Effect of different dietary concentrations of brown marine algae (Sargassum dentifebium) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens

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Abstract

The effect of different concentrations (0%, 3% and 6%) of brown marine algae (BMA, Sargassum dentifebium) prepared according to different methods (sun-dried, SBMA; boiled, BBMA; autoclaved, ABMA) on plasma and yolk lipid profiles, carotene, and lutein plus zeaxanthin in egg yolks was studied in hens aged from 23 to 42 weeks (30 hens per treatment). We determined the fatty acid profiles in BMA and in the egg yolk of hens fed different levels of BMA prepared according to different methods. In addition, plasma and yolk lipid profiles, yolk total carotene, and lutein plus zeaxanthin were determined at week 42 of age. Plasma and yolk cholesterol were significantly lower in BMA than in the control group, but high-density lipoprotein (HDL) significantly decreased as BMA concentration increased. There was a significant similar decline in yolk triglycerides with inclusion of either 3% or 6% BMA in the laying hen diet. Palmitic acid was the main saturated fatty acid (SFA) found in BMA and oleic acid (omega-9) and linoleic acid (omega-6) were the main unsaturated fatty acids (UFA), while there was a significant increase in palmitic acid in egg yolk when BMA was included at 6%. There was a significant increase in oleic acid (omega-9) when feed containing 3% BMA was given compared to the control group, but this decreased with a further increase in BMA. Linoleic acid (omega-6) also significantly decreased with inclusion of either 3% or 6% BMA. There was a significant increase in total carotene and lutein plus zeaxanthin in the laying hen eggs as a result of feeding diets containing 3% and 6% BMA.

Introduction

Marine algae are considered to be potential source of nutrients, containing high amounts of proteins, amino acids, carbohydrate, lipids, vitamins A, B (especially B12) and C, colourants, antioxidants and antimicrobial substances (Serviere-Zaragoza et al., 2002; Rimber, 2007, Abd El-Baky et al., 2008; Al-Harthi and El-Deek, 2011, 2012). They also have an important economic profits impact (FAO, 2003). Seaweeds have total dietary fibre contents ranging between 32.7% and 74.6% (dry matter, DM) of which 51.6-85.0% are water soluble (Castro-Gonzalez et al., 1991; Lahaye, 1991; Wong et al., 2000), but their crude lipid contents are very low (ranging from 1.42% to 1.64% DM).

Gracilaria changii (G. changii) algae contain a greater amount of UFA (74%), mainly omega-3 fatty acids, and 26% of SFA, mainly palmitic acid. They also contain relatively high levels of Ca and Fe (Mohd et al., 2000). Brown algae contain 8.4-14.2% crude protein (CP), 25.3-37.5% ash, 4.8-7.2% crude fibre (CF), 0.4-1.0% ether extract (EE) and 45.6-59.0% nitrogen free extract (NFE). Levels of these components depend on the season and the geographical location (Serviere-Zaragoza et al., 2002).

David (2001) reported that 100 g DM of Ulva sp. algae contained 960 U vitamin A (retinal), 0.06 mg vitamin B1, 0.03 mg vitamin B2, 8.0 mg vitamin B6, 6.3 mg vitamin B12, 11.8 mg folic acid and 10.0 mg vitamin C. Algae have been found to be a valuable feed resource in poultry diets and could be added to feed in proportions of up to 2-3% without having any adverse effects on egg production quality traits or broiler performance (Al-Harthi and El-Deek, 2011, 2012; El-Deek et al., 2011a). Algae may have a beneficial impact on human health and can improve the quality of eggs by enhancing the carotene, lutein and zeaxanthin contents, and improving antimicrobial activity and antioxidant levels (Abd El-Baky et al., 2003; El-Deek et al., 2003; El-Deek and A.A. El-Deek, 2012). They also have an important economic profits impact (FAO, 2003). Brown algae have been considered as potential sources of nutrients, containing high amounts of proteins, amino acids, carbohydrate, lipids, vitamins A, B (especially B12) and C, colourants, antioxidants and antimicrobial substances (Serviere-Zaragoza et al., 2002; Rimber, 2007, Abd El-Baky et al., 2008; Al-Harthi and El-Deek, 2011, 2012). They also have an important economic profits impact (FAO, 2003).

Materials and methods

This study was carried out at the Hada Al-Sham Agriculture Research Station, the Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

Harvesting and processing

Brown marine algae Sargassum dentifebium were the most prevalent BMA on the Red Sea shore in Saudi Arabia, near Jeddah. Algae were harvested, transported to the research station, exposed to the sun (up to 40°C) for most of the day, and continuously stirred until dried (con-
stant weight). They were then crushed to a dry powder, sieved in a special container to reach an appropriate (0.5 mm) size for feeding, and then stored in dark bags until use. One portion of the sun-dried brown marine algae (SBMA) was taken and boiled (BBMA) in water in a cooking unit using indirect methods (1 part algae: 4 parts water, w/w) for 20 min while stirring. They were then transferred directly onto trays, continuously stirred for 36 h in the drying unit, and ground again before being sieved. Another portion of the SBMA was processed by autoclaving (ABMA) under 115 bar/inc2 for 15 min. BBMA

tion of the SBMA was processed by autoclaving

ground again before being sieved. Another por-

uously stirred for 36 h in the drying unit, and

were then transferred directly onto trays, contin-

stant weight). They were then crushed to a dry

powder, sieved in a special container to reach

an appropriate size (approximately 0.5 mm) for

feeding, and then stored in dark bags (as with the SBMA) until use.

**Feeding trial**

The SBMA, BBMA and ABMA at concentra-

tions of 3.0% and 6.0% were included in the

Hy-line laying hen diets (Table 1) formulated

according to nutrient recommendations for

laying hens 23-42 weeks of age (NRC, 1994). These combinations resulted in 3 processing

treatments (30 birds/group) of 6 replicates of 5 hens each. Hens were housed in individual
cages (520 cm2/hen) in an environmentally

controlled house. Feed and water were provided

ad libitum throughout the experimental period. Vaccinations and medical programmes were

followed at different time points according to

age under the supervision of a veterinarian.

The hen house was lit according to a 14:10 h light-dark cycle.

**Data collection**

Samples from BMA, processed according to different methods, and egg yolk were extracted according to Folch et al. (1957). The methyl esters of fatty acids, obtained from the standard acids and various samples under study, were analyzed according to Radwan (1978). Blood samples (12/treatment) were collected (5 ml) from the brachial vein and placed into heparinized tubes at 42 weeks of age and plasma was collected after centrifugation of blood samples at 1500 x g. Yolk cholesterol (7 samples/treatment) was determined after lipid extraction with a mixture of chloroform: methanol (2:1 v/v) using the procedure

### Table 1. The composition of the experimental diets containing different levels of brown marine algae according to different processing methods.

| Ingredients, g/kg | Control | Sun-dried 3% | Sun-dried 6% | Boiled 3% | Boiled 6% | Autoclaved 3% | Autoclaved 6% |
|------------------|---------|--------------|--------------|-----------|-----------|--------------|--------------|
| Maize            | 629.5   | 577.7        | 491.6        | 577.9     | 488.8     | 577.7        | 490.0        |
| Soybean meal, 48% CP | 231.8   | 233.2        | 233.0        | 233.0     | 232.5     | 233.1        | 232.8        |
| Wheat bran       | 8.30    | 10.7         | 52.7         | 18.8      | 55.3      | 18.8         | 54.0         |
| Palm oil         | 1.1     | 14.5         | 38.4         | 14.4      | 39.2      | 14.5         | 38.9         |
| Dical phosphate  | 25.2    | 24.9         | 24.4         | 24.9      | 24.4      | 24.9         | 24.4         |
| Limestone        | 94.6    | 94.6         | 94.8         | 94.6      | 94.6      | 94.6         | 94.8         |
| Sodium chloride  | 4.5     | 1.4          | 0.0          | 1.4       | 0.0       | 1.4          | 0.0          |
| Vit+Min premixc  | 2.0     | 2.0          | 2.0          | 2.0       | 2.0       | 2.0          | 2.0          |
| DL-methionine    | 1.5     | 1.5          | 1.6          | 1.5       | 1.5       | 1.5          | 1.6          |
| Choline C70      | 0.5     | 0.5          | 0.5          | 0.5       | 0.5       | 0.5          | 0.5          |
| Antioxidant      | 1.0     | 1.0          | 1.0          | 1.0       | 1.0       | 1.0          | 1.0          |
| Brown marine algae | 0.0   | 30           | 60           | 30        | 60        | 30           | 60           |
| Total            | 1000    | 1000         | 1000         | 1000      | 1000      | 1000         | 1000         |

Calculated or determined analysis

| Dry matter, g/kg | 907     | 909          | 913          | 909       | 914       | 909          | 914          |
| Crude protein, g/kg | 170    | 170          | 170          | 170       | 170       | 170          | 170          |
| Arginine, g/kg   | 10.54   | 10.71        | 10.94        | 10.73     | 10.97     | 10.73        | 10.98        |
| Lysine, g/kg     | 8.55    | 8.64         | 8.74         | 8.63      | 8.73      | 14.9         | 14.7         |
| Methionine + Cystine, g/kg | 4.2 | 4.2          | 4.2          | 4.2       | 4.2       | 4.2          | 4.2          |
| Methionine + Cystine, g/kg | 7.0 | 7.0          | 7.0          | 7.0       | 7.0       | 7.0          | 7.0          |
| Threonine, g/kg  | 6.2     | 6.3          | 6.4          | 6.3       | 6.4       | 6.4          | 6.5          |
| Tryptophan, g/kg | 2.11    | 2.12         | 2.14         | 2.11      | 2.14      | 2.11         | 2.14         |
| Ether extract, g/kg | 27.6   | 39.5         | 61.2         | 39.4      | 62.0      | 39.5         | 61.8         |
| Linoleic acid, g/kg | 15.02 | 22.96        | 31.5         | 22.91     | 31.45     | 22.62        | 30.86        |
| Crude fibre, g/kg | 23.8   | 26.2         | 30.4         | 24.0      | 26.2      | 26.2         | 30.4         |
| Calcium, g/kg    | 4.3     | 4.3          | 4.3          | 4.3       | 4.3       | 4.3          | 4.3          |
| Available phosphorus, g/kg | 6.5 | 6.5          | 6.5          | 6.5       | 6.5       | 6.5          | 6.5          |
| Chlorine, g/kg   | 3.11    | 1.25         | 0.38         | 1.26      | 0.38      | 0.12         | 0.38         |
| Sodium, g/kg     | 2.0     | 2.0          | 2.85         | 2.00      | 2.64      | 2.00         | 2.66         |
| Metabolizable energy, MJ/kg | 11.30 | 11.30        | 11.30        | 11.30     | 11.30     | 11.30        | 11.30        |

CP: crude protein. °Provided the following per kg of diet: vitamin A, 12,000 U; vitamin D3, 7200 U; vitamin E, 20 U; vitamin B1, 2.5 mg; vitamin B2, 5 mg; vitamin K, 3 mg; vitamin B12, 1.5 ppb; pyridoxine, 0.225 ppb; pantothenic acid, 10 mg; niacin, 35 mg; folic acid, 1.5 mg; biotin 125 mg; Mn, 90 mg; Cu, 7.5 mg; Zn, 85 mg; Fe, 50 mg; Se, 0.1 mg.
plasma and yolk triglyceride, total cholesterol, plasma high density lipoprotein (HDL) and plasma calcium were determined by colorimetric methods using commercial kits (Diamond Diagnostics, Holliston, MA, USA). Low-density lipoprotein was calculated according to Fridewald et al. (1957) as follows:

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LDL = \text{Total cholesterol} - \text{High density lipoprotein} - \text{Triglycerides}/5
\]

Plasma alkaline phosphatase (ALP, IU/L) was determined according to Yan et al. (1972).

In addition, total carotene contents in the egg yolk were determined (5 samples/treatment) according to the AOAC (1995), whereas lutein and zeaxanthin fractions in egg yolk (5 samples/treatment) were analyzed by HPLC according to Handelman et al. (1999).

**Statistical analysis**

Data were analyzed using the GLM procedure of SAS (2001) using factorial analyses (3x2) plus the control group. Before analysis, all percentages were subjected to logarithmic transformation \((\log(x + 1))\) to normalize data distribution. Mean difference at \(P < 0.05\) was tested using the Student-Newman-Keuls Test.

**Results**

**Fatty acids content**

Fatty acids (FAs) profiles of BMA processed according to different preparation methods are shown in Table 2. The omega-9, -6 and -3 in the BMA ranged from 27.1% to 28.7%, from 22.2% to 25.4% and from 4.4% to 5.6%, respectively. Palmitic acid was the main SFA (22.1-26.6%). Content of SFA, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and unsaturated fatty acids (UFA) ranged from 32.5% to 35.9%, 35.8% to 36.7%, 27.8% to 30.4% and from 64.1% to 67.5%, respectively. The SFA/UFA ranged from 0.482 to 0.560.

**Blood plasma components**

Blood plasma components, as influenced by different dietary BMA concentrations processed according to different preparation methods, are shown in Table 3. The different processing methods had no significant effect on plasma total lipid, triglycerides, total cholesterol, HDL and LDL, calcium or alkaline phosphatase levels. Dietary concentrations of BMA at 3% and 6% significantly reduced plasma total cholesterol compared to the control group. On the other hand, LDL showed a stepwise significant decrease with increasing BMA concentrations. There was no significant effect of BMA concentrations on plasma total lipids, triglycerides, HDL, calcium or alkaline phosphatase. There was a significant interaction between the processing method and BMA concentrations on plasma total cholesterol, but no change was seen for other traits. The results show that increasing BMA concentrations from 3% to 6% in groups fed SBMA and ABMA significantly decreased plasma cholesterol, while increasing plasma cholesterol in groups fed BBMA.

**Yolk lipid profiles and selected fatty acids**

Yolk lipid profiles as affected by different dietary BMA concentrations processed according to different preparation methods are shown in Table 4. Methods of processing had no significant effects on yolk triglycerides, total cholesterol, palmitic acid, stearic acid, oleic acid (omega-9) or linoleic acid (omega-6). Concentration of BMA had a significant effect on all yolk lipid profiles. Yolk triglycerides, total cholesterol, stearic acid and linoleic acid (omega-6) were significantly and similarly lower in groups fed diets containing either 3% or 6% BMA than in the control group.

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**Table 2. Fatty acid profiles of brown marine algae processed by different methods as compared to values in the literature and chicken requirements.**

| Fatty acid, g/100g | Fatty acids profile | Chickens requirements | Autoclaved | Dried brown algae | Boiled | Sun-dried |
|-------------------|---------------------|----------------------|------------|-------------------|--------|----------|
| Lauric, 12:0      | 0.03-0.8            | -                    | 0.726      | 0.521             | 1.079  |
| Myristic, 14:0    | 5.0-6.9             | -                    | 3.047      | 1.822             | 3.453  |
| Palmitic, 16:0    | 41.7-49.5           | -                    | 26.6       | 22.1              | 25.9   |
| Palmitoleic, 16:1 | 2.8-4.0             | -                    | 7.26       | 6.38              | 6.12   |
| Heptadecanoic, 17:0 | 0.3-0.6         | -                    | 1.451      | 1.822             | 1.511  |
| Stearic, 18:0     | 1.2-1.9             | -                    | 2.54       | 5.74              | 2.88   |
| Oleic 18:1 (omega-9) | 6.1-11.7        | 1.0                  | 27.1       | 28.7              | 27.3   |
| Linoleic 18:2 (omega-6) | 3.4-4.1      | -                    | 24.5       | 25.4              | 22.2   |
| Linolenic 18:3 (omega-3) | 0.2-2.2    | -                    | 4.40       | 4.99              | 5.61   |
| Arachidic, 20:0   | 1.0-2.0             | -                    | 0.967      | 0.521             | 1.079  |
| Eicosanoic, 20:1  | -                   | -                    | 1.452      | 1.594             | 2.878  |
| SFA              | 48.3-61.7           | -                    | 35.3       | 32.5              | 35.9   |
| MUFA             | 12.5-22.0           | -                    | 64.7       | 67.5              | 64.1   |
| SFA/PUFA         | 3.66-2.80           | -                    | 0.546      | 0.482             | 0.560  |
| PUFA             | 8.9-15.7            | -                    | 35.8       | 37.0              | 36.5   |
| MUFA/PUFA        | 3.6-6.3             | -                    | 28.9       | 30.4              | 27.8   |
| MUFA/PUFA        | 2.47-2.49           | -                    | 1.239      | 1.265             | 1.308  |

*Heba et al., 1997; NRC, 1994. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.*
but induced the opposite trend in hens fed BBMA and ABMA. Increasing BMA concentration from 3% to 6% in hens fed BBMA and ABMA significantly decreased oleic acid (omega-9) levels when BMA concentrations were increased from 3% to 6% in hens fed BBMA and ABMA. A significant decrease in yolk total cholesterol was shown only in hens fed SBMA when the concentration was increased from 3% to 6%, but there was no effect on the other groups fed BBMA and ABMA. Increasing BMA concentration from 3% to 6% in hens fed SBMA significantly increased palmitic acid levels but resulted in the opposite trend in hens fed BBMA and ABMA. Sstearic acid was significantly increased when BMA concentration increased from 3% to 6% in hens fed SBMA but stearic acid was not detected in the other groups. Increasing BMA concentrations from 3% to 6% in hens fed SBMA and ABMA significantly decreased oleic acid (omega-9) levels but induced the opposite trend in hens fed BBMA. There was a significant decrease in linoleic acid (omega-6) levels when BMA concentrations of either 3% or 6% were included in the diet.

**Total carotene and lutein plus zeaxanthin**

Total carotene and lutein plus zeaxanthin, as influenced by different dietary BMA concentrations prepared according to different processing methods are shown in Table 3. There was no significant effect of different processing method on total carotene or lutein plus zeaxanthin content in the egg yolk. On the other hand, a significant increase in total carotene in egg yolk was observed with increasing BMA concentration to 6% and a significant increase in lutein plus zeaxanthin was achieved at 3 and 6% BMA concentrations when compared with the control group.

Increasing BMA concentrations from 3% to 6% in hens fed SBMA significantly decreased total carotene but induced the opposite trend in hens fed BBMA and ABMA. Increasing BMA concentrations from 3% to 6% in hens fed SBMA and ABMA significantly decreased oleic acid (omega-9) levels but induced the opposite trend in hens fed BBMA and ABMA. There was a significant decrease in linoleic acid (omega-6) levels when BMA concentrations of either 3% or 6% were included in the diet.

**Discussion**

In the present study, we investigated the health benefits of BMA from its ability to reduce fatty acid profiles and cholesterol, and due to its antioxidant properties and pigmentation profiles. Reports in the literature found algae was a valuable ingredient in human and animal nutrition because of its protein, amino acid, dietary fibre, mineral, fatty acid and bioactive content (Serviere-Zaragoza et al., 2002; Rimber, 2007; Al-Harthi and El-Deek, 2011). The present results (Table 2) indicate that BMA is a good source of UFA and PUFA which offer several health benefits (Abd El-Baky et al., 2008). In the literature, algae was reported to contain UFA, mainly omega-3 fatty acids, and relatively high amounts of Ca and Fe (Mohd et al., 2000). These results indicate that different processing methods had a small effect on BMA fatty acid profiles. Similarly, G. changgi (C. changii) contained a greater amount of UFA (74%), mainly omega-3 fatty acids, and 26% of SFA, mainly palmitic acid, whereas oleic acid and linoleic were the main MUFA and PUFA, respectively (Heiba et al., 1997). Brown algae contained 8.4-14.2% CP, 25.3-37.5% ash, 4.8-7.2% CF, 0.4-1.0% EE and 45.6-59.0% NFE (Serviere-Zaragoza et al.,

### Table 3. Effect of different processing methods and concentrations of brown marine algae on plasma lipid profiles, calcium and alkaline phosphatase at 42 weeks of age.

| Dietary treatments | Alkaline phosphatase, U/L | Calcium, mg/dL | LDL, mg/dL | HDL, mg/dL | Total cholesterol, mg/dL | Triglycerides, mg/dL | Total lipids, mg/dL |
|--------------------|--------------------------|----------------|------------|------------|--------------------------|---------------------|-------------------|
| **Preparing ways**  |                          |                |            |            |                          |                     |                   |
| Sun-dried          | 172                      | 10.2           | 54.8       | 46.7       | 130                      | 176                 | 712               |
| Boiled            | 190                      | 10.9           | 54.5       | 42.0       | 122                      | 224                 | 898               |
| Autoclaved        | 170                      | 12.8           | 58.8       | 48.7       | 133                      | 214                 | 605               |
| Concentrations of brown marine algae, % |                  |                |            |            |                          |                     |                   |
| 0.0                | 178                      | 7.28           | 70.9       | 44.2       | 166                      | 201                 | 760               |
| 3.0                | 175                      | 13.7           | 55.9       | 50.8       | 115                      | 217                 | 659               |
| 6.0                | 181                      | 12.9           | 41.3       | 42.4       | 103                      | 197                 | 797               |
| **Interaction between preparing ways and brown marine algae concentrations** |                  |                |            |            |                          |                     |                   |
| PW                  |                          |                |            |            |                          |                     |                   |
| Sun-dried          | 166                      | 14.8           | 52.4       | 43.6       | 121<sup>a</sup>          | 168                 | 719               |
| Boiled            | 169                      | 8.58           | 44.1       | 55.1       | 105<sup>b</sup>          | 167                 | 589               |
| Autoclaved        | 189                      | 12.1           | 57.4       | 52.6       | 86.9<sup>b</sup>         | 233                 | 629               |
| Concentrations of brown marine algae, % |                  |                |            |            |                          |                     |                   |
| 0.0                | 193                      | 13.3           | 38.0       | 31.9       | 117<sup>b</sup>          | 247                 | 1212              |
| 3.0                | 164                      | 14.1           | 58.0       | 56.2       | 137<sup>b</sup>          | 251                 | 629               |
| 6.0                | 181                      | 16.8           | 41.9       | 40.1       | 87.2<sup>b</sup>         | 177                 | 590               |
| **SEM**            | 0.47                     | 45.6           | 17.5       | 9.5        | 17.3                     | 2.6                 | 4.03              |
| **Analysis of variance** |              |                |            |            |                          |                     |                   |
| PW                  | ns                       | ns             | ns         | ns         | ns                       | ns                  | ns                |
| C                   | ns                       | ns             | 0.0004     | ns         | ns                       | 0.0001              | ns                |
| PWxC                | ns                       | ns             | ns         | ns         | 0.04                     | ns                  | ns                |

PW, preparing way; C, concentration. *a,b,c* Means in a column under similar treatment not sharing the same superscript are significantly different at P<0.05; ns, not significant.
Table 4. Effect of different processing methods and concentrations of brown marine algae on yolk lipid profiles and selected fatty acids at 42 weeks of age.

| Dietary treatments | Omega 3, mg/g (Linoleic) | Omega 6, mg/g (Linoleic) | Omega 9, mg/g (Oleic) | Stearic acid, mg/g | Palmitic acid, mg/g | Total cholesterol, mg/g | Triglycerides, mg/g |
|--------------------|--------------------------|--------------------------|-----------------------|--------------------|--------------------|------------------------|----------------------|
| Preparing ways     |                          |                          |                       |                    |                    |                        |                      |
| Sun-dried          | 0                        | 0.533                    | 8.12                  | 0.983              | 4.02               | 15.2                   | 68.1                 |
| Boiled             | 0                        | 0.433                    | 7.15                  | 0.433              | 3.98               | 12.7                   | 74.8                 |
| Autoclaved         | 0                        | 0.250                    | 5.55                  | 0.250              | 5.23               | 12.5                   | 122.0                |
| Concentrations of brown marine algae, % |                          |                          |                       |                    |                    |                        |                      |
| 0.0                | 0                        | 0.517^b                 | 7.617^b               | 0.550^a            | 3.42^a             | 15.5^a                 | 111.1^a              |
| 3.0                | 0                        | 0.350^b                 | 8.283^a               | 0.183^b            | 3.63^b             | 13.7^b                 | 106.0^b              |
| 6.0                | 0                        | 0.367^b                 | 6.567^b               | 0.250^b            | 4.45^b             | 12.8^b                 | 101.5^b              |

Interaction between preparing ways and brown marine algae concentrations

| PW | C | SEM |
|----|---|-----|
| PW | ns| ns  |
| C  | ns| ns  |
| PW×C | ns| ns  |

Analysis of variance

| PW | C | SEM |
|----|---|-----|
| PW | ns| ns  |
| C  | 0.01| 0.05|
| PW×C | 0.01| 0.05|

PW, preparing way; C, concentration. * Measured in a column under similar treatment not sharing the same superscript are significantly different at P<0.05; ns, not significant.

Table 5. Effect of different processing methods and concentrations of brown marine algae on egg yolk total carotenoids and Lutein plus Zeaxanthin at 42 weeks of age.

| Dietary treatments | Total carotene, mg/kg | Lutein plus Zeaxanthin, mg/kg |
|--------------------|-----------------------|------------------------------|
| Preparing ways     |                       |                              |
| Sun-dried          | 23.5                  | 15.5                         |
| Boiled             | 24.1                  | 16.0                         |
| Autoclaved         | 23.7                  | 15.9                         |
| Concentrations of brown marine algae, % |                       |                              |
| 0.0                | 22.7^a                | 15.5^a                       |
| 3.0                | 24.0^b                | 16.8^b                       |
| 6.0                | 25.0^c                | 16.2^c                       |

Interaction between preparing ways and brown marine algae concentrations

| PW | C | SEM |
|----|---|-----|
| PW | ns| ns  |
| C  | ns| ns  |
| PW×C | ns| ns  |

Analysis of variance

| PW | C | SEM |
|----|---|-----|
| PW | ns| ns  |
| C  | 0.01| 0.05|
| PW×C | 0.05| 0.05|

PW, preparing way; C, concentration. * Measured in a column under similar treatment not sharing the same superscript are significantly different at P<0.05; ns, not significant.

2002). The differences in fatty acid composition reported in the literature could be attributed to different species and sources of algae, geographical location, season, environmental factors and physiological conditions (Abd El-Baky et al., 2008; Chakraborty et al., 2008). The effect of BMA in reducing plasma and yolk cholesterol, and yolk triglycerides was confirmed in the present study with either 3% and 6% BMA. The positive effect of BMA on plasma and yolk total cholesterol, and yolk triglycerides could be attributed to the dietary fibre contents of BMA and/or higher PUFA content. Reports in the literature indicated that BMA has a beneficial effect on plasma cholesterol, HDL, LDL and triglycerides which could be attributed to algae sterols (Kritchevsky et al., 1999; Hassan et al., 2005). Furthermore, algae supplementation to human and animal diets has been reported to significantly improve lipid profile (Venkataraman et al., 1994; Schiavone et al., 2007). However, dietary BMA concentrations from 0 to 6% in broiler diets had no constant impact on plasma lipid profiles (total lipid, triglycerides, cholesterol, HDL and LDL) (El-Deek et al., 2011a). These
inconsistencies in reports of the effect of algae on lipid profile in blood plasma could be due to different species and sources of algae, geographical location, season, environmental factors, growth media and physiological conditions (Abd El-Baky et al., 2008; Chakraborty et al., 2008).

The fatty acid profiles in egg yolk indicated that oleic acid (omega-9) and palmitic acid were significantly increased when hens were fed 3% and 6%, respectively. BMA compared to the control group. Stearic acid and linoleic acid (omega-6) showed a similar significant decrease with addition of either 3% or 6% BMA. There was an apparent association between egg yolk fatty acid profiles and fatty acid profiles of BMA. The relationship between dietary intake of lipids/fatty acids and lipid profile of meat and eggs has been reported (Attia et al., 1995, El-Deek et al., 1997).

The natural colourant in the egg yolk (such as total carotenes which have antioxidant properties, and lutein plus zeaxanthin, which are pigments) was enhanced as a result of feeding diets containing 3% and 6% BMA. In literature, the total carotene and lutein and zeaxanthin in algae was reported to be 12.73 mg/d DM Ulva lactu tus grown in normal water and 23.91 mg/d DM, in artificial sea water. Lutein had 0.51% and 1.18% as a relative area peak in HPLC profile while zeaxanthin was found at 0.16 and 2.32% in the Ulva lactu tus grown in either normal or artificial sea water, respectively. These results indicate that colourant and antioxidant contents of algae depend on several environmental factors (Abd El-Baky et al., 2008; Chakraborty et al., 2008).

In this regard, algae is a natural product that may have a beneficial impact on human health and that can improve the quality of eggs by enhancing the total carotene, lutein and zeaxanthin, and antimicrobial and antioxidant contents (Abd El-Baky et al., 2004, 2008; El-Baz et al., 2002; Athukorala et al., 2006; Chakraborty et al., 2008). In line with this, BMA was found to be a valuable feed resource for laying hens and broiler chickens; concentrations of up to 2-3% could be added to their diet without adverse effects on egg production quality traits or broiler performance (Al-Harthi and El-Deek 2011, 2012; El-Deek et al., 2011a). It should be mentioned that the processing method did not influence plasma and yolk lipid profiles, Ca and alkaline phosphatase in plasma and yolk total carotene and lutein plus zeaxanthin, indicating that sun-drying, boiling or autoclaving did not affect the nutrient availability of BMA (Attia et al., 2003; El-Deek et al., 2011a, 2011b; Al-Harthi and El-Deek 2011, 2012). The results show that the changes in plasma cholesterol and lipid profiles in egg yolk depend on the processing method of BMA and its concentration in the laying hen diets. For example, the lowest plasma cholesterol level was observed with hens fed 6% ABMA and this was 36.4% less than those observed in hens fed 3% ABMA, while the decrease in plasma cholesterol level seen with SBMA at 6% was 13.2% less than the group fed 3% SBMA. There was a 15.3% decrease in egg yolk cholesterol when BMA concentration in SBMA was increased from 3% to 6% while no changes were observed in the other levels. There was a significant decrease in yolk triglyceride in groups fed SBMA (13.9%) and BBMA (7.8%). These results indicate that BMA had a greater effect on plasma cholesterol than on yolk triglycerides which showed, in turn, a greater response to BMA than the yolk cholesterol. This could be explained by several factors, such as diet and ova synthesis, that contribute to the production of yolk cholesterol (Elkin, 2006). On the other hand, the highest omega-6 fatty acid level was observed in hens fed 3% SBMA and 6% ABMA. It was reported that 6% ABMA in laying hen diet increased total carotene by 15.3% compared to concentration found in hens fed 3% ABMA, while the corresponding increase in total carotene concentration due to supplementation of 6% BBMA in laying hens was 4.6%. On the other hand, there was a significant decrease in lutein plus zeaxanthin in egg yolk with increasing algae concentration from 3% to 6% in SBMA. However, there was a slight increase in the BBMA and ABMA groups when BMA concentrations were increased.

Conclusions

Palmitic acid was the main saturated fatty acid while oleic and linoleic acids were the main unsaturated fatty acids in BMA. There was a significant increase in palmitic acid levels in egg yolk when BMA was added at 6%. In addition, up to 6% BMA in the laying hen diet resulted in a significant decrease in plasma and yolk cholesterol and triglycerides, while enhancing total carotene and lutein plus zeaxanth in laying hen eggs. This implies a beneficial health impact on humans.

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