Sexual maturity and performance of pullets fed different preparations and concentrations of brown marine algae (Sargassum dentifebium) in pre-laying and early laying periods

Mohammed A. Al-Harthi
Arid Land Agriculture Department, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

The effect of brown marine algae (BMA; Sargassum dentifebium) as alternative feed source for pullets was studied in three processed and at three concentrations in the pullets from 14-42 weeks. The processing forms were sundried brown marine algae (SBMA), sundried and boiled brown marine algae (BBMA) and sundried and autoclaved brown marine algae (ABMA). The concentrations of BMA were 2%, 4% and 6% that composed 10 treatments along with control. Each treatment was replicated 6 times using 30 pullets per treatment. Different criteria on pullets and eggs, including feed intake, body weight, feed conversion efficiency, laying rate and egg mass and quality were studied. Results indicated that BMA could be used up to 6% in the pullets diets from 14 to 42 weeks without adverse effects (P>0.05) on sexual maturity (139-142 days), laying rate (80.7-87.9%), egg mass (44.99-51.86 g/ hen/day), feed conversion ratio (2.468-2.868 kg feed/kg egg) and Haugh unit (82.9-90.6) and shell percentage (8.61-9.87%). Furthermore, egg yolk color and calcium content in eggshell were improved (P≤0.05) by 12.31% and 9.1%, respectively.

Introduction

Research for alternative feed resource during the last decade has become essentially important due to the shortage of feedstuffs for animal feeding as some ingredients as corn and soybean meal were used for biofuel production (Attia et al., 2003; 2008; Al-Harthi et al., 2011). In the tropical areas, the problem is even more serious than in other parts of the world as feed resources availability for animal production is low (Al-Harthi et al., 2009; Al-Saffar et al., 2002). Utilization of algae as valuable feed resource seems a possible alternative. Algae are rich source of trace elements, proteins, lipids, polysaccharides, vitamins, enzymes (Rimber, 2007; Al-Harthi and El-Deek, 2011; 2012) and have also an antimicrobial activity (Rizvi and Shameel, 2004; Abdel-Wahab et al., 2006; Abd El-Baky et al., 2008).

The current algae world production (30-50%) is sold for use in animal feed and feed additives (Yamaguchi, 1997; Becker, 2004). Limited research has been done on algae as feed source that has recommended its feeding safely between 5-10% and an adverse effect may be excepted when using it in high concentrations for long periods (El-Harthi and El-Deek, 2012). However, pigmentation of poultry products (Becker, 2004; Al-Harthi and El-Deek, 2011) and n-3 fatty acids (Schiavone et al., 2007; Al-Harthi and El-Deek, 2012) could be increased by algae addition. Al-Harthi and El-Deek (2011) and El-Deek et al. (2011) have reported that brown marine algae (BMA) may be fed to laying hens and broiler chicks up to 3% without adverse effects on productive performance. Some studies (Yoshie et al., 1994; Al-Harthi and El-Deek, 2011; Al-Harthi et al., 2010, 2011; El-Deek et al., 2011) have reported that the quality and feeding value of feedstuffs may be improved by autoclaving, boiling in water and freezing and that result in increasing nutrient availability and thus animal performance.

This study aimed to investigate the effects of various processed forms and different dietary concentrations of brown marine algae (Sargassum dentifebium) on sexual maturity and performance of pullets during pre-laying and early laying periods, 14-42 weeks of age.

Materials and methods

Brown marine algae source

This work was done in the Agriculture Research Station at the Hada Al-Sham area, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Collection and processing of brown marine algae

Algae were harvested from the red sea and brought to the Agriculture Research Station, then sundried at an average temperature of 40°C with continuous stirring until dry. Algae were crushed to dry powder of 0.5 mm size and then stored in airtight plastic bags, until use in diet formulation.

Preparation of treatments

A part of the sundried brown marine algae (SBMA) was boiled in a cooking unit (1:4, algae:water, w:w) for 20 min with stirring, then transferred to big trays and kept for 36 h in the drying unit and dried algae were then ground again to prepare sundried and boiled brown marine algae (BBMA). A further part of SBMA was processed in autoclave under 115 barfin2 for 15 min to prepare sundried and autoclaved brown marine algae (ABMA).
Procedure for data collection

Individual body weight was recorded at week 14 and 42 of age as well as age at sexual maturity (10% laying rate). Laying rate (egg number/hen/d), egg weight (g/hen/day), egg mass and feed intake (g/hen/d) were measured daily for each replicate. Feed conversion ratio was calculated as the amount of feed required to produce 1 g of egg. Survival rate (%) was calculated as the number of live hens at the end of the experiment divided by number of hens at start of the experiment. At week 42 of age, six hens per treatment were slaughtered after being fasted overnight to obtain inner organs. They were weighed and their relative weights to live body weight were recorded.

At 28, 35 and 42 weeks of age, 20 eggs per treatment were collected for determination of egg and shell quality (Attia et al., 1994; Attia et al., 2012). The collected eggs were divided into two portions of 10 eggs each. The 1st group was used for determination of the quality of fresh eggs (on the same day of laying), while the other portion (10 eggs) was stored in the refrigerator at 5°C for 21 days, after which it was broken for egg quality evaluation. Shells of the fresh eggs (six per treatment) were used for determination of Ca, Na and K using an automatic absorption spectrophotometer (Model YL AAS-8000) according to AOAC (2004). Phosphorus was determined by Flame photometry according to Davis et al. (1964).

The mineral contents of egg shell were expressed as a percent of shell ash content. Shell percentage was expressed as a percentage of egg weight, while shell dry matter was expressed as a percentage of shell weight.

Statistical analysis

Data were analyzed using the GLM proce-

---

Table 1. Composition of the pullets’ diets containing different levels of brown marine algae prepared by different ways fed during 14-20 weeks of age.

| Ingredients, g/kg | Control | Sundried | Boiled | Autoclaved |
|-------------------|---------|----------|--------|------------|
| Maize, %          | 554.5   | 501.1    | 443.8  | 554.5      | 501.1      | 443.8    | 554.5 |
| Soybean meal, 48% CP | 164.9   | 171.9    | 179.4  | 164.9      | 171.9      | 179.4    | 164.9 |
| Wheat bran        | 194.3   | 202.5    | 211.3  | 194.3      | 202.5      | 211.3    | 194.3 |
| Palm oil          | 22.0    | 40.2     | 59.8   | 22.0       | 40.2       | 59.8     | 22.0  |
| Dicalcophosphate  | 28.0    | 28.1     | 28.1   | 28.0       | 28.1       | 28.1     | 28.0  |
| Limestone         | 8.4     | 8.2      | 8.1    | 8.3        | 8.2        | 8.1      | 8.3   |
| Sodium chloride   | 3.2     | 3.2      | 4.7    | 3.2        | 3.2        | 4.7      | 3.2   |
| Vitamin + mineral premix° | 2.0 | 2.0     | 2.0    | 2.0        | 2.0        | 2.0      | 2.0   |
| DL-methionine     | 1.2     | 1.3      | 1.3    | 1.3        | 1.3        | 1.3      | 1.3   |
| L-Lysine          | 0.2     | 0.0      | 0.0    | 0.0        | 0.0        | 0.0      | 0.0   |
| Choline chloride  | 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       | 20      | 60     | 20         | 40         | 60       | 20    |
| Total             | 1000    | 1000     | 1000   | 1000       | 1000       | 1000     | 1000  |

Calculated and determined analysis

| ME, MJ/kg | Dry matter, g/kg | Crude protein, g/kg | Lysine, g/kg | Methionine, g/kg | Methionine + Cystine, g/kg | Ether extract, g/kg | Linoleic acid, g/kg | Crude fibre, g/kg | Calcium, g/kg | Total phosphorus, g/kg | Chlorine, g/kg | Sodium, g/kg |
|-----------|------------------|---------------------|--------------|------------------|--------------------------|-------------------|-------------------|------------------|---------------|------------------------|----------------|--------------|
| 11.30     | 896.9            | 160                 | 7.50         | 3.80             | 6.62                    | 34.1              | 17.5              | 40.0             | 11.0          | 7.5                    | 2.4             | 1.5          |

CP: crude protein; ME, metabolizable energy; °Provided the following per kg of diet: vitamin A, 12,000 U; vitamin D₃, 7200 U; vitamin E, 20 U; vitamin B₁₂, 2.5 mg; vitamin B₃, 5 mg; vitamin K, 3 mg; vitamin B₂, 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, 65 mg; Fe, 50 mg; Se, 0.1 mg.
Results and discussion

Productive traits and sexual maturity

There was no significant effect of BMA concentration and/or preparing way on BW, BWG, age at sexual maturity and feed intake of pullets (Table 3). This indicated that using BMA up to 6% had no negative effect on BW and sexual development and this was confirmed by the lack of effect on feed intake during 14-20 weeks of age, too.

Laying rate, egg weight, feed intake and FCR from weeks 21 to 42 of age were also not significantly affected by preparing way and concentration of BMA (Table 4). However, egg mass was significantly greater in hens fed 2% BMA in respect of hens fed 4%.

In addition, there was a significant interaction between preparing way and concentration of BMA on only egg weight and egg mass. Sundried and autoclaved algae did not change egg weight due to increase of inclusion level, but boiled algae increased (P≤0.01) the egg weight at 6% in respect of 2%. However, both values were not different from the control group. When boiled or autoclaved algae were used, the 4% of inclusion level reduced (P≤0.01) egg mass more than 2%, and together 2% and 6%, respectively. Sundried algae at 6% reduced (P≤0.01) egg mass than only the 4% group; in addition, SBMA at 6% showed a lower egg mass than the control group.

The lack of significant effect of preparing way on productive performance of pullets from weeks 21 to 42 of age indicated that sundried was adequate way for preparing BMA for pullets' diets. Similar results were reported by...

Table 2. Composition of the pullets' diets containing different levels of brown marine algae prepared by different ways fed during 21-42 weeks of age.

| Ingredients, g/kg | Control | Sundried | Boiled | Autoclaved |
|-------------------|---------|----------|--------|------------|
|                    | 2%      | 4%       | 6%     | 2%         | 4%       | 6%       | 2%     | 4%       | 6%       |
| Maize              | 616.8   | 575.8    | 569.4  | 530.6      | 583.8    | 535.7    | 484.3  | 583.8    | 535.7    |
| Soybean meal, 48% CP | 257.8   | 257.3    | 256.5  | 260.0      | 263.5    | 270.9    | 278.0  | 263.5    | 270.9    |
| Wheat bran         | 0       | 0        | 4.6    | 12.1       | 0        | 4.1      | 10.7   | 0        | 4.1      |
| Palm oil           | 0       | 28.0     | 10.8   | 21.1       | 11.6     | 28.2     | 45.8   | 11.6     | 28.2     |
| Dicalcium phosphate | 25.1    | 24.9     | 24.7   | 24.6       | 25.2     | 25.2     | 25.3   | 25.2     | 25.2     |
| Limestone          | 91.1    | 86.6     | 86.7   | 86.7       | 86.5     | 86.4     | 86.3   | 86.5     | 86.4     |
| Sodium chloride    | 4.5     | 2.5      | 0.40   | 0.0        | 4.5      | 4.6      | 4.6    | 4.5      | 4.6      |
| Vitamin + mineral premix | 2.0  | 2.0      | 2.0    | 2.0        | 2.0      | 2.0      | 2.0    | 2.0      | 2.0      |
| DL-methionine      | 1.4     | 1.4      | 1.4    | 1.4        | 1.4      | 1.4      | 1.4    | 1.4      | 1.4      |
| Choline chloride   | 0.5     | 0.5      | 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    | 1.0      | 1.0      |
| Brown marine algae | 0       | 20       | 40     | 60         | 20       | 40       | 60     | 20       | 40       |
| Total              | 1000    | 1000     | 1000   | 1000       | 1000     | 1000     | 1000   | 1000     | 1000     |

Calculated and determined analysis

|                  | 11.30   | 11.30    | 11.30   | 11.30      | 11.30    | 11.42    | 11.54   | 11.42    | 11.54    |
|                  | Dry matter, g/kg | 906.4    | 906.7   | 908.7    | 911.0     | 908.0    | 911.2    | 914.4   | 908.4    | 911.4    |
|                  | Crude protein, g/kg | 180     | 180     | 180     | 180       | 182      | 183      | 185     | 182      | 183      |
|                  | Lysine, g/kg  | 9.2      | 9.3     | 9.4     | 9.4       | 9.4      | 9.6      | 9.8     | 9.4      | 9.6      |
|                  | Methionine, g/kg | 4.2     | 4.2     | 4.2     | 4.2       | 4.3      | 4.3      | 4.4     | 4.2      | 4.3      |
|                  | Methionine + Cystine, g/kg | 7.2  | 7.2     | 7.1     | 7.1       | 7.2      | 7.3      | 7.3     | 7.2      | 7.3      |
|                  | Ether extract, g/kg | 26.0    | 28.3    | 35.3    | 44.6      | 36.5     | 51.5     | 57.6    | 36.5     | 51.5     |
|                  | Linoic acid, g/kg | 14.6    | 19.6    | 24.9    | 30.2      | 20.0     | 25.7     | 31.3    | 19.6     | 25.2     |
|                  | Crude fibre, g/kg | 23.6    | 24.8    | 26.2    | 27.8      | 24.7     | 25.9     | 27.4    | 24.1     | 25.7     |
|                  | Calcium, g/kg | 41.7     | 40.0    | 40.0    | 40.0      | 40.0     | 40.0     | 40.0    | 40.0     | 40.0     |
|                  | Total phosphorus, g/kg | 6.5    | 6.5     | 6.5     | 6.5       | 6.5      | 6.6      | 6.6     | 6.6      | 6.6      |
|                  | Chlorine, g/kg | 3.12     | 18.8    | 0.64    | 0.38      | 3.12     | 3.12     | 3.11    | 3.12     | 3.12     |
|                  | Sodium, g/kg | 2.0      | 2.0     | 2.0     | 2.6       | 2.80     | 3.60     | 4.41    | 2.51     | 3.62     | 4.43

CP: crude protein; ME, metabolizable energy. °Provided the following per kg of diet: vitamin A, 12,000 IU; vitamin D3, 7200 IU; vitamin E, 20 IU; vitamin B12, 1.5 mg; vitamin B6, 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, 65 mg; Fe, 50 mg; Se, 0.1 mg.
Yoshie et al. (1994) and Al-Harthi and El-Deek (2011, 2012). However, autoclaving significantly improved laying performance in hens fed dried whole eggs than boiling and/or freezing (Al-Harthi et al., 2010, 2011). These discrepancies of the effect of preparing way on nutritive values of feedstuffs could be attributed to the nature of feedstuffs and its anti-nutritional factors. Al-Harthi and El-Deek (2012) showed that preparing way such as boiling or autoclaving had a small effect on neutral detergent fibre (27.95-30.21%), acid detergent fibre (21.18-23.54%), hemicellulose (6.41-7.73%) and tannins (0.733-0.815 mg/gm protein) of BMA. Thus, the positive effect of autoclaving could be attributed to its effect on microorganisms of feed.

As noted, no significant negative impact on the productive performance of pullets during the pre-laying and laying periods BMA was fed up to 6%. The lack of the effect of BMA concentration on most of the productive traits except, greatest egg mass at 2%, is in agreement with those reported by Venkataramanan et al. (1994) and Schaivone et al. (2007) with chickens when fed algae up to 10%. Survival rate ranged from 94 to 100% and differences among different experimental groups were not significant (not shown). This indicated that level of BMA up to 6% did not negatively affect on survival of pullets from 14 to 42 weeks of age. It is important to mention that algae have an antimicrobial activity which might positively affect animal health (Rizvi and Shameel, 2004; Abdel-Wahab et al., 2006; Abd El-Baky et al., 2008). In addition, the mineral contents of BMA used in this study were already reported by Al-Harthi and El-Deek (2012) whose results were Ca 0.126-0.144%, P 0.239-0.255%, Na 4.01-4.05%, Zn 215-345 ppm, Mn 216-270 ppm, I 12.72-13.51 ppm, Fe 11.2-11.5 ppm and Cu 4.6-4.81 ppm. In addition, the heavy metals of BMA are in acceptable range (Pb 0.001 to 0.002 ppm), while no Cd was detected in the samples. The low level of heavy metals in the BMA samples indicated that BMA could be used safely in laying hen diets without deleterious effects on laying hen health and quality of eggs for human consumption.

**Quality of fresh and stored eggs**

Generally, there was no significant negative effect of preparing ways, levels of BMA and their interactions on quality of fresh (Table 5) and stored eggs (Table 6) and mineral content in eggshell (Table 7). Furthermore, a significant (P≤0.01) increase in yolk colour (Table 5) and shell Ca (Table 7) were observed when 2%, 4% and 6% BMA were included in pullets’ diets. This indicated a beneficial effect of BMA as a source of pigmentation (lutein and zeaxanthin) and mineral (Abd El-Baky et al., 2008; Al-Harthi and El-Deek, 2011).

**Table 3. Effect of different preparing ways and concentrations of brown marine algae on productive performance of pullets during weeks 14–42 of age.**

| Preparing way | Initial body weight, g | Final body weight, g | Body weight gain, g | Age at sexual maturity, d | Feed intake, g/h/d 14-20 wk of age |
|---------------|------------------------|----------------------|---------------------|--------------------------|-----------------------------------|
|               |                        |                      |                     |                          |                                   |
| SUNDRIED      | 1061                   | 1664                 | 603.3               | 141.5                    | 82.8                              |
| BOILED        | 1057                   | 1629                 | 571.9               | 140.2                    | 83.6                              |
| AUTOCLOAVED   | 1068                   | 1667                 | 599.7               | 141.7                    | 85.9                              |
| SEM           | 2.70                   | 22.2                 | 19.0                | 0.791                    | 1.07                              |
| Concentration of brown marine algae, % | | | | | |
| 0.0           | 1063                   | 1668                 | 604.6               | 142.5                    | 83.8                              |
| 2.0           | 1061                   | 1638                 | 577.4               | 140.2                    | 83.1                              |
| 4.0           | 1059                   | 1648                 | 588.6               | 141.5                    | 85.4                              |
| 6.0           | 1065                   | 1661                 | 596                 | 140.5                    | 84.1                              |
| SEM           | 6.58                   | 25.6                 | 21.9                | 0.913                    | 1.24                              |
| Interaction between preparing way and brown marine algae level | | | | | |
| PW×C          |                        |                      |                     |                          |                                   |
| CONTROL       | 1059                   | 1671                 | 612                 | 143                      | 82.5                              |
| 2             | 1069                   | 1656                 | 587                 | 139                      | 82.5                              |
| 4             | 1065                   | 1662                 | 607                 | 141                      | 82.2                              |
| 6             | 1060                   | 1667                 | 607                 | 141                      | 84.0                              |
| 2             | 1041                   | 1563                 | 523                 | 139                      | 83.7                              |
| BOILED        | 1068                   | 1628                 | 560                 | 141                      | 82.7                              |
| 4             | 1065                   | 1681                 | 617                 | 139                      | 84.2                              |
| 6             | 1072                   | 1694                 | 622                 | 142                      | 83.0                              |
| 2             | 1054                   | 1653                 | 599                 | 142                      | 91.2                              |
| 6             | 1070                   | 1633                 | 564                 | 141                      | 84.0                              |
| SEM           | 11.4                   | 44.4                 | 38.0                | 1.58                     | 2.15                              |

P-value

| PW          | ns                      | ns                    | ns                   | ns                       | ns                                 |
| C           | ns                      | ns                    | ns                   | ns                       | ns                                 |
| PW×C        | ns                      | ns                    | ns                   | ns                       | ns                                 |

PW, preparing way; C, concentration. ns, not significant.
Table 4. Effect of different preparing ways and concentrations of brown marine algae on productive performance of pullets during weeks 21-42 of age.

| Preparing way | Hen daily laying rate, % | Egg weight, g | Egg mass | Feed intake, g/h/d | Feed conversion ratio, g feed/g egg |
|---------------|--------------------------|---------------|----------|-------------------|-----------------------------------|
| SEM           |                         |               |          |                   |                                   |
| Control       | 85.8                     | 58.9          | 49.7     | 129.4             | 2.572                             |
| 2%            | 87.9                     | 59.2          | 50.8     | 129.6             | 2.572                             |
| 6%            | 86.5                     | 58.9          | 49.7     | 129.4             | 2.572                             |
| SEM           | 1.48                     | 0.192         | 22.6     | 1.97              | 0.11                              |

Interaction between preparing way and brown marine algae level

| PW | C | 6%                      | 84.8                     | 59.1          | 50.12b        | 129.3                     | 2.572                             |
|----|---|-------------------------|--------------------------|---------------|---------------|--------------------------|-----------------------------------|
|    |   | 2%                      | 85.8                     | 58.8          | 50.45b       | 128.0                     | 2.536                             |
|    |   | 4%                      | 83.3                     | 58.8          | 48.99b       | 127.9                     | 2.619                             |
|    |   | 6%                      | 83.2                     | 59.1          | 49.17bc      | 128.1                     | 2.605                             |
| SEM|   | 1.48                     | 0.192                     | 22.6           | 1.97          | 0.11                     |

P values

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

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

Reproductive and inner organs

Different concentrations of BMA prepared by different ways showed no significant effect on the reproductive (Table 8) and inner organs (Table 9) of pullets at 42 weeks of age except for number of follicles, which were significantly (P<0.05) greater in pullets of the SBMA than those of the ABMA. In addition, pullets of 2% and 4% BMA indicated greater number of follicles than those of the control diet. Liver percentage was significantly greater in pullets of the ABMA than those of the SBMA. Also, gizzard percentage was significantly heavier on pullets fed 2% and 6% of BMA than those fed 0% and 4% of BMA. However, no effect was observed on the intestinal measurements (Table 9), and no adverse effects on nutrient utilization was noted (Table 4). Similar results were reported by Al-Harthi et al. (2011). However, in literature lower gizzard percent was associated with lower feed digestibility and thus less nutrient availabilities (Hetland et al., 2003; Biggs and Parsons, 2009).

Conclusions

Sundried, boiled or autoclaved brown marine algae up to 6% could be included in the pullets’ diets from 14 to 42 weeks of age without adverse impacts on productive performance, sexual maturity, fresh and stored eggs quality, reproductive and inner organs. Furthermore, yolk colour and Ca content in eggs were improved.
### Table 5. Effect of different preparing ways and concentrations of brown marine algae on quality of fresh eggs.

| Preparing way | Haugh unit score | Yolk index | Yolk colour | Shape index | Shell thickness, mm | Shell weight, % | Shell dry matter, % |
|---------------|-----------------|------------|-------------|-------------|---------------------|----------------|---------------------|
| Sundried      | 87.2            | 46.7       | 9.02        | 0.762       | 0.478               | 13.9           | 75.8                |
| Boiled        | 85.9            | 46.8       | 9.34        | 0.757       | 0.497               | 13.7           | 73.4                |
| Autoclaved    | 86.7            | 46.9       | 9.52        | 0.577       | 0.563               | 14.1           | 72.0                |
| SEM           | 1.11            | 4.47       | 0.166       | 0.004       | 0.01               | 0.151          | 0.863               |

**Concentration of brown marine algae, %**

| SEM | P value | Interaction between preparing way and brown marine algae level |
|-----|---------|--------------------------------------------------------------|
| 0.0 | 88.4    | ns                                                          |
|     | 88.0    | ns                                                          |
|     | 87.5    | ns                                                          |
|     | 86.5    | ns                                                          |
| 2.0 | 88.4    | ns                                                          |
| 6.0 | 87.5    | ns                                                          |
|     | 88.5    | ns                                                          |

- SEM: Standard error of means
- PW: Preparing way
- C: Concentration
- P value: Probability value
- ns: Not significant
- *: Means in a column under similar treatment not sharing the same superscript are significantly different at P<0.05.

### Table 6. Effect of different preparing ways and concentrations of brown marine algae on quality of stored eggs.

| Preparing way | Yolk index | Egg loss, % | Haugh unit score | Yolk colour | Shape index | Shell thickness, mm | Shell weight, % |
|---------------|------------|-------------|-----------------|-------------|-------------|---------------------|----------------|
| Sundried      | 57.1       | 3.61        | 56.2            | 7.31        | 0.430       | 12.9                |
| Boiled        | 57.6       | 3.92        | 58.9            | 7.82        | 0.429       | 13.1                |
| Autoclaved    | 59.4       | 3.73        | 58.5            | 8.07        | 0.427       | 12.8                |
| SEM           | 0.720      | 0.02        | 2.75            | 0.221       | 0.006       | 0.132               |

**Concentration of brown marine algae, %**

| SEM | P value | Interaction between preparing way and brown marine algae level |
|-----|---------|--------------------------------------------------------------|
| 0.0 | 59.2    | ns                                                          |
|     | 57.6    | ns                                                          |
|     | 57.5    | ns                                                          |
|     | 57.1    | ns                                                          |
| 2.0 | 56.4    | ns                                                          |
| 4.0 | 55.4    | ns                                                          |
| 6.0 | 57.5    | ns                                                          |
|     | 59.0    | ns                                                          |
|     | 57.9    | ns                                                          |

- SEM: Standard error of means
- PW: Preparing way
- C: Concentration
- P value: Probability value
- ns: Not significant
- *: Means in a column under similar treatment not sharing the same superscript are significantly different at P<0.05.
Table 7. Effect of different preparing ways and concentrations of brown marine algae on mineral contents of fresh eggshell.

| Preparing way | Calcium | Eggshell mineral contents, % | Phosphorus | Sodium | Potassium |
|---------------|---------|-------------------------------|------------|--------|-----------|
| Sun-dried     | 78.2    | 0.017                         | 11.1       | 3.00   |
| Boiled        | 78.7    | 0.015                         | 11.4       | 3.25   |
| Autoclaved    | 77.7    | 0.015                         | 11.7       | 3.00   |
| SEM           | 1.05    | 0.001                         | 0.338      | 0.102  |

Concentration of brown marine algae, %

| Concentration | PW, preparing way; C, concentration. a-bMeans in a column under similar treatment not sharing the same superscript are significantly different at P<0.05. ns, not significant. |
|---------------|--------------------------------------------------------------------------------------------------|
| 0.0           | 72.7                                          | 0.019                     | 11.5   | 3.17   |
| 2.0           | 80.7†                                         | 0.016                     | 11.5   | 3.17   |
| 4.0           | 79.7†                                         | 0.014                     | 10.8   | 3.00   |
| 6.0           | 80.0†                                         | 0.014                     | 11.8   | 3.00   |
| SEM           | 1.21                                          | 0.001                     | 0.391  | 0.118  |

Interaction between preparing way and brown marine algae level

| PW           | SEM | P value |
|--------------|-----|---------|
| Control      | 2.10| 0.003   |
| 0            | 75.0| ns      |
| 4            | 78.0| ns      |
| 6            | 81.0| ns      |
| Sun-dried    | 3.95| ns      |
| 2            | 80.0| 0.014   |
| 4            | 83.0| 0.018   |
| 6            | 81.0| 0.014   |
| 4            | 81.0| 0.011   |
| 6            | 81.0| 0.014   |
| 2            | 81.0| 0.014   |
| 4            | 79.0| 0.014   |
| 6            | 78.0| 0.014   |
| SEM          | 2.10| 0.003   |

P value

| PW | SEM | P value |
|----|-----|---------|
| C  | 0.002| ns      |
| PW×C| ns  | ns      |

Table 8. Effect of different preparing ways and concentrations of brown marine algae on reproductive organs.

| Preparing way | Oviduct length | Oviduct weight | Ovary weight | Bursa of Fabricius | Thyroid weight | Adrenal of follicles |
|---------------|----------------|----------------|--------------|--------------------|----------------|----------------------|
| Sun-dried     | 3.95           | 0.785           | 0.098        | 0.057              | 0.082          | 6.48*                |
| Boiled        | 4.07           | 0.801           | 0.099        | 0.060              | 0.041          | 6.16**               |
| Autoclaved    | 4.11           | 0.815           | 0.337        | 0.057              | 0.041          | 5.63*                |
| SEM           | 0.180          | 0.017           | 0.096        | 0.002              | 0.024          | 0.155                |

Concentration of brown marine algae, %

| Concentration | PW, preparing way; C, concentration. a-bMeans in a column under similar treatment not sharing the same superscript are significantly different at P<0.05. ns, not significant. |
|---------------|--------------------------------------------------------------------------------------------------|
| 0.0           | 72.7                                          | 0.019                     | 11.5   | 3.17   |
| 2.0           | 80.7†                                         | 0.016                     | 11.5   | 3.17   |
| 4.0           | 79.7†                                         | 0.014                     | 10.8   | 3.00   |
| 6.0           | 80.0†                                         | 0.014                     | 11.8   | 3.00   |
| SEM           | 1.21                                          | 0.001                     | 0.391  | 0.118  |

Interaction between preparing way and brown marine algae level

| PW | SEM | P value |
|----|-----|---------|
| Control | 2.10| 0.003   |
| 0   | 75.0| ns      |
| 2   | 78.0| ns      |
| 4   | 81.0| ns      |
| Sun-dried | 3.95| ns      |
| 2   | 80.0| 0.014   |
| 4   | 83.0| 0.018   |
| 6   | 81.0| 0.014   |
| 4   | 81.0| 0.011   |
| 6   | 81.0| 0.014   |
| 2   | 81.0| 0.014   |
| 4   | 79.0| 0.014   |
| 6   | 78.0| 0.014   |
| SEM | 2.10| 0.003   |

P value

| PW | SEM | P value |
|----|-----|---------|
| C  | 0.002| ns      |
| PW×C| ns  | ns      |

PW, preparing way; C, concentration. **Means in a column under similar treatment not sharing the same superscript are significantly different at P<0.05; ns, not significant.
### Table 9. Effect of different preparing ways and concentrations of brown marine algae on inner organs.

| Preparing way | Liver, % | Gizzard, % | Heart, % | Spleen, % | Pancreas, % | Intestinal weight,% | Intestinal length,% |
|---------------|----------|------------|----------|-----------|-------------|----------------------|---------------------|
| SEM           |          |            |          |           |             |                      |                     |
| control       | 0.105    | 0.040      | 0.024    | 0.008     | 0.026       | 0.186                | 6.04                |
| sundried      | 0.121    | 0.046      | 0.027    | 0.009     | 0.030       | 0.214                | 6.97                |
| boiled        | 0.105    | 0.040      | 0.024    | 0.008     | 0.026       | 0.186                | 6.04                |
| autoclaved    | 0.121    | 0.046      | 0.027    | 0.009     | 0.030       | 0.214                | 6.97                |

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

**References**

Abd El-Baky, H.H., El Baz, F.K, Baroty, G.S.E., 2008. Evaluation of marine alga Ulva lactuca L. as a source of natural preservative ingredient. American-Eurasian J. Agric. Environ. Sci. 3:434-444.

Abdel-Wahab, M.A., Ahmed, H.H., Hagazi, M.M., 2006. Prevention of aflatoxin B1 initiated hepatotoxicity in rat by marine algae extracts. J. Appl. Toxicol. 26:229-238.

Al-Harthi, M.A., El-Deek, A.A., 2011. The effects of preparing methods and enzyme supplementation on the utilization of brown marine algae (Sargassum dentifebium) meal in the diet of laying hens. Ital. J. Anim. Sci. 10:48.

Al-Harthi, M.A., El-Deek, A.A., 2012. Nutrient profiles of brown marine algae (Sargassum dentifebium) as affected by different processing methods for chicken. J. Food Agric. Environ. 10:475-480.

Al-Harthi, M.A., El-Deek, A.A., Attia, Y.A., 2010. Utilization of dried whole eggs processed by different methods with or without growth promoting mixture on performance and body weight of laying hens. Int. J. Poult. Sci. 9:511-520.

Al-Harthi, M.A., El-Deek, A.A., Attia, Y.A., 2011. Impacts of dried whole eggs on productive performance, quality of fresh and stored eggs, reproductive organs and lipid metabolism of laying hens. Brit. Poult. Sci. 52:333-344.

Al-Harthi, M.A., El-Deek, A.A., Attia, Y.A., Bovera, F., Qota, E.M., 2009. Effect of different dietary levels of mangrove (Laguncularia racemosa) leaves and spices supplementations on productive performance, egg quality, lipids metabolism and metabolic profiles in laying hens. Brit. Poult. Sci. 50:700-708.

Alsaif, A.A., Attia, Y.A., Mahmoud, M.B., Zewell, H.S., Bovera, F., 2012. Productive and reproductive performance and egg quality of laying hens fed diets containing different levels of date pits with enzyme supplementations. Trop. Anim. Health Prod. 45:327-334.

AOAC, 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Attia, Y.A., Abd El-Hamid, A.E., Ellakany, H.F., Bovera, F., Al-Harthi, M.A., Ghazaly, S.A., 2013. Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. Ital. J. Anim. Sci. 12:e37.

Attia, Y.A., Al-Harthi, M.A., El-Deek, A.A., 2003. Nutritive value of unde hull sunflower meal as affected by multienzymes supplementation to broiler diets. Arch. Geflügelk. 67:97-106.

Attia, Y.A., Burke, W.H., Yamani, K.A., Jensen, L.S., 1994. Response of broiler breeder hens to forced molting by hormonal and dietary manipulations. Poult. Sci. 73:245-268.

Attia, Y.A., Tag El-Din, A.E., Zeweil, H.S., Hussein, A.H., Afatef, M.A., 2008. The effect of supplementation of enzyme on laying and reproductive performance in Japanese Quail hens fed nigella seed meal. J. Poultry Sci. 45:110-115.

Becker, W., 2004. Microalgae in human and animal nutrition. In: A. Richmond (ed.) Handbook of microalgal culture. Blackwell.
Brown marine algae in pre- and laying hens

Biggs, P., Parsons, C.M., 2009. The effects of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. Poultry Sci. 88:1893-1905.

Davis, A., Dinan, F.J., Lobbett, E.J., Chazin, J.D., Tufts, L.E., 1964. Phosphorus determination by Flame photometry. Anal. Chem. 36:1066-1068.

Duncan, D.B., 1955. Multiple range and multiple test. Biometrics 11:1-42.

Hetland, H., Svibus, B., Kroghdahl, A., 2003. Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Brit. Poultry Sci. 44:275-282.

National Research Council, 1994. Nutrient Requirements of Poultry, 9th rev. ed. National Academy Press, Washington, DC, USA.

Rimber, I.I., 2007. Why seaweeds. Degree Diss., Sam Ratulangi University, Manado, Indonesia.

Rizvi, M.A., Shameel, S., 2004. Studies on the bioactivity and elementology of marine algae from the coast of Karachi, Pakistan. Phytotherapy Res. 18:865-872.

SAS, 2001. User's guide: Statistics, 10th ed. SAS Institute Inc., Cary, NC, USA.

Schiavone, A., Chiarini, R., Marzoni, M., Castillo, A., Tassone, S., Romboli, I., 2007. Breast meat traits of Muscovy ducks fed on a microalgae Cryptophycodinium cohnii meal supplemented diet. Brit. Poultry Sci. 48:573-579.

Venkataraman, L.V., Somasekaran, T., Becker, E.W., 1994. Replacement value of blue green algae Spirulina platensis for fish meal and a vitamin-mineral premix for broiler chicks. Brit. Poultry Sci. 35:373-381.

Yamaguchi, K., 1997. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. J. Appl. Phycol. 8:487-502.

Yoshie, Y., Suzuki, T., Shirai, T., Hirano, T., 1994. Changes in the contents of dietary fibers, minerals, free amino acids, and fatty acids during processing of dry Nori. Nippon Suisan Gakk. 60:117-123.