resistant acid phosphatase (TRAP) activity was determined using the methods of Lau et al. [25]. Plasma inorganic phosphorus (PIP) was analyzed using the method of Gomori [26]. Kits for total cholesterol (TC), triglyceride (TG) and non-esterified fatty acid (NEFA) were purchased from Wako Chemicals (Richmond, VA, USA). The kit for determining plasma alanine aminotransferase (ALT) was obtained from Thermo Scientific, Inc. (Waltham, MA, USA). The kit for determining glucose concentration was purchased from Sigma Aldrich (St. Louis, Missouri, USA).

2.3. Astaxanthin analysis

Astaxanthin in the diets, plasma, liver, and egg yolk were extracted using the method of Lopez et al. [27] with modification. Concentrations of the extracted astaxanthin from the samples were measured using the methods of Sowell et al. [28], Breithaupt et al. [29], and Rohrle et al. [30] with modifications. Briefly, extracted astaxanthin was eluted isocratically with methanol and acetonitrile (50:50) with 0.1% triethylamine (TEA) at a flow rate of 1 mL/min, carried on a Agilent Eclipse plus C18 reverse phase column (5 μm, 4.6 × 250 mm) using a HPLC system (Shimadzu, Japan) (LC-10AD vp pumps, an SIL-10Ai auto injector, and an SPD-10 AV vp UV–vis detector). Column temperature was set up at 30 °C. Mobile phase was sonicated at room temperature for 15 min before using. Chromatographic peaks were identified by comparison of the retention time of standard astaxanthin. To validate the results, sample extracts were spiked with standard astaxanthin to determine its appearance on the chromatogram in relation to the sample peak being identified. Astaxanthin and β-carotene standards were purchased from Sigma Aldrich (St. Louis, Missouri, USA).

2.4. Oxygen radical absorbance capacity assay

Oxygen radical absorbance capacity of plasma, liver, and egg yolk were measured using an adapted method of Ou et al. [31]. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard. All results were expressed as μM of trolox equivalence.

2.5. Determinations of malondialdehyde, glutathione, and antioxidant enzyme activities

Malondialdehyde (MDA) levels were determined by the method of Esterbauer et al. [32], using 2-thiobarbituric acid assay and1,1,3,3-tetraethoxypropane as a standard. Glutathione peroxidase (GPX), glutathione transferase (GST), glutathione reductase (GR), and superoxide dismutase (SOD) activities were determined using previously described