

Nutritive Value of Treated Brown Marine Algae in Pullet and Laying Diets

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Summary

The study aim was to determine the chemical composition and the nutritive value of the treated (raw-boiling-autoclaved) dry meal of Brown Marine Algae (BMA) (*Sargassum spp.*) cultivated from red sea shore. BMA were fed to commercial hens in two trials. The effect of different levels 0, 1, 2, 3, 4,5 or 6% of treated BMA (- or +) of feed was included in pullets and consequently laying hens diets from 14-42 wk of age in the first trial. A factorial arrangement 7x3x2 was used resulting in a total of 42 experimental treatments, thus the total hens were 672. In the second trial 600 hens received levels of treated BMA 0,3,6,9 or 12 % without or with enzyme preparations from 23-42 wks of age. A factorial arrangement 5x3x2 was used resulting in a total of 30 experimental treatments. Chemical, amino acids, fatty acids and trace minerals analysis were determined for treated BMA. Pullet and laying hens performance, slaughter test, lymphoid organs, blood constituents, yolk color and components were determined.

General results of the early periods for both of the 2 trials showed that there were no significant ($p < 0.05$) effects of dietary algae on body weight, egg production, egg weight, egg mass, feed consumption, feed conversions, mortality rate and egg quality. Ovary, oviduct, lymphoid glands as well as titer of NDV were significantly affected by including BMA. Thus, BMA at the levels tested can be useful for enhancing pullets' performance, laying productivity and egg quality. Enzyme addition had benefits when compared to un-supplemented groups.

Keywords: Brown marine algae – laying hens – pullets – enzymes – egg production – immune organs – yolk color

Introduction

There is high interest regarding the world wide shortage of available food quantities. Scientists have increased their efforts to explore new and non-conventional natural resources. There is a certain type of marine algae that is promising to be a non-conventional source for both human and livestock. Some species of BMA were distributed in the Red Sea shore at Saudi Arabia (Khafaji and Meinesz, 1984; Khafaji *et al.*, 1992); and different coastal areas of Karachi. (Rizvi and Shameel, 2004).

Recently interest on the enrichment of eggs and poultry meat with omega-3 Fatty acid has increased given their important role in human metabolism. (Farrell, 1998; Simopoulos, 1994; Simopoulos, 2000 and Gonzalez and Leeson, 2001; Schiavone *et al.*, 2007). The inclusion of omega fatty acids into eggs and poultry meat is achieved by feeding ingredients such as marine algae (Herber and van Elswyk, 1996 and 1998; Abril and Barclay, 1998).

Therefore, the development of dietary formulations will allow locally available new ingredients such as marine algae to be used for reducing feed cost plus using enzyme technology partridge (2008) as well as saving the imports from some feed ingredients (Taher, 1986; Beraka, 1993; Jimenez-Escrig and Goni, 1999). Moreover, these materials have been previously successfully evaluated in poultry diets by scientists. This was done to try introducing better performing poultry and optimize egg production and quality especially pigments and omega 3, fatty acid content (Chapman and Chapman, 1980; Eldeek *et al.*, 1985; Nimruzi, 2002; Kang *et al.*, 2004; Sim *et al.*, 2004).

The objective of this study is to evaluate the nutritive values of BMA (*Sargassum Sp.*) processed by different methods on pullet and subsequent laying performance on yolk, color, fatty acid composition and cholesterol concentration of yolk lipids. The addition of enzyme preparations to diets containing treated BMA was observed.

Materials and Methods

BMA were obtained from southern Jeddah Red Sea shore. It was either sun dried for 48 h until constant weight or boiled in water (1 algae :4 water) for 20 minutes or autoclaved under 115 bar/inc for 15 minutes then air dried at 45° c for 48 h. The samples were then analyzed for proximate chemical composition according to A.O.A.C. (1990) for dry matter, crude protein, crude fat, crude fiber and ash, while NFE was calculated by differences.

Natural detergent fiber and acid detergent fiber were determined according to the methods of Van Soest and Wine (1967), while hemicelluloses was estimated by difference Total soluble sugars and starch were determined according to Egan *et al.* (1981). Tannins were determined according to the methods of Khokhar and Chauhan (1986).

Minerals were determined by atomic absorption (GBC Avanta Z) using standard curve. Amino acids were determined by using Beckman amino acid analyzer Model 118\119CL. The methyl esters of fatty acids were obtained from standard acids and various samples under study were analyzed using Unicom Gas-Liquid Chromatography Model: Shimadzu - 4CM (PFE) equipped with PID detector and glass column 2.5 m X 3 mm.

In Experiment 1 a total of 672 pullets aged 14 weeks, were used. All hens were randomly assigned to 42 treatment groups consisting of 4 replicates each and birds were additionally fed on a two-phase (pre-laying and laying) standard commercial layer mash and all had the same daily access (given from the age of 14 weeks onwards). There was a constant lighting period of 16h (06:00h to 22:00h) light and 8h dark, for all treatments.

In experiment two, 600 of Lohman laying hens starting from the age of 23 weeks were well into egg laying stage (76% production percentage). Birds were housed in cages in the research farm, Hada El-Sham Faculty of Metrology, King AbdulAziz University. Birds were divided into 30 treatments and were fed ad libitum either as a control diet containing 0% algae or the control diet incorporated with 3, 6, 9, 12 treated brown marine algae meal from 23 to 42 wk of age with or without enzyme preparations.

Egg quality parameters were recorded. Every four weeks (26, 30, 34, 38 and 42 weeks of age), 10 eggs were taken at random from each treatment to determine egg quality characteristics, percent yolk, percent albumen and percent

shell. Yolks were carefully separated without albumen for colorimetric cholesterol assay. A pooled sample of five yolks from each 42 treatment was used to measure yolk fatty acids by using gas chromatography.

All eggs were broken on to a flat surface and Haugh units were measured by E.Q. Instruments (Technical Services and Supplies Ltd, Top Lane, Copmanthorpe, UK). Yolk color was determined using the Roche color fan (1979). Shell thickness was measured (average of three measurements after the removal of shell membranes) using an AMES micrometer (Waltham, Massachusetts 02254). The weight of the albumen was calculated as the difference between the egg weight and the weight of the shell.

The effect of storage time was studied on the eggs collected on the 30th and 42nd week of lay. The eggs were stored for 21 days at storage temperature of 22°C. The decrease weight of eggs, albumen height, Haugh unit, thickness of eggshell, eggshell percentage, yolk and albumen percentage, color of yolk were determined. At the end of the both experiments 42 wk of age four hens from each treatment were euthanized. Giblets and lymphoid glands were weighed, ovary, oviduct, intestine were weighted.

The birds of each group were housed in a single poultry house maintained at 21°C through the production cycle, during the study feed consumption, mortality, egg production and weight were recorded. All data were analyzed by ANOVA using the general linear model (GLM) procedures of the SAS Institute (SAS Institute, 2000). The differences between means were determined using the Duncan test (1955).

Results and Discussion

The study aimed to evaluate the nutritional value of BMA as feed ingredient for pullet and laying hens. Productivity, yolk components and immune organs were investigated. BMA were collected from southern of Jeddah shore of Saudi Arabia and sun-dried and ground with hammer mill. In this studies algae meal processing by cooking or autoclaved is often darker than the sun-dried algae. Larbier and Leclercq, 1992; stated that the more intense the heating cause the darker the color of materials.

The nutritional values of tested BMA (raw, boiling and autoclaves) were evaluated by analyzing chemical composition and fatty acids and amino acids contents. Results indicated that chemical composition of the three samples of BMA processed by different method. The results indicated that method of processing had small effect on chemical composition for algae. The value ranged from 94.1 to 94.7% for DM, from 7.54 to 7.77% for CP, from 0.41 to 0.47% for EE, from 7.65 to 7.90% for crude fiber, from 47.15 to 48.31% for ash, from 29.95 to 31.38% for NFE, 1.666 to 1.88% for total soluble sugars and from 1523 to 1543 kcal for ME. The analyses didn't detect presence of starch in the samples. These results indicated that different processing methods have insignificant effect on proximate chemical composition of algae.

The values ranged from 27.95 to 30.21% for natural detergent fiber (NDF), from 21.18 to 23.54 for acid detergent fiber (ADF), from 6.41 to 7.73% for hemicellulose, the data showed higher tannins contents, the values ranged from 0.733 to 0.815 mg/g protein. Tannins are considered as well known ant nutritional factors (Potter and Fuller, 1968; Scott *et al.*, 1982, Attia, 1998). The values for calcium, phosphorus and sodium ranged from 0.126 to 0.144, 0.246 to 0.255 and from 4.01 to 4.05%. The results showed also higher content of sodium. The values for Zn, Mn, I, Fe and Cu ranged from 215 to 345, 216 to 265, 12.72 to 13.51, 11020 to 11495 and from 4.60 to 4.81 ppm respectively. These indicated that BMA contain considerable amount of trace minerals that could stratify poultry requirements based on the recommendations of NRC (1994). Furthermore, heavy metals of BMA are in acceptable range since the values for Pb ranged from 0.001 to 0.002 ppm, while no Cad was detected in the samples. These suggest that BMA may be used as mineral supplement in laying hens or broiler chicks to enhance egg shell quality and bone mineralization.

Sim *et al.*, (2004) found that BMA (*Ecklonia cava kjellman*) contained an average 10.29% of crude protein, 0.73% of ether extract, 36.41% of crude fiber, 27.23% of ash and 10.85% of NaCl as fed basis. The values of TME were 1.849 Kcal/kg. The average (TAAA) values of 13 amino acids for BMA was 31.99% Ec contained 99.6, 56.3, 7.65 and 2.0mg/kg of V.E, V/B2, Mg and Cd respectively. Marine algae generally contain Na, K, Ca, Mg and Fe in large quantities up to 15-25% of dry weight (Rizvi and Shameel, 2004). Tucker (2008) found that organic minerals fed for layers at 25-33% of NRC maintained laying performance and body weight. This confirmed our results in Exp. 1 & Exp. 2 concerning body weight and performance of hens. The essential e.g. methionine, lysine, threonine, and arginine as the 1st, 2nd, 3rd, and 4th limiting amino acids of 3 samples indicated that the values ranged from 0.17 to 0.25, 0.30 to 0.40, 0.63 to 0.74 and 0.71 to 0.74%, respectively. The results indicated that BMA containing considerable amount of amino acids that could

meet most of the requirements for chickens except for methionine and lysine. These amino acids profiles are similar to those of alfalfa meal and wheat bran, and better than corn (NRC, 1994).

It was found that saturated fatty acids of BMA ranged from 32.543 to 35.899% with the palmitic acid is the dominant fatty acids, monounsaturated fatty acids ranged from 35.793 to 37.023% with the oleic acid is the major fatty acids, meanwhile PUFA ranged from 27.77 to 30.434% with the linoleic is the main fatty acids. The ratio of SEA to USFA ranged from 0.482 to 0.560. These results indicated that different processing techniques had small effect on fatty acid profiles of BMA.

Egg weight tended to decrease slightly in laying hens fed BAM compared to control, Kang *et al.*, (2004). Numerous factors have been identified that affect broiler skin and egg yolk pigmentation. Factors such as xanthophyll source, xanthophyll concentration, type of bird, storage of feed, age of birds, and disease condition of the bird have been extensively reviewed (Marusich and Bauernfeind, 1981; Fletcher, 1989). The egg processing industry requires eggs with intense golden yellow yolks, which will impart a good color to their end- products. The consumer also prefers eggs with an attractive golden yellow color. Our results showed that all levels of BMA used in Exp. 1 and exp. 2 caused a deep yellow yolk color of acceptable appearance.

El-Boushy (1989) revealed that the use of the linear programming results in obtaining the least cost range of feedstuffs that may have low xanthophylls content. The inclusion of the synthetic carotenoids as feed additives will be of great value. This statement was confirmed by our results of Exp. 1 which showed that using natural sources of algae increased yolk color instead of using synthetic carotenoids.

Grigorova, (2005) concluded that adding 2% and 10% of dry biomass of fresh water algae of *Chlorella* genus to the combined forages for laying hens led to the improvement of the bird productivity and the morphological characteristics of the eggs and the egg yolk pigmentation was more intensive by 2,5 units by the Roche's scale. However, Strand *et al.*, (1998) feeding experiments were conducted with white leghorn laying hens in which they were fed a carotenoid depleted control diet (containing some zeaxanthin and lutein) supplemented with 15% seaweed meal of established carotenoid composition. Their results confirmed that fucoxanthin, the major carotenoid in seaweed meal, is not transferred on the yolk. However, fucoxanthin gave rise to the metabolites fucoxanthinol, fucoxanthin 3- sulphate and paracentrone that are ascribed to enzymatic modifications occurring in the hens. The

difuranoid auroxanthin encountered in the egg yolk was ascribed to violaxanthin and/or its furanoid derivatives present in the seaweed meal. The overall value of mortality was 1.5% and is in agreement with the industry level. Also, mortality was not much different among the experimental groups, indicating that BMA had no adverse effect on livability. Most results of these experiments are unpublished in this article as they are still under way at the laboratory.

According to the early investigation of some results summarized here. It is suggested that BMA processed by different methods is a good source of nutrients and acts as biologically active substances, which in the last years attracted the interest of the specialists in their search for natural, ecologically and healthy feed for the poultry.

Table 1: Nutrient composition of different samples of raw and treated algae

Nutrient	Brown algae (air dried)	Brown algae (boiled in water)	Brown algae (autoclaved)
DM%	94.1	94.4	94.7
Crude protein %	7.54	7.77	7.61
Crude fat %	0.45	0.47	0.41
Crude fiber %	7.77	7.90	7.65
Ash %	47.15	48.31	47.65
NEF %	31.19	29.95	31.38
Total Soluble Sugars %	1.848	1.666	1.880
Starch	0.0	0.0	0.0
ME (Kcal/kg)	1530	1543	1523
NDF	27.95	30.21	28.91
ADF	21.54	23.54	21.18
Hemicellulose	6.41	6.67	7.73
Tannins (mg/g protein)	0.775	0.733	0.815
Calcium %	0.126	0.144	0.139
Phosphorus %	0.246	0.239	0.255
Na %	4.03	4.01	4.05
Zinc ppm	215	330	345
Manganese ppm	216	270	265
Iodine ppm	13.51	12.72	13.18

Ferrous ppm	11020	11495	11212
Copper ppm	4.81	4.79	4.60
Pb ppm	0.001	0.002	0.001
Cad ppm	ND	ND	ND
Alanine	0.87	0.64	0.87
Arginine	0.71	0.77	0.74
Aspartic	1.61	1.54	1.80
Cystine	0.07	0.10	0.05
Glutamic	1.98	2.01	2.22
Glycine+serine	0.77+0.59	0.71+0.63	0.72+0.73
Histidine	0.18	0.43	0.21
Isoleucine	0.47	0.49	0.57
Leucine	0.92	0.88	0.96
Lysine	0.40	0.40	0.30
Methionine	0.17	0.25	0.18
Phenylalanine+tyrosine	0.70+0.52	0.64+0.48	0.80+1.00
Proline	0.53	0.72	1.26
Threonine	0.63	0.65	0.74
Valine	1.03	1.00	0.94
Ammonia	0.28	0.16	0.19
Total	12.41	12.48	14.29
Luric 12:0	1.079	0.521	0.726
Myristic 14:0	3.453	1.822	3.047
Palmitic 16:0	25.899	22.119	26.602
Palmitoleic 16:1	6.115	6.739	7.255
Heptadecanoic 17:0	1.155	1.822	1.451
Stearic 18:0	2.878	5.738	2.539
Oleic 18:1	27.338	28.690	27.086
Linoleic 18:2	22.158	25.438	24.474
Linolenic 18:2	5.612	4.996	4.401
Arachidic 20:0	1.079	0.521	0.967
Eicosenoic 20:1	2.878	1.549	1.452
SFA	35.899	32.543	35.332
MUFA	36.331	37.023	35.793
PUFA	27.77	30.434	28.875
MUFA:PUFA	0.988	0.879	1.240
USFA	64.101	67.457	64.668
SFA/USFA	0.561	0.482	0.546

Table 2: Experimental Laying diets used in Experiment 1

INGREDIENTS	0%	1%	2%	3%	4%	5%	6%
Corn grain	61.69	61.29	60.83	59.3	57.3	55.31	53.32
Soybean	25.76	25.67	25.6	25.71	25.8	25.88	25.96
Wheat barn	0	0	0	0.03	0.41	0.79	1.17
Palm oil	0	0	0.02	0.42	0.96	1.49	2.03
Dical phos	2.51	2.5	2.49	2.48	2.47	2.46	2.46
Limestone	9.66	9.15	8.67	8.67	8.67	8.67	8.67
Common salt	0	0	0	0	0	0	0
Vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Minral premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL Metionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14
LLysine HCl	0	0	0	0	0	0	0
Choline C170	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutrients analysis							
Dry matter	90.614	90.632	90.631	90.726	90.836	90.946	91.056
Me Kcal/kg	2700	2700	2700	2700	2700	2700	2700
CP%	18	18	18	18	18	18	18
Arginine%	1.131	1.133	1.136	1.142	1.148	1.154	1.161
Glycine	0.732	0.734	0.737	0.741	0.745	0.749	0.753
Serine	0.867	0.871	0.875	0.88	0.885	0.89	0.895
Glycine+ Serine%	1.856	1.862	1.869	1.878	1.888	1.898	1.907
Hisidine%	0.472	0.471	0.471	0.471	0.471	0.471	0.471
Isoleucine%	0.725	0.727	0.729	0.731	0.734	0.736	0.739
Leucine%	0.158	1.582	1.584	1.583	1.579	1.575	1.571
Laysine%	0.923	0.923	0.924	0.927	0.931	0.935	0.938
Methionine%	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Cystine	0.296	0.296	0.295	0.294	0.293	0.292	0.291
Methionine+ Cystine	0.716	0.716	0.715	0.714	0.713	0.712	0.711

Table 3: Experimental laying diets used in Experiment 2

INGREDIENTS	0%	3%	6%	9%	12%
Corn grain	60.82	57.83	52.81	46.86	40.91
Soybean	26.95	27.02	27.31	27.54	27.76
Wheat barn	0	0	0.43	1.58	2.73
Palm oil	0	0.63	1.95	3.54	5.14
Dical phos	2.5	2.48	2.46	2.43	2.4
Limestone	9.34	8.66	8.66	8.67	8.67
Common salt	0	0	0	0	0
Vitamin premix	0.1	0.1	0.1	0.1	0.1
Minral premix	0.1	0.1	0.1	0.1	0.1
DL Metionine	0.13	0.13	0.13	0.13	0.13
LLysine HCl	0	3	6	9	12
Choline C170	0.05	0.05	0.05	0.05	0.05
Nutrients analysis					
Dry matter age	90.614	90.769	91.076	91.414	91.751
Me Kcal/kg	2700	2700	2700	2700	2700
CP%	18.5	18.5	18.5	18.5	18.5
Arginine%	1.169	1.183	1.202	1.222	1.242
Glycine	0.753	0.763	0.774	0.786	0.798
Serine	0.893	0.905	0.918	0.931	0.943
Glycine+ Serine%	1.916	1.938	1.965	1.992	2.019
Hisidine%	0.485	0.492	0.499	0.506	0.514
Isoleucine%	0.748	0.755	0.764	0.771	0.779
Leucine%	1.616	1.615	1.606	1.593	1.579
Laysine%	0.956	0.962	0.972	0.983	0.993
Methionine%	0.42	0.42	0.42	0.42	0.42
Cystine	0.304	0.302	0.299	0.297	0.294
Methionine+ Cystine	0.724	0.722	0.719	0.717	0.714

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