The effects of preparing methods and enzyme supplementation on the utilization of brown marine algae (Sargassum dentiferium) meal in the diet of laying hens

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Abstract

Brown marine algae (BMA; Sargassum dentiferium) were collected from Jeddah on the shores of the Red Sea and sun dried at an average daily temperature of 40°C until constant weight was obtained. Part of the sun dried brown marine algae was subsequently processed by boiling (BBMA; boiled brown marine algae) in water and by autoclaving (ABMA; autoclaved brown marine algae). The SBMA, BBMA and ABMA were included in laying hen diet during weeks 23-42 of age at concentrations of 0.0%, 3.0% and 6.0%. The diets were given with or without enzyme supplementation. This resulted in 3 (preparation methods) × 2 (concentrations of supplemented BMA, i.e. 3 and 6%) × 2 (with and without enzyme supplementation) diet programs plus two control groups (with and without enzyme supplementation) for a total of 14 treatments. Each treatment was represented by six replicates of five hens each. Sun dried or autoclaved brown marine algae at 3% without enzyme supplementation in the laying hen diet could be fed to laying hens without any adverse effect on laying performance. However, enzyme supplementation to a diet containing 6% autoclaved brown marine algae improved productive performance and eggshell quality.

Introduction

Algae are valuable sources of food and trace elements. They are considered the most important food supplement of the 21st century as a source of proteins, lipids, polysaccharides, minerals, vitamins, and enzymes (Rimber, 2007). Brown marine algae (BMA; Sargassum dentiferium) has a significant activity against bacteria (Rizvi and Shamee, 2004). Abdel-Wahab et al. (2006) pointed out that the extract of some types of marine algae in the Red Sea, such as Laurencia obtusa and Caulerpa prolifera, have an obvious effect against fungal toxins AFB1 which are responsible for initiating and promoting the emergence of cancer in the liver. Becker (2004) stated that 30% of the current production of algae in the world is sold for use in animal feed. Moreover, Yamaguchi (1997) reported that 50% of current production of algae Arthrospira is used as a feed additive.

Algae could have unlimited use in dried food and feed (Becker, 2004). Mohd et al. (2000) postulated that G. changii contained a high composition of unsaturated fatty acids (74%), mainly omega 3 fatty acids and 26% of saturated fatty acids (mainly palmitic acid) and also had relatively high concentrations of calcium and iron. David (2001) and Becker (2004) reported that algae are a good source of fat and water soluble vitamins and pigments, such as chlorophyll. Sim et al. (2004) found that Ecklonia acva Kjellman (EC) of brown algae, which are aerobically dried, contained 10.5% protein, 0.73% fat, 36.4% fibre, 27.2% mineral salts and 10.6% sodium chloride. The average energy value was 1849 kcal/kg while the average value of the 13 amino acids was approximately 32%.

In poultry, 5-10% algae can be safely given in the diet but the use of algae in high concentrations for long periods produces adverse effects (El-Deek et al., 1985). Algae can improve pigmentation of poultry products (Becker, 2004) and could provide an alternative to the traditional sources (fish oils) of n-3 fatty acids in the broiler diet (Schiaiwone et al., 2007).

Preparation methods such as autoclaving, boiling in water and freezing could improve the nutritive value of algae by affecting nutrient availability. Indeed, autoclaving resulted in superior nutritive value and chicken performance (Yoshie et al., 1994; El-Deek and Al-Harthi, 2009; Al-Harthi et al., 2010; 2011).

Nowadays, enzymes are employed to improve the use of animal feeds and increase animal performance. These enzymes increase nutrient digestibility, improve nutrient utilization and reduce nutrient wastes, and limit antinutritional factors (Attia and Abd El-Rahman, 2001; Attia et al., 2003; 2008; Madrid et al., 2010; Flores-Cervantes et al., 2011). The aim of this study was to evaluate different methods of preparation and enzyme supplementation in the use of brown algae meal as a feed resource in the diet of laying hens, and their effects on productive performance, egg and shell quality, and reproductive organs.

Materials and methods

Brown marine algae source

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

Gathering and preparation of brown marine algae

Algae were harvested, transported to the Hada Al-Sham Research Station, and exposed to the sun up to 40°C during most of the day with continuous stirring until dry. They were then crushed to dry powder, sieved in a special container in order to reach the appropriate size for feeding (approximately 0.5 mm) and then stored in dark bags until use in diet formulation.
Preparation of treatments
A part of the sundried brown marine algae was taken and boiled in a cooking unit using indirect methods (1:4; algae:water, w/w) for 20 min while stirring (boiled brown marine algae; BBMA), transferred directly to drying trays, and continuously stirred for 36 h in the drying unit. The dried algae were then ground again and then sieved. Another part of the sundried brown marine algae (SBMA) was processed by autoclaving (ABMA, autoclaved brown marine algae) at 115 bar/15° C for 15 min.

Multienzyme complex

The multienzyme complex used in the experimental diets consisted of two multienzyme complexes: Xylem 500 and Amcozyme 2x enzyme. Xylem 500 (extracted from the Baccilus subtilis bacteria; components are X-amylose 8000 U/gm and 1,4 B-xylanase 1260 U/gm) and Amcozyme 2x enzyme (components include: Amylase 2,500,000 U/gm, Protease 2,000,000 U/gm, Lipase 150,000 U/gm, Beta-glucanase 30,000 U/gm, Xylanase 500,000 U/gm, Cellulase 15,000 U/gm, Phytase and X-glucocidase) were used in the ratio 1:1 and supplemented at 1 g/kg feed to improve BMA utilization.

Birds and experimental design

The SBMA, BBMA and AMBA were included in the Hy-line laying hen diets formulated according to NRC (1994) nutrient recommendation for laying hens (Table 1) during weeks 23-42 of age at 3% and 6%. The diets were given with or without enzyme supplementation. These combinations resulted in 3 (preparation methods) × 2 (3% and 6% levels) × 2 (with and without multienzyme complex) feeding programs. An additional 2 control diets without algae inclusion with or without enzyme supplementation completed the 14 treatments. Thus, a total number of 420, 23-week old Hy-line laying hens were distributed randomly among the 14 treatments (30 birds in each group) of 6 replicates of five hens for each group. Hens were housed in individual cages (space available for each hen 320 cm²) in an environmentally controlled chicken house. Feed and water were provided ad libitum throughout the experimental period. Vaccination and medical programs were carried out according to age under supervision of a veterinary surgeon. Laying hens were kept in a controlled environment with 14:10 h light-dark cycle.

Table 1. Composition of the experimental diets containing different levels of brown marine algae prepared by different methods.

| Ingredients, g/kg | Control | Sundried 3% | Sundried 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% crude protein | 231.8   | 233.2       | 233.0       | 233.0     | 232.5     | 233.1        | 232.8        |
| Wheat bran        | 8.30    | 18.7        | 52.7        | 18.8      | 55.3      | 18.8         | 54.0         |
| Palm oil          | 1.1     | 14.5        | 38.4        | 14.4      | 39.2      | 14.5         | 35.9         |
| 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      | 94.8      | 94.6         | 94.8         |
| Sodium chloride, %| 4.5     | 1.4         | 0.0         | 1.4       | 0.0       | 1.4          | 0.0          |
| Vitamin + mineral premix° | 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          | 1.5          |
| Choline Cl70      | 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 and determined analysis

| Metabolizable energy, MJ/kg | 11.30 | 11.30 | 11.30 | 11.30 | 11.30 | 11.30 | 11.30 |
| Dry matter, g/kg           | 909   | 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  | 14.14 | 14.7 |
| Methionine, 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             | 43.0  | 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  | 0.124 | 0.38  |
| Sodium, g/kg              | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   |

°Provided the following per kg of diet: vitamin A, 12,000 U; vitamin D3, 7200 CU; vitamin E, 20 U; vitamin B1, 2.5 mg; vitamin B2, 5 g; 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.
treatment were collected for determination of egg and shell quality (Attia et al., 1995). Eggs were divided into two sets of 12 eggs each. One set was used to determine the quality of fresh eggs (on the same day of laying) while the other set (12 eggs) was stored in a refrigerator at 5°C for 21 days after which the eggs were broken to evaluate egg quality. In addition, 8 fresh shells per treatment were separated and dried to determine Ca, P, Na and K using an automatic absorption spectrophotometer and to carry out chemical analyses of the diet (AOAC, 1985). The mineral contents of the egg shells were expressed as a percentage of shell ash contents.

Statistical analysis
Data were analyzed using the GLM procedure of SAS® (SAS, 2001) using factorial analyses (3×2×2) plus the 2 control groups (with or without multienzyme complex) and type of eggs for egg quality data. Before analysis, all percentages were subjected to logarithmic transformation (log10 x + 1) to normalize data distribution. Mean difference at P≤0.05 was tested using Duncan’s New Multiple Range Test (Duncan, 1955).

Table 2. Effect of different preparation methods, concentration of brown marine algae and enzyme supplementation on productive performance of laying hens during weeks 23–42 of age.

| Dietary treatments | Laying rate, %/hen/day | Egg weight, g | Egg mass, g | Feed intake, g/hen | FCR | BWG | Survival rate, % |
|--------------------|------------------------|---------------|-------------|-------------------|-----|-----|------------------|
| **Preparation methods** |                        |               |             |                   |     |     |                  |
| Autoclaved          | 62.0                   | 60.5          | 5245        | 111.5             | 2.98| 299a | 99.0             |
| Boiled              | 61.6                   | 60.5          | 5208        | 111.0             | 2.98| 297a | 99.0             |
| Sundried            | 62.0                   | 60.5          | 5245        | 111.5             | 2.98| 299a | 99.0             |
| Concentration of brown marine algae, % |                        |               |             |                   |     |     |                  |
| 0.0                 | 62.7a                  | 61.2a         | 5365a        | 116.0a            | 3.02| 329a | 100              |
| 3.0                 | 62.9a                  | 60.7a         | 5340a        | 112.0a            | 2.93| 326a | 99.3             |
| 6.0                 | 60.4a                  | 60.6a         | 5124a        | 115.0a            | 3.15| 316a | 99.3             |
| Multienzyme complex effect |                          |               |             |                   |     |     |                  |
| Without (-)         | 61.4a                  | 60.8          | 5215a        | 113.0             | 3.03| 348a | 99.6             |
| With (+)            | 62.6a                  | 60.9          | 5338a        | 116.0             | 3.04| 300a | 99.6             |

Interaction between preparation method, brown marine algae level and multienzyme complex addition.

| PM | C | E | SEM |
|----|---|---|-----|
| Control | 0 | - | 62.3 | 60.8 | 5289 | 117 | 3.10 | 328 | 100 |
| 0 | + | 63.0 | 61.6 | 5441 | 115 | 2.95 | 329 | 100 |
| 3 | - | 62.8 | 60.7 | 5325 | 114 | 2.99 | 302 | 100 |
| Sundried | 3 | + | 59.5 | 59.8 | 4964 | 110 | 3.10 | 303 | 96 |
| 6 | - | 62.5 | 61.4 | 5375 | 107 | 2.78 | 188 | 100 |
| 6 | + | 61.5 | 60.1 | 5166 | 112 | 3.04 | 355 | 100 |
| 3 | - | 61.2 | 59.9 | 5142 | 117 | 3.19 | 330 | 96 |
| Boiled | 3 | + | 57.5 | 60.8 | 4889 | 114 | 3.25 | 366 | 100 |
| 6 | - | 58.2 | 58.8 | 4761 | 120 | 3.53 | 313 | 100 |
| 6 | + | 58.7 | 62.1 | 5095 | 120 | 3.30 | 398 | 100 |
| Autoclaved | 3 | + | 65.1 | 61.5 | 5591 | 117 | 2.93 | 276 | 100 |
| 6 | - | 64.4 | 61.3 | 5522 | 121 | 3.16 | 419 | 100 |
| 6 | + | 66.7 | 60.1 | 5606 | 118 | 2.95 | 233 | 100 |

Analysis of variance

| PM | C | E | SEM |
|----|---|---|-----|
| C | 0.01 | 0.05 | 0.05 | 0.05 | 0.05 | 0.01 | ns |
| E | 0.05 | 0.01 | 0.01 | ns | 0.01 | 0.01 | ns |
| PM×C | 0.05 | 0.05 | 0.01 | 0.05 | 0.01 | 0.01 | ns |
| PM×E | 0.01 | ns | ns | 0.05 | ns | 0.01 | ns |
| C×E | 0.01 | ns | ns | 0.05 | ns | 0.01 | ns |
| PM×C×E | 0.05 | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | ns |

*Means in a column under similar treatment not sharing the same superscript are significantly different at (P<0.05); PM, preparation method; C, concentration; E, enzymes; ns, not significant.
performance indicated that the sun drying approach was an adequate method for preparing BMA for the diet of laying hens but body weight was lower. Similar results were reported by Yoshie et al. (1994). On the other hand, Al-Harthi et al. (2010; 2011) found that autoclaving dried whole eggs significantly increased laying performance compared to boiling and/or freezing. The differences among the abovementioned results in response to preparation methods are those expected on the basis of feedstuffs and their limiting factors. El-Deek and Al-Harthi (2009) showed that preparation methods 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.

Level of BMA had a significant effect on most productive traits, except for survival rate, with a significant 3% improvement in FCR, although increasing BMA to 6% had a negative affect on most of the productive traits and BWG of laying hens. Venkataraman et al. (1994) found that FCR and productive performance in general were not affected by the addition of dried Spirulina algae powdered to broiler diet. Moreover, 5 g algae/kg feed did not affect the performance of the Muscovy ducks (Schiavone et al., 2007). Furthermore, up to 10% sea grass in the diet of broiler, quail, ducks and laying hens did not affect feed intake or FCR (El-Deek et al., 1985; 2009). These differences in the recommended level of algae in poultry diets could be attributed to different species of algae and their tannin contents and/or bird species.

The reduction in productive performance traits of hens fed 6% BMA meal could be attrib-

| Preparation methods   | Haugh unit | Albumen, % | Yolk, % | Yolk index | Yolk color | Shape index |
|-----------------------|------------|------------|---------|------------|------------|-------------|
| Sun dried             | 84.5       | 61.2       | 25.6    | 43.2       | 6.81       | 75.4        |
| Boiled                | 84.3       | 61.2       | 25.4    | 43.4       | 7.01       | 75.7        |
| Autoclaved            | 83.7       | 61.1       | 25.3    | 43.2       | 6.94       | 75.2        |

| Concentration of brown marine algae, % | Haugh unit | Albumen, % | Yolk, % | Yolk index | Yolk color | Shape index |
|---------------------------------------|------------|------------|---------|------------|------------|-------------|
| 0.0                                   | 82.6       | 61.0       | 25.1    | 44.8       | 6.17       | 75.5        |
| 3.0                                   | 83.4       | 60.8       | 25.6    | 45.4       | 6.97       | 75.3        |
| 6.0                                   | 83.8       | 61.0       | 25.6    | 45.1       | 7.12       | 75.2        |

| Multienzyme complex effect | Haugh unit | Albumen, % | Yolk, % | Yolk index | Yolk color | Shape index |
|----------------------------|------------|------------|---------|------------|------------|-------------|
| Without (-)               | 84.0       | 60.7       | 25.5    | 45.4       | 6.83       | 75.6        |
| With (+)                  | 82.8       | 61.2       | 25.4    | 44.8       | 6.88       | 75.1        |

| Egg type | Haugh unit | Albumen, % | Yolk, % | Yolk index | Yolk color | Shape index |
|----------|------------|------------|---------|------------|------------|-------------|
| Fresh    | 83.3a      | 60.9b      | 25.4b   | 45.1b      | 6.75       | 75.3b       |
| Stored   | 56.1b      | 66.3a      | 26.3a   | 53.1a      | 6.82       | 76.3a       |

Analysis of variance

| PM     | C   | E   | ns    | ns   | ns    | ns    | ns    | ns    |
|--------|-----|-----|-------|------|-------|-------|-------|-------|
| Control| 0   | -   | 83.3  | 60.8 | 25.2  | 44.3  | 6.56  | 75.9  |
|         | 0+  | +   | 82.0  | 61.2 | 25.0  | 45.2  | 5.79  | 75.0  |
| Sun dried| 3   | -   | 83.5  | 60.5 | 25.9  | 42.9  | 6.40  | 76.0  |
|         | 3+  | +   | 83.7  | 62.0 | 25.4  | 43.5  | 6.92  | 75.4  |
|         | 6   | -   | 85.5  | 61.3 | 25.7  | 43.6  | 6.96  | 74.6  |
|         | 6+  | +   | 85.3  | 60.9 | 25.3  | 42.7  | 6.96  | 75.6  |
| Boiled  | 3   | -   | 84.8  | 60.8 | 25.4  | 47.5  | 6.92  | 75.2  |
|         | 3+  | +   | 81.8  | 60.4 | 25.4  | 46.4  | 7.00  | 75.0  |
|         | 6   | -   | 83.9  | 60.6 | 25.6  | 47.6  | 7.32  | 74.6  |
|         | 6+  | +   | 82.0  | 61.9 | 25.7  | 45.1  | 7.08  | 74.6  |
| Autoclaved| 3   | -   | 83.9  | 60.5 | 25.3  | 47.2  | 6.84  | 74.8  |
|         | 3+  | +   | 82.8  | 60.7 | 26.1  | 44.8  | 7.72  | 75.4  |
|         | 6   | -   | 84.4  | 60.4 | 25.6  | 46.8  | 7.32  | 74.4  |
|         | 6+  | +   | 82.0  | 60.9 | 25.7  | 45.1  | 7.08  | 74.6  |

SEM: 3.45 2.52 1.84 3.56 1.21 4.31

Means in a column under similar treatment not sharing the same superscript are significantly different at (P<0.05); PM, preparation method; C, concentration; E, enzymes; ns, not significant.
uted to higher Na intake as well as tannins, which was shown to adversely affect protein/amino acid digestibility and Ca and P availability (Scott et al., 1982; Lesson and Summers, 1997; Balnave and Zhang; 1993; 1998; Attia, 1998; El-Deek and Al-Harthi, 2009).

Multienzyme complex supplementation significantly increased laying rate (+1.95%) and egg mass (+2.4%) but reduced BWG (-13.3%) and had no significant effect on the other laying performance traits. These findings showed that the multienzyme complex improved nutrient availability for egg formation and played a role in nutrition repartitioning between egg formation and BWG (Attia et al., 2008). These results are in agreement with those reported by Madrid et al. (2010) who found that multienzyme complex significantly improved nutrient digestibility in broilers under different rearing conditions. On the other hand, Flores-Cervantes et al. (2011) showed that multienzymes had a minor effect on sorghum based diet for laying hens. This contradiction in responses to multienzyme complex supplementation could be attributed to type and age of birds, type of diets and dietary composition (Attia et al., 2003; 2008).

A significant interaction between preparation method and level of BMA or multienzyme complex supplementation was observed in laying rate, feed intake and BWG. The interaction between preparation method, level of BMA and multienzyme complex supplementation was significant (P<0.05) for most of the productive traits, except for survival rate. The results indicated that the highest laying rate was obtained with hens fed 6% ABMA supplemented with multienzyme complex and the lowest laying rate was found with hens fed 3% BBMA supplemented with multienzyme complex; there was a 16% difference between these groups. The highest and the lowest egg weights were observed with hens fed 6% BBMA with or without multienzyme complex supplementation, with a difference of 5.6%, respectively. Combining the data from laying rate and egg size to obtain egg mass, it was clear that the highest egg mass was shown with hens fed 6% ABMA supplemented with multienzyme complex while the lowest egg mass was reported with hens fed 6% BBMA without multienzyme complex supplementation; the difference between these groups reached 17.7%. These results indicate that the effect of the multienzyme complex used in this study on laying performance depends on preparation method and dietary level of BMA, as the multienzyme complex resulted in a lower increase in egg mass in the control group (2.9%).

Feed intake was the greatest with hens fed 6% ABMA without multienzyme complex supplementation and the least feed intake was observed for hens fed 6% SBMA without multienzyme complex supplementation with a difference of 13.1%. The best FCR was recorded for hens fed 6% SBMA without multienzyme complex supplementation, while the worst was recorded for hens fed 6% BBMA without multienzyme complex supplementation; the difference between these groups was 21.2%. These results indicate that the sundried method of preparation is adequate for hens fed 6% BMA without enzyme supplementation.

It was observed that multienzyme complex supplementation had different effects on FCR of laying hens according to processing methods and level of BMA. For example, the effect of multienzyme complex on FCR of hens fed 6% BBMA or ABMA was 6.5 and 3.9%, respectively. Also, multienzyme complex improved FCR of the control group by 4.8%. However, multienzyme complex induced a negative effect (-3.4%) on FCR of the group fed 6% SBMA. These results indicated that multienzyme complex not only affected BMA but also the other dietary components of the diets, such as enzyme supplementation which markedly improved laying performance of the control group (Attia and Abd El-Rahman, 2001; Attia et al., 2003).

The biggest increase in BWG of laying hens was recorded for hens fed 3% ABMA supplemented with multienzyme complex in comparison to hens fed 6% SBMA without multienzyme complex supplementation. Survival rate ranged from 96 to 100% and differences among different experimental groups were not significant. This indicated that levels of BMA of up to 6% did not have a negative effect on survival of laying hens.

**Egg quality traits**

The data reported in Table 3 show that neither preparation methods, level of BMA or multienzyme complex supplementation and their interactions have any significant effect on egg quality traits. Stored eggs were of a significantly lower quality to that of fresh eggs. Similarly, El-Deek and Al-Harthi (2009) and Al-Harthi et al. (2011) found that preparation methods had a negligible effect on egg quality measurements; however, fresh eggs were of a better quality to stored eggs except for yolk color which was not affected by storage. The present results indicate that BMA had no harmful effect on quality of fresh and stored eggs in terms of Haugh unit score, yolk index, yolk color and percentage of albumen and yolk.

**Egg shell quality**

The data reported in Table 4 show that neither preparation methods, level of BMA or multienzyme complex supplementation and their interactions had any significant effect on eggshell quality or eggshell mineral content. This indicated that preparation methods did not affect mineral availability for egg shell formation. Similar findings were reported by El-Deek and Al-Harthi (2009) and Al-Harthi et al. (2011).

In spite of this, level of BMA had a significant effect on shell percentage and shell Ca and Na contents. Here data indicated that 3% BMA did not significantly affect shell percentage; in contrast, an increase in BMA to 6% significantly reduced shell percentage. The decrease in shell percentage coincided with lower productive performance traits of 6% BMA and a significant reduction in Ca when BMA was given. Inclusion of BMA at 6% resulted in a significant reduction in shell percentage compared to control. The decrease in Ca percentage and the increase in shell Na indicated a lower Ca availability with increasing BMA and the negative effect of Na on eggshell Ca deposition and, therefore, egg shell quality (Pourreza and Edriss, 1992). NaCl, ranging from 200 to 2,000 mg/L, increased the incidence of egg shell defects (Pourreza and Edriss, 1992). Feeding BMA at 6% in the present study increased Na by 276 mg above laying hen requirements: 150 mg/day for white and 110 mg/day for brown egg-producing hens (NRC, 1994). Similarly, Balnave and Zhang (1998) showed that increasing NaCl level significantly reduced laying performance and eggshell quality due to the decrease in activity of carbonic anhydrase in the shell gland (Yoselewitz and Balnave, 1989; Balnave and Zhang, 1993). This influence could be explained by the fact that an excess of chloride ions inhibits the carbonic anhydrase activity (Dionisio-Sese and Miyachi, 1992) which limits the supply of bicarbonate ions to the lumen of the shell gland and, therefore, the uptake of Ca to the shell gland (Chen and Balnave, 2001). Furthermore, an increase in BMA level was associated with an increase in tannin intake which was shown to adversely affect protein/amino acid digestibility and Ca and P availability (Scott et al., 1982; Leeson and Summers, 1997; Attia, 1998): This could, therefore, also contribute to the reduction in laying hen performance and egg shell quality of hens fed 6% BMA. It should be mentioned that tannin content of the present samples of BMA ranged from 0.733 to 0.815 mg/g protein with an overall mean of 0.774 mg/g protein (El-Deek and Al-Harthi, 2009).
Shell Ca and P percentage were significantly increased while shell Na and K were significantly reduced with multienzyme complex supplementation. Furthermore, shell Ca percentage was significantly affected by: i) the interaction between preparation method and level of BMA; ii) multienzyme complex supplementation and level of BMA; and iii) multienzyme complex supplementation, and the interaction between the three. The three way interaction also had significant effects on shell percentage, shell P, shell Na and shell K.

The highest shell percentage and Ca was observed with hens fed 3% BBMA supplemented with multienzyme complex and the lowest with hens fed 3% SBMA given without multienzyme complex. However, the lowest shell percentage was from hens fed 3% SBMA supplemented with multienzyme complex. The results indicate that multienzyme complex supplementation to either 3% SBMA or 3% BBMA tended to increase shell Na content while it induced the opposite trend when added to either 6% SBMA or 6% BBMA. On the other hand, multienzyme complex supplementation to 3% ABMA reduced shell Na and K and reduced shell K only in the group fed 6% ABMA and the control group, respectively. The effect of multienzyme complex was seen in eggshell quality of groups fed 6% SBMA and 3% BBMA, and these coincided with an increase in shell Ca percentage of these groups. This indicated that the effect of multienzyme complex supplementation on nutrient availability included an effect on minerals (Attia and Abd El-Rahman, 2001; Attia et al., 2003, 2008; Madrid et al., 2010).

**Inner organs**

The data shown in Table 5 show the effect of preparation method, level of BMA and multienzyme complex supplementation and their interaction on inner organs and the number of follicles of laying hens at week 42 of age. Preparation methods had a significant effect only on gizzard, ovary and pancreas percentages. Boiling significantly reduced gizzard percentage. On the other hand, ABMA signifi-

| Table 4. Effect of different preparation methods, concentration of brown marine algae and enzyme supplementation on eggshell quality criteria and mineral of eggshell during weeks 23-42 of age. |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Dietary treatments | Eggshell quality criteria | Eggshell mineral contents, % |
| | Thickness, µm | Dry matter, % | Calcium | Phosphorus | Sodium | Potassium |
| Preparations methods | | | | | |
| Sundried | | | | | |
| Boiled | | | | | |
| Autoclaved | | | | | |
| Concentration of brown marine algae, % | | | | |
| 0.0 | 427 | 13.8 | 75.5 | 86.2 | 0.018 | 10.8 | 3.67 |
| 3.0 | 439 | 13.6 | 75.1 | 84.7 | 0.019 | 10.8 | 3.83 |
| 6.0 | 426 | 13.4 | 77.2 | 84.3 | 0.018 | 11.3 | 3.50 |
| Multienzyme complex effect | | | | |
| Without (+) | 432 | 13.8 | 76.2 | 84.5 | 0.019 | 11.2 | 3.89 |
| With (-) | 429 | 13.4 | 77.0 | 85.8 | 0.019 | 10.9 | 3.44 |

Interaction between preparation method, brown marine algae level and multienzyme complex addition

| PM | C | E |
|---|---|---|
| Control | 0 - | 425 | 13.9 | 78.5 | 84.5 | 0.020 | 10.9 | 4.00 |
| 0 + | 430 | 13.7 | 76.5 | 87.9 | 0.020 | 10.7 | 3.33 |
| 3 - | 439 | 13.6 | 73.4 | 86.0 | 0.018 | 10.0 | 4.00 |
| Sundried | 3 + | 409 | 12.5 | 77.2 | 84.0 | 0.018 | 11.0 | 4.00 |
| 6 - | 414 | 13.0 | 80.4 | 82.0 | 0.018 | 12.0 | 4.00 |
| 6 + | 398 | 13.8 | 85.0 | 78.2 | 0.018 | 11.0 | 4.00 |
| 3 - | 464 | 13.8 | 75.6 | 79.0 | 0.018 | 11.0 | 4.00 |
| Boiled | 3 + | 454 | 14.2 | 76.0 | 87.0 | 0.025 | 12.0 | 4.00 |
| 6 - | 430 | 13.8 | 75.6 | 86.0 | 0.018 | 12.0 | 3.00 |
| 6 + | 418 | 12.4 | 79.8 | 83.0 | 0.018 | 11.0 | 3.00 |
| 3 - | 427 | 14.2 | 70.6 | 86.0 | 0.018 | 11.0 | 4.00 |
| Autoclaved | 3 + | 442 | 13.2 | 77.8 | 86.0 | 0.018 | 10.0 | 3.00 |
| 6 - | 439 | 14.0 | 74.6 | 86.0 | 0.018 | 11.0 | 4.00 |
| 6 + | 455 | 13.4 | 74.8 | 84.0 | 0.018 | 11.0 | 3.00 |

SEM | 3.08 | 1.0 | 6.4 | 3.5 | 0.003 | 0.98 | 0.09 |

Analysis of variance

| PM | C | E |
|---|---|---|
| ns | ns | ns |
| ns | 0.05 | ns |
| ns | ns | 0.05 |
| ns | ns | ns |
| ns | ns | ns |
| ns | ns | ns |

*Means in a column under similar treatment not sharing the same superscript are significantly different at (P<0.05); PM, preparation method; C, concentration; E, enzymes; ns, not significant.
cantly increased gizzard and pancreas percentages while reducing percentage of ovary weight in comparison with the other BMA levels.

It was quoted that feeding 6% BMA resulted in a decrease in intestinal and gizzard percentage and number of follicles which may reflect a lower nutrient availability for egg formation. It has been demonstrated that lower gizzard percentage was associated with lower feed digestibility and thus less nutrient availabilities (Hetland et al., 2003; Biggs and Parsons, 2009).

Multienzyme complex supplementation increased intestinal length while decreasing pancreatic weight and could, therefore, contribute to improving nutrient availability, and could explain the positive effect of multienzyme complex on laying rate, egg mass (Table 2), shell Ca and P, and lower shell Na and K (Table 4). The increase in intestinal length could be beneficial because this increases the absorptive capacity of the gut (Attia and Abd El-Rahman, 2001; Attia et al., 2003; 2008).

Multienzyme complex supplementation significantly reduced percentage of ovary weight but did not affect gizzard, ovudict or thyroid percentages or the number of follicles.

There was a significant interaction between preparation methods and level of BMA on percentages of most of the organs except the ovary. A significant interaction between preparation method and multienzyme complex supplementation was only observed with regard to pancreas percentage. A significant interaction between level of BMA and enzyme supplemen-

Table 5. Effect of different preparation methods, concentration of brown marine algae and enzyme supplementation on inner organs of 42-week old hens.

| Dietary treatment | Intestinal length | Gizzard weight | Ovary weight | Organs, % | Pancreas weight | Thyroid weight | N. of follicles |
|-------------------|-------------------|----------------|--------------|-----------|----------------|---------------|---------------|
|                   |                   |                |              |           |                |               |               |
| Preparation methods |                  |                |              |           |                |               |               |
| Sundried          | 146               | 2.00          | 0.918        | 4.29      | 0.345         | 0.074         | 6.94          |
| Boiled            | 143               | 1.85          | 0.925        | 4.23      | 0.365         | 0.077         | 7.00          |
| Autoclaved        | 146               | 1.99          | 0.888        | 4.22      | 0.375         | 0.077         | 7.13          |
| Concentration of brown marine algae, % |                  |                |              |           |                |               |               |
| 0.0               | 162              | 1.95          | 0.785        | 4.05      | 0.345         | 0.076         | 7.00          |
| 3.0               | 156              | 1.48          | 0.783        | 4.00      | 0.373         | 0.068         | 6.46          |
| 6.0               | 143              | 1.56          | 0.873        | 4.00      | 0.340         | 0.074         | 5.88          |
| Multienzyme complex effect |              |                |              |           |                |               |               |
| Without (+)       | 151             | 1.61          | 0.832        | 4.04      | 0.378         | 0.073         | 6.36          |
| With (-)          | 157             | 1.71          | 0.799        | 4.00      | 0.327         | 0.070         | 6.54          |

Interaction between preparation method, brown marine algae level and multienzyme complex addition

| PM | E   | C   | Interactions | SEM  |
|----|-----|-----|--------------|------|
| C  | 0.05| 0.05| ns           | 0.22 |
| E  | 0.01| 0.05| ns           | 0.22 |
| PM×C| 0.05| 0.05| ns           | 0.22 |
| PM×E| 0.05| 0.05| ns           | 0.22 |
| C×E| 0.01| 0.05| ns           | 0.22 |
| PM×C×E| 0.05| 0.05| ns           | 0.22 |

**Means in a column under similar treatment not sharing the same superscript are significantly different at (P<0.05); PM, preparation method; C, concentration; E, enzymes; ns, not significant.
tation was also observed in percentages of most of the organs except for oviduct percentage and in the number of follicles.

There was a significant three-way interaction between preparation method, BMA level and multienzyme complex supplementation on percentage of intestinal length, gizzard, ovary weight, pancreas weight and the number of follicles. The longest intestinal length was observed in hens fed 6% BBMA supplemented with multienzyme complex and the shortest with hens fed 6% BBMA supplemented with enzyme. The biggest gizzard percentage was observed with hens fed 3% BMA without enzyme addition and the smallest with hens fed 3% ABMA supplemented with enzyme. Feeding with 6% BBMA with or without enzyme supplementation resulted in the biggest ovary percentage while feeding 3% BBMA supplemented with enzyme had the smallest ovary percentage. It seems that multienzyme complex supplementation reduced hen pancreatic hypertrophy when supplemented to 6% but not in those fed sun-dried or boiled BMA at 6% in which an increase in pancreas percentage was seen. Percentage of thyroid weight was increased when enzyme was added to groups fed 6% BBMA or BBMA. On the other hand, percentage of thyroid decreased in the control group (12.3%) and in groups fed ABMA at 3% (25.4%) or 6% (5.5%) when multienzyme complex was supplemented. It seems that the effect of multienzyme complex on inner organs and the number of follicles depends on preparation methods and level of BMA, but some adaption responses were reflected in an increase in the traits of laying hens fed 6% BBMA or ABMA.

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Conclusions

Sun dried or autoclaved brown marine algae at 3% without enzyme supplementation in the laying hen diet could be fed to laying hens without adverse effects on laying performance. However, enzyme supplementation to a diet containing 6% autoclaved brown marine algae improved productive performance and eggshell quality.

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