Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Jul - Sept 2018 / v.20 / n.3 / 487-496

http://dx.doi.org/10.1590/1806-9061-2017-0666

# The Effects of Fucoxanthin Dietary Inclusion on the Growth Performance, Antioxidant Metabolism and Meat Quality of Broilers

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■Keywords

Antioxidant, broiler, fucoxanthin, meat quality, performance.



Submitted: 30/January/2018
Approved: 09/March/2018

## ABSTRACT

Fucoxanthin is a major carotenoid found in marine brown algae. This study investigated the impact of fucoxanthin on the growth performance, antioxidant metabolism and meat quality of broilers. Overall, 180 one-day-old male broiler chicks (Ross 308) were assigned to one control group (CONT) and 2 treatment groups (FUCO1 and FUCO2), with six replicates of 10 birds each. The CONT, FUCO1 and FUCO2 birds were fed a basal diet supplemented with 0, 100 and 200 mg/kg of fucoxanthin, respectively. Average body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were similar among the groups. Fucoxanthin increased catalase (CAT) and superoxide dismutase (SOD) activities and glutathione (GSH) levels ($p$<0.01), and reduced malondialdehyde (MDA) levels ($p$<0.01) in the liver, breast and drumstick tissues. The effects of fucoxanthin on drumstick yellowness (b*) on day 3 and water activity ($a_w$) on day 5 and breast lightness (L*) on day 3 b* values days 2 and 5 were limited and variable. While fucoxanthin showed antimicrobial effect against *Staphylococcus* spp. in the breast meat on days 5 and 6 of storage ($p$<0.05), its effects at different time periods and against other microorganisms varied. In conclusion, fucoxanthin did not affect performance parameters, but had a significant impact on antioxidant metabolism, and showed a limited effect on the microbial quality of meat.

## INTRODUCTION

Fucoxanthin is a major natural carotenoid found in the chloroplasts of marine brown algae *(Phaeophyceae)*, and accounts for almost 10% of the total carotenoid production in the world (Hosakawa *et al.,* 2006; Matsuno, 2001). Fucoxanthin has several physiological effects, including proton donation and free-radical scavenging (Sasaki *et al.,* 2008; Yan *et al.,* 1999), and has anticarcinogenic (Kotake *et al.,* 2005) and anti-inflammatory effects (Heo *et al.,* 2010; Sasaki *et al.,* 2008). In a previous study carried out with broilers, an oral daily dose of 10 mg of fucoxanthin per bird for two weeks improved plasma antioxidant status and meat color but did not affect their performance parameters (Sasaki *et al.,* 2010).

The amount of free radicals generated by oxidation, which is a natural process that occurs during metabolic activities, may increase excessively due to various stress factors (Surai, 2015). Oxidative stress causes major cell damage, which leads to animal health problems and reduced yields (Fang *et al.,* 2002; Halıcı *et al.,* 2012). Antioxidants are needed to prevent the generation of free radicals and to allow their elimination from the body without any harm to the body (Gumus *et al.,* 2017; Surai, 2015). The enzymes superoxide dismutase (SOD) and catalase (CAT) and glutathione (GSH) play a major role in the antioxidant defense system of the body, and natural feed additives

have a positive impact on this system (Gümüş & Imik, 2016; Halıcı *et al.,* 2012). Antioxidant additives are also used in the food industry for the prevention of oxidation reactions such as oil rancidity color change, as well as of microbiological spoilage, and thereby, for the extension of the shelf life of food (Alimentarius, 2010). It has been shown that antioxidant vitamins positively affect both the quality and shelf life of meat (Imik *et al.*, 2012a; Imik *et al.,* 2012b).

There are only very few studies on the use of fucoxanthin as a feed additive in animal nutrition. In most of the published studies, fucoxanthin was applied *postmortem* and its effects on meat were investigated (Sasaki *et al.,* 2008). This study investigated the dietary inclusion of different levels of fucoxanthin on the growth performance, antioxidant metabolism in the liver, breast and drumstick tissues, and the quality of breast and drumstick meat of broilers.

## MATERIALS AND METHODS

### Birds, experimental design, and diet

The research protocol of the current study was approved by the local Ethics Committee for Animal Experiments of the Sivas Cumhuriyet University (Approval number: 2016/71).

The experiment was conducted in the Research and Application Center of the Faculty of Veterinary Medicine, Atatürk University. A total of 180 one-day-old male Ross 308 broilers were divided into three treatments with six replicates of 10 birds each. The feeding period was divided in starter diets, fed from 1 to 21 days of age, and finisher diets, fed from 22 to 42 days of age (Table 1). The experimental diets were based on a standard commercial feed used as control (CONT group), which was supplemented with 100 mg/kg fucoxanthin (FUCO1 group) and 200 mg/kg fucoxanthin (FUCO2 group). The birds were housed in 18 three-storey cages measuring 100x55x35 cm. Feed and water were supplied *ad libitum*. The ambient temperature was gradually decreased from 33 °C in first week to 22 °C on day 14 and was then kept constant afterwards. The lighting program applied was a continuous 23 h light. Diets formulated and considered as control according to the recommendation of NRC (1994) (Table 1). The nutritional composition of the diets was determined according to the AOAC (2005).

### Feed additives

Fucoxanthin was added as the commercial product ThinOgen™ (BBG Company, Algae Health Sciences, USA) derived from *Laminaria japonica* seaweed, containing 5% fucoxanthin oil as tested by HPLC. The (ThinOgen™) was added to the diets of the FUCO1 and FUCO2 groups at 100 and 200 mg fucoxanthin/kg diet, respectively.

**Table 1 -** Ingredients and chemical composition of the basal diets, g/kg

| | Starter (1 to 21 d) | Finisher (22 to 42 d) |
|---|---|---|
| *Ingredients g/kg* | | |
| Corn | 514.6 | 562.0 |
| Soyabean meal (%44) | 393.5 | 316.5 |
| Soybean oil | 31.8 | 66.0 |
| Dicalcium phosphate | 21.1 | 17.3 |
| Wheat bran* | 15.0 | 15.0 |
| Calcium carbonate | 9.6 | 8.6 |
| Sodium bicarbonate | 2.8 | 2.7 |
| Salt | 2.7 | 2.8 |
| Vit-Min. Premix** | 5 | 5 |
| DL-methionine | 2.4 | 2.6 |
| L-lysine HCL | 0.8 | 0.6 |
| L-threonine | 0.7 | 0.9 |
| *Calculated analysis* | | |
| Metabolizable energy, MJ/kg | 12.22 | 13.40 |
| Crude protein, % | 22.21 | 19.21 |
| Ether extract% | 3.48 | 5.65 |
| Crude fibre, % | 2.8 | 2.65 |
| Lysine, % | 1.19 | 1.02 |
| Methionine, % | 0.57 | 0.52 |
| Calcium, % | 0.96 | 0.81 |
| Phosphorous, % | 0.64 | 0.56 |

*The fucoxanthine was added in replacement of wheat bran.

** Contents per kilogram: vitamin A, 3,600,000 U; vitamin D3, 800,000 U; vitamin E, 7200 U; vitamin K3, 800 mg; thiamine, 720 mg; riboflavin, 2640 mg; calcium pantothenate, 4000 mg; niacin, 12,000 mg; pyridoxine, 1200 mg; folic acid, 400 mg; vitamin B12, 6 mg; biotin, 40 mg; choline, 100,000 mg; Mn, 39680 mg; Fe, 20000 mg; Zn, 33880 mg; Cu, 4000 mg; I, 400 mg; Se, 80 mg.

### Performance parameters

Body weight (BW), body weight gain (BWG) and feed intake (FI) were measured at 3, 10, 17, 24, 31 and 42 days of age. Feed conversion ratio (FCR) was calculated as total FI (g) / total BWG (g). Mortality was recorded when it occurred.

### Biochemical analyses

Ten randomly selected male broilers per group were fasted for 10 h, and then sacrificed. Liver, breast and drumstick tissues were collected, homogenized, and frozen in liquid nitrogen at -80°C, and stored until biochemical analyses.

The activities of the enzymes SOD and CAT and the levels of GSH and MDA in the liver, breast and drumstick tissues were determined. To prepare the tissues homogenates, the liver, breast and drumstick tissues

were ground in liquid nitrogen using a tissuelyser device (QIAGEN, Tissuelyser II, Germany) at frequency 1/s for 30 seconds. These tissue samples were then used for biochemical analyses. All biochemical measurements were carried out using commercial test kits (Cayman, USA) in ELISA reader (BioTek, µQuant, USA). For SOD determination, 200 µL of the diluted Radical Detector were added in the SOD standard wells and 10 µL of the standard in the designated wells, after which 10 µL of the sample were added to wells. Reactions were initiated by adding 20 µL diluted xanthine oxidase to all wells. The plate was placed on shaker and incubated for 30 min at room temperature. Measurements were made using a spectrophotometer at 440-460 nm absorbance. For CAT determination,100µL assay buffer, 30 µL methanol and 20µLsamples were added to the standard wells, and 100µL diluted assay buffer, 30µL methanol and 20µL diluted catalase were added to positive control wells. The reaction was initiated by adding 20µL diluted hydrogen peroxide to all wells. The plate was placed on shaker and incubated for 20 min at room temperature. Measurements were made using a spectrophotometer at 540 nm absorbance. For GSH determination, 50µL of the samples were added to each sample well. The plate cover was removed and 150 µL of freshly-prepared assay cocktail were added to both standard and sample wells. The plate cover was replaced and the plate was incubated for 20 min in the dark. Measurements were made using a spectrophotometer at 405-414 nm absorbance. For MDA determination, 100µL of the samples or malondialdehyde standard were added to each vial. Vials were boiled for one hour, placed on ice for 10 min and then centrifuged for 10 min at 1600xg at 4 °C. Vials stabilized at room temperature for 30 min. Measurements were made using a spectrophotometer at 530-550 nm absorbance.

## Determination of meat quality

At the end of the experimental period (42 days), 10 birds per group were slaughtered after 10-h fasting. Birds were bled for 120 s, manually plucked, and washed. Carcasses were stored at +4±1 °C for 24 h, when drumstick meat and breast meat were separated. The meat from the drumsticks and breast were placed on polyethylene plates, covered with stretch film, and stored at 4±1 °C for 6 d. Subsequently, the samples were analyzed on d 1, 2, 3, 4, 5 and 6 for pH, water activity ($a_w$), and color [L*(lightness), a*(redness), b*(yellowness)] and microbial counts [*Enterobacteriaceae*, total psychrotrophic aerobic bacteria *(TPAB)*, total mesophilic aerobic bacteria *(TMAB)*, *Staphylococcus* spp., *Lactobacillus* spp. and *Pseudomonas* spp.]. Microbiological analyses of the samples preceded the other analyses.

Water activity values were measured using an Aqualab 4TE (USA) device. Meat samples were placed in the container of the device for the reading of the $a_w$ values.

The pH values of the samples were measured as described by Gökalp *et al.* (2001). Accordingly, 10 g-portions of the homogenized samples were weighed and 100 mL of distilled water were added. Samples were homogenized for 1 min using an Ultra-Turrax (IKA Werk T 25, Germany) homogenizer and the pH values were measured using a pH meter (WTW Inolab, Germany).

The color intensities (L*, a*, b*) of the cross-sectional areas of the drumstick and breast meat samples were determined using a Minolta colorimeter (CR-200, Minolta Co, Osaka, Japan). Color measurements were performed directly on the surface of muscle tissue, by removing the skin.

The microbiological analyses of the samples were performed in compliance with the method described by Baumgart *et al.* (1993). Accordingly, 25 g of the meat samples were homogenized in 225 mL of sterile Ringer's solution. Subsequently, serial dilutions of the homogenates were prepared. Inoculations were made using the spread plate technique. The *TMAB* count was determined on Plate Count Agar (PCA, Merck) incubated under aerobic conditions at 30±1°C for 72±1 h. The *TPAB* count was also determined on Plate Count Agar (PCA, Merck), but incubated under aerobic conditions at 7 ± 1°C for 10 days. For *Enterobacteriaceae* enumeration, 1 mL of the appropriate dilutions was seeded on Violet Red Bile Dextrose Agar (VRBDA, Merck), and incubated at 30 °C under anaerobic conditions for 2 d. *Staphylococcus* spp. counts were determined on mannitol-salt agar (MSA) incubated under aerobic conditions at 30±1°C for 48±1 h. *Pseudomonas* spp. counts were determined on *Pseudomonas* Agar (Oxoid CM 0559) supplemented with CFC supplement (Oxoid SR 0103) and incubated under aerobic conditions at 25±1°C for 48±1 hours. *Lactobacillus* spp. counts were determined on MRS Agar (De Man Rogosa and Sharpe) (Oxoid CM 1153) incubated under anaerobic conditions at 37±1°C for 48±1 h. Bacterial counts were expressed in log cfu $g^{-1}$.

## Statistical Analysis

The data obtained was analyzed using the SPSS 20 software (SPSS, 2011) using one-way analysis of variance (ANOVA) test. Differences among means

were determined by Duncan's post-test. The data were expressed as mean±standard error of mean (SEM). Differences were considered with significant at $p<0.05$ and $p<0.01$.

## RESULTS

Growth performance results (BW, BWG, FI and FCR) are presented in Table 2. Significantly higher BW ($p<0.05$) on days 10 and 17and BWG between days 3-10 and11-17were obtained in the of the FUCO1 and FUCO2 birds compared with the CONT group. During other time periods, no differences were detected among treatments ($p>0.05$). There was no effect of treatments on FI and FCR ($p>0.05$)(Table 2).

It was determined that, in the liver and breast tissues of FUCO1andFUCO2 groups, the activities of both antioxidant enzymes SOD and CAT ($p<0.01$) and the levels of GSH significantly increased ($p<0.01$), whilst the level of MDA decreased ($p<0.05$) compared with

**Table 2 –** Effects of dietary fucoxanthin supplementation on the growth performance in broiler chickens

| Items | Groups | | | *p-value* |
|---|---|---|---|---|
| | CONT | FUCO1 | FUCO2 | |
| BW, g | | | | |
| 3 d | 73.71±0.11 | 73.53±0.85 | 73.90±0.38 | ns |
| 10 d | 261.87±2.35$^{b}$ | 276.06±6.19$^{a}$ | 281.67±0.88$^{a}$ | * |
| 17 d | 561.44±7.81$^{b}$ | 618.16±18.48$^{a}$ | 639.26±36.85$^{a}$ | * |
| 24 d | 1123.89±32.72 | 1079.58±74.33 | 1212.96±18.79 | ns |
| 31 d | 1658.76±21.08 | 1581.03±57.62 | 1638.94±56.50 | ns |
| 42 d | 2417.78±117.15 | 2331.67±54.03 | 2358.33±173.76 | ns |
| BWG, g | | | | |
| 3-10 d | 26.74±0.35$^{b}$ | 29.04±0.76$^{a}$ | 29.68±0.15$^{a}$ | * |
| 11-17 d | 42.80±0.10$^{b}$ | 48.87±0.73$^{a}$ | 51.09±3.16$^{a}$ | * |
| 18-24 d | 80.35±3.94 | 65.92±9.56 | 81.96±0.52 | ns |
| 25-31 d | 76.41±2.67 | 71.64±13.03 | 70.44±9.35 | ns |
| 32-42 d | 84.34±10.69 | 83.40±8.99 | 81.29±13.34 | ns |
| 3-42 d | 63.32±3.17 | 61.05±1.45 | 62.18±4.71 | ns |
| FI, g | | | | |
| 3-10 d | 40.48±2.14 | 42.95±1.34 | 44.29±0.00 | ns |
| 11-17 d | 89.05±5.24 | 91.43±2.86 | 93.61±0.68 | ns |
| 18-24 d | 164.80±11.26 | 164.13±5.85 | 174.50±1.20 | ns |
| 25-31 d | 139.87±2.73 | 140.36±2.81 | 134.57±2.70 | ns |
| 32-42 d | 135.15±5.5 | 123.38±11.32 | 121.41±5.84 | ns |
| 3-42 d | 113.87±5.03 | 112.44±1.97 | 113.68±1.16 | ns |
| FCR, g/g | | | | |
| 3-10 d | 1.52±0.08 | 1.48±0.03 | 1.49±0.01 | ns |
| 11-17 d | 2.08±0.09 | 1.87±0.03 | 1.85±0.12 | ns |
| 18-24 d | 2.07±0.21 | 2.60±0.36 | 2.13±0.03 | ns |
| 25-31 d | 1.84±0.09 | 2.10±0.39 | 2.21±0.03 | ns |
| 32-42 d | 1.66±0.24 | 1.54±0.27 | 1.52±0.12 | ns |
| 3-42 d | 1.81±0.15 | 1.84±0.03 | 1.83±0.06 | ns |

All values are given as mean ± SEM, (n=60). a, b: Means in the same row with different superscripts differ (*: $p<0.05$).

ns: not significant ($p>0.05$). BW: Body weight, BWG: Body weight gain, FI; Feed intake, FCR; Feed conversion ratio.

CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin.

the CONT group (Table 3). In the drumstick tissue, CAT activity increased only in FUCO2, whilst SOD activity and GSH levels were significantly increased both in FUCO1and FUCO2 ($p<0.01$) relative to the CONT group. Similarly, in the drumstick tissue, MDA levels were decreased in FUCO1 and FUCO2 groups ($p<0.01$) relative to the CONT group (Table 3).

In the drumstick meat, the *TMAB* count was significantly lower on the 5$^{th}$ day of storage in the FUCO1 and FUCO2 groups and on the 6$^{th}$ day of storage only in the FUCO2 group ($p<0.05$) relative to the CONT group (Table 4). Furthermore, *Staphylococcus* spp. counts were also significantly lower in the drumstick meat on the 5$^{th}$ and 6$^{th}$ days of storage both in FUCO1 and FUCO2 groups ($p<0.01$) relative to the CONT group (Table 4). On the other hand, lower *Pseudomonas* spp. counts in the drumstick meat were determined on the 3$^{rd}$ day of storage in groupFUCO1 and on the 6$^{th}$ day

**Table 3 –** Effects of dietary fucoxanthin supplementation on antioxidant metabolism in liver, breast and drumstick tissues of broiler chickens

| Items | Groups | | | *p*-value |
|---|---|---|---|---|
| | CONT | FUCO1 | FUCO2 | |
| CAT, *nmol/min/g protein* | | | | |
| Liver | 4725.92 ±155.23$^{b}$ | 5792.78±115.68$^{a}$ | 5474.15±237.37$^{a}$ | ** |
| Breast | 693.87±34.38$^{b}$ | 1547.70±215.08$^{a}$ | 1106.49±167.29$^{a}$ | ** |
| Drumstick | 703.22±60.96$^{b}$ | 1191.53±137.75$^{ab}$ | 1316.58±128.43$^{a}$ | ** |
| SOD, *U/mg protein* | | | | |
| Liver | 20.31±1.36$^{b}$ | 26.32±4.32$^{a}$ | 27.50±0.84$^{a}$ | ** |
| Breast | 20.64±1.35$^{b}$ | 31.55±1.69$^{a}$ | 34.32±2.30$^{a}$ | ** |
| Drumstick | 27.22±2.01$^{b}$ | 40.15±3.19$^{a}$ | 41.41±2.93$^{a}$ | ** |
| GSH, *µmol/g protein* | | | | |
| Liver | 24.19±2.64$^{b}$ | 37.04±3.86$^{a}$ | 36.71±4.23$^{a}$ | * |
| Breast | 3.56±0.41$^{b}$ | 8.80±0.62$^{a}$ | 9.56±0.83$^{a}$ | ** |
| Drumstick | 12.18±1.34$^{b}$ | 26.93±2.24$^{a}$ | 28.05±2.52$^{a}$ | ** |
| MDA, *µmol/g protein* | | | | |
| Liver | 8.39±0.83$^{a}$ | 5.72±0.50$^{b}$ | 4.49±0.38$^{b}$ | ** |
| Breast | 2.73±0.98$^{a}$ | 1.39±0.17$^{b}$ | 1.56±0.30$^{b}$ | * |
| Drumstick | 4.64±0.65$^{a}$ | 2.45±0.34$^{b}$ | 2.00±0.30$^{b}$ | ** |

All values are given as mean ± SEM, (n=10). a, b: Means in the same row with different superscripts differ (*: $p<0.05$), (**: $p<0.01$). ns: not significant ($p>0.05$). CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione. CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin.

**Table 4 –** Effects of dietary fucoxanthin supplementation and storage time on *TMAB*, *Enterobacteriaceae*, *Lactobacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *TPAB* counts in chicken drumstick meat (log cfu g$^{-1}$)

| Days | Groups | *TMAB* | *Enterobacteriaceae* | *Lactobacillus* spp. | *Staphylococcus* spp. | *Pseudomonas* spp. | *TPAB* |
|---|---|---|---|---|---|---|---|
| 1 | CONT | 4.76±0.02$^{b}$ | 5.02±0.32 | 5.48±0.34 | 4.20±0.12 | 4.16±0.12$^{a}$ | 2.69±0.09$^{b}$ |
| | FUCO1 | 4.51±0.01$^{b}$ | 4.32±0.32 | 4.75±0.18 | 3.45±0.45 | 3.35±0.05$^{b}$ | 2.84±0.01$^{b}$ |
| | FUCO2 | 6.12±0.16$^{a}$ | 4.94±0.10 | 5.13±0.11 | 3.92±0.08 | 4.34±0.22$^{a}$ | 4.06±0.28$^{a}$ |
| | *p*-value | ** | ns | ns | ns | * | * |
| 2 | CONT | 6.02±0.54 | 5.49±0.55 | 5.34±0.26 | 5.02±0.26 | 4.88±0.16 | 4.52±0.00 |
| | FUCO1 | 6.18±0.18 | 5.37±0.01 | 5.77±0.03 | 4.25±0.21 | 5.53±0.12 | 4.90±0.04 |
| | FUCO2 | 6.65±0.03 | 6.05±0.07 | 5.55±0.23 | 3.95±0.47 | 5.73±0.28 | 4.67±0.28 |
| | *p*-value | ns | ns | ns | ns | ns | ns |
| 3 | CONT | 5.18±0.18$^{c}$ | 5.49±0.17 | 5.11±0.11$^{b}$ | 4.59±0.11 | 6.08±0.00$^{a}$ | 6.18±0.06 |
| | FUCO1 | 6.28±0.19$^{b}$ | 5.32±0.48 | 6.54±0.06$^{a}$ | 4.54±0.06 | 5.61±0.10$^{b}$ | 5.87±0.01 |
| | FUCO2 | 7.03±0.08$^{a}$ | 5.46±0.02 | 5.40±0.10$^{b}$ | 4.15±0.15 | 6.21±0.13$^{a}$ | 6.32±0.32 |
| | *p*-value | ** | ns | ** | ns | * | ns |
| 4 | CONT | 6.99±0.12 | 5.95±0.38 | 6.90±0.00 | 5.13±0.16 | 6.61±0.01$^{b}$ | 6.62±0.11 |
| | FUCO1 | 7.96±0.27 | 5.63±0.93 | 6.80±0.16 | 5.26±0.08 | 6.91±0.01$^{a}$ | 6.92±0.06 |
| | FUCO2 | 7.46±0.18 | 5.76±0.02 | 6.66±0.18 | 4.39±0.27 | 6.90±0.03$^{a}$ | 5.66±0.70 |
| | *p*-value | ns | ns | ns | ns | * | ns |
| 5 | CONT | 7.88±0.01$^{a}$ | 6.95±0.39 | 6.41±0.01 | 5.93±0.30$^{a}$ | 7.67±0.03 | 7.48±0.18 |
| | FUCO1 | 7.45±0.15$^{b}$ | 6.59±0.81 | 6.44±0.48 | 4.78±0.18$^{b}$ | 7.79±0.09 | 7.70±0.13 |
| | FUCO2 | 7.04±0.04$^{c}$ | 5.20±0.60 | 6.42±0.06 | 4.46±0.02$^{b}$ | 7.58±0.29 | 7.32±0.24 |
| | *p*-value | * | ns | ns | * | ns | ns |
| 6 | CONT | 8.19±0.05$^{a}$ | 7.16±0.06$^{b}$ | 7.01±0.00 | 6.35±0.20$^{a}$ | 8.72±0.12$^{a}$ | 8.53±0.08 |
| | FUCO1 | 8.01±0.05$^{a}$ | 7.71±0.10$^{a}$ | 6.66±0.19 | 4.69±0.09$^{c}$ | 8.79±0.06$^{a}$ | 8.48±0.03 |
| | FUCO2 | 7.70±0.00$^{b}$ | 6.93±0.01$^{b}$ | 6.73±0.15 | 5.32±0.02$^{b}$ | 8.23±0.06$^{b}$ | 8.19±0.21 |
| | *p*-value | ** | ** | ns | ** | * | ns |

All values are given as mean ± SEM, (n=10). a-d: Means in the same column with different superscripts differ (*: $p<0.05$), (**: $p<0.01$). ns: not significant ($p>0.05$).

CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin. *TMAB*; total mesophilic aerobic bacteria, *TPAB*; total psychrotrophic aerobic bacteria.

of storage in group FUCO2 ($p<0.05$) of the FUCO1 and FUCO2 birds (Table 4). In the breast meat, a significant decrease in *TMAB* counts was detected on the 5$^{th}$ and 6$^{th}$ days of storage and in the *Enterobacteriaceae* counts on the 6$^{th}$ day of storage in group FUCO2 ($p<0.05$) relative to the CONT group (Table 5). *Staphylococcus*

**Table 5 –** Effects of dietary fucoxanthin supplementation and storage time on *TMAB*, *Enterobacteriaceae*, *Lactobacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *TPAB* counts in chicken breast meat (log cfu $g^{-1}$)

| Days | Groups | *TMAB* | *Enterobacteriaceae* | *Lactobacillus*spp. | *Staphylococcus*spp. | *Pseudomonas* spp. | *TPAB* |
|---|---|---|---|---|---|---|---|
| 1 | CONT | 4.42±0.03$^{c}$ | 3.98±0.50 | 4.89±0.01 | 4.25±0.07 | 4.26±0.22 | 2.31±0.01 |
| | FUCO1 | 5.11±0.01$^{b}$ | 4.39±0.39 | 4.27±0.04 | 4.08±0.04 | 3.49±0.01 | 3.02±0.02 |
| | FUCO2 | 5.37±0.03$^{a}$ | 5.77±0.65 | 5.40±0.88 | 3.54±0.24 | 3.30±0.30 | 3.15±0.85 |
| | *p*-value | ** | ns | ns | ns | ns | ns |
| 2 | CONT | 6.24±0.44 | 6.30±0.04 | 5.94±0.71 | 4.98±0.07$^{a}$ | 4.06±0.02$^{b}$ | 4.28±0.13 |
| | FUCO1 | 6.44±0.21 | 4.86±0.26 | 6.44±0.06 | 3.95±0.49$^{b}$ | 5.17±0.06$^{a}$ | 4.60±0.36 |
| | FUCO2 | 5.72±0.24 | 5.78±0.43 | 5.53±0.10 | 4.05±0.10$^{b}$ | 5.25±0.05$^{a}$ | 4.55±0.40 |
| | *p*-value | ns | ns | ns | ** | ** | ns |
| 3 | CONT | 5.62±0.22 | 5.35±0.03 | 5.04±0.04$^{b}$ | 4.15±0.15 | 5.95±0.01$^{b}$ | 5.94±0.01 |
| | FUCO1 | 6.54±0.24 | 5.71±0.29 | 6.20±0.12$^{a}$ | 4.53±0.01 | 6.11±0.01$^{a}$ | 5.82±0.01 |
| | FUCO2 | 6.36±0.08 | 5.04±0.44 | 6.03±0.25$^{a}$ | 4.41±0.03 | 6.07±0.04$^{a}$ | 5.78±0.24 |
| | *p*-value | ns | ns | * | ns | * | ns |
| 4 | CONT | 6.60±0.30$^{b}$ | 5.54±0.08$^{b}$ | 5.72±0.12 | 3.15±0.15$^{b}$ | 6.57±0.12 | 6.50±0.14 |
| | FUCO1 | 7.93±0.20$^{a}$ | 6.59±0.39$^{a}$ | 5.78±0.78 | 4.55±0.01$^{a}$ | 6.48±0.16 | 6.86±0.34 |
| | FUCO2 | 7.49±0.06$^{ab}$ | 5.08±0.00$^{b}$ | 7.17±0.06 | 4.64±0.01$^{a}$ | 6.64±0.13 | 6.69±0.24 |
| | *p*-value | * | * | ns | ** | ns | ns |
| 5 | CONT | 7.43±0.03$^{a}$ | 6.97±0.41 | 6.97±0.03 | 5.68±0.01$^{a}$ | 7.62±0.01 | 7.50±0.10 |
| | FUCO1 | 7.45±0.15$^{a}$ | 6.82±0.46 | 5.40±0.50 | 5.20±0.01$^{c}$ | 7.54±0.22 | 7.66±0.12 |
| | FUCO2 | 6.15±0.15$^{b}$ | 5.04±0.74 | 6.53±0.13 | 5.29±0.01$^{b}$ | 7.54±0.06 | 7.68±0.00 |
| | *p*-value | ** | ns | ns | ** | ns | ns |
| 6 | CONT | 7.94±0.09$^{a}$ | 7.28±0.10$^{b}$ | 6.62±0.20 | 6.20±0.08$^{a}$ | 8.08±0.50 | 8.51±0.17 |
| | FUCO1 | 7.94±0.01$^{a}$ | 7.58±0.04$^{a}$ | 6.99±0.07 | 4.54±0.06$^{c}$ | 8.75±0.13 | 8.46±0.12 |
| | FUCO2 | 7.45±0.05$^{b}$ | 6.34±0.01$^{c}$ | 7.05±0.05 | 5.53±0.03$^{b}$ | 7.25±0.26 | 7.92±0.20 |
| | *p*-value | * | ** | ns | ** | ns | ns |

All values are given as mean ± SEM, (n=10). a-d: Means in the same column with different superscripts differ (*: $p<0.05$), (**: $p<0.01$). ns: not significant ($p>0.05$).

CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin. *TMAB*; total mesophilic aerobic bacteria, *TPAB*; total psychrotrophic aerobic bacteria.

spp. counts in the breast meat were significantly lower on days 5and 6 of storage in groups FUCO1 andFUCO2 ($p<0.01$) relative to the CONT group (Table 5).

Drumstick the values of meat color parameters L* and a* were not affected by fucoxanthin, whereas the b* value significantly increased on the 1st day of storage in only group FUCO1 ($p<0.01$) relative to the other groups (Table 6). Fucoxanthin supplementation had no effect on the pH value of the drumstick meat. However, it significantly increased $a_w$ on the 5th day of storage only in group FUCO2 ($p<0.05$) (Table 6). Furthermore, fucoxanthin had no effect on the pH, $a_w$ and a* values of the breast meat, but caused a significant increase in the L* value on the 3rd day of storage in group FUCO1, and in the b* value on the 2nd and 5th days of storage in both groups FUCO1 and FUCO2 ($p<0.05$) relative to the CONT group (Table 7).

## DISCUSSION

Due to the adverse effects of synthetic antioxidants on organisms, the search for alternative natural antioxidants have been researched. Studies have shown that fucoxanthin, which is found in marine brown algae, is a natural and safe antioxidant substance that leaves no residues (Yan *et al.,* 1999).

Similar to other carotenoid-containing substances, due to its hydrophobic structure, fucoxanthin is hydrolyzed into fucoxanthinol by digestive enzymes in the gastrointestinal tract, and is absorbed by intestinal cells (Beppu *et al.,* 2009;Sugawara *et al.,* 2002). In the present study, fucoxanthin supplementation to feeds had no effect on BW (except for days 10 and 17), BWG (except for days 3-10 and 11-17), FI, and FCR of broiler chicks (Table 2). The results obtained in the present study are in agreement with a previous studies that showed that fucoxanthin (10 mg/day/animal) (Sasaki *et al.,* 2010), marigold extract containing varying levels of several carotenoid types(added at percentages of 0.075%, 0.15%, 0.30%and 0.60% to the feed ration) (Wang *et al.,* 2017), and astaxanthin-rich yeast *(Phaffiarhodozyma)* (added at concentrations of 10 and 20 mg/kg to the feed ration) did not affect BWG, FI and FCR of broiler chickens (Perenlei *et al.,* 2014).

Antioxidants significantly reduce lipid peroxidation (Halıcı *et al.,* 2012). Due to its allenic bonds, epoxide and hydroxyl groups, fucoxanthin has a specific

**Table 6 –** Effects of dietary fucoxanthin supplementation and storage period on colour parameters, water holding capacity and pH in chicken breast meat

| Days | Groups | L* | a* | b* | WHC | pH |
|---|---|---|---|---|---|---|
| 1 | CONT | 52.07±1.68 | 5.14±0.59 | 6.32±0.15 | 0.988±0.001 | 5.81±0.01 |
| | FUCO1 | 56.52±3.87 | 4.03±0.47 | 7.96±1.16 | 0.989±0.001 | 5.97±0.08 |
| | FUCO2 | 54.37±3.69 | 4.81±0.70 | 10.25±1.52 | 0.989±0.002 | 5.81±0.08 |
| | *p*-value | ns | ns | ns | ns | ns |
| 2 | CONT | 49.49±1.15 | 5.00±0.89 | 6.79±0.48$^{b}$ | 0.987±0.001 | 5.95±0.13 |
| | FUCO1 | 51.45±1.06 | 3.27±0.49 | 8.63±0.33$^{a}$ | 0.988±0.001 | 5.83±0.11 |
| | FUCO2 | 51.53±0.52 | 3.59±0.29 | 9.44±0.55$^{a}$ | 0.988±0.001 | 5.86±0.01 |
| | *p*-value | ns | ns | ** | ns | ns |
| 3 | CONT | 51.21±1.49$^{b}$ | 3.82±0.55 | 8.49±0.32 | 0.986±0.001 | 5.82±0.06 |
| | FUCO1 | 56.08±0.34$^{a}$ | 2.41±0.57 | 8.64±1.61 | 0.983±0.001 | 5.65±0.05 |
| | FUCO2 | 49.33±0.70$^{b}$ | 3.32±0.36 | 8.62±0.69 | 0.985±0.002 | 6.00±0.08 |
| | *p*-value | ** | ns | ns | ns | ns |
| 4 | CONT | 51.00±1.63 | 3.71±1.01 | 6.97±1.36 | 0.986±0.000 | 5.83±0.06 |
| | FUCO1 | 53.18±1.45 | 2.41±0.49 | 7.46±1.35 | 0.989±0.000 | 5.75±0.13 |
| | FUCO2 | 51.50±1.38 | 3.66±0.16 | 10.14±0.82 | 0.987±0.001 | 5.86±0.01 |
| | *p*-value | ns | ns | ns | ns | ns |
| 5 | CONT | 51.33±0.98 | 3.30±0.40 | 6.06±1.21$^{b}$ | 0.987±0.002 | 5.90±0.05 |
| | FUCO1 | 52.39±1.14 | 4.22±1.56 | 9.16±0.59$^{a}$ | 0.992±0.010 | 5.96±0.09 |
| | FUCO2 | 51.47±0.95 | 2.86±0.30 | 10.09±0.58$^{a}$ | 0.992±0.006 | 5.84±0.04 |
| | *p*-value | ns | ns | * | ns | ns |
| 6 | CONT | 56.30±2.45 | 3.46±0.93 | 10.04±0.74 | 0.987±0.003 | 6.07±0.16 |
| | FUCO1 | 50.82±0.91 | 5.28±0.35 | 11.48±0.66 | 0.989±0.002 | 5.93±0.01 |
| | FUCO2 | 50.77±2.00 | 4.83±0.50 | 10.00±0.11 | 0.990±0.001 | 5.83±0.12 |
| | *p*-value | ns | ns | ns | ns | ns |

All values are given as mean ± SEM, (n=10). a-d: Means in the same column with different superscripts differ (*: $p<0.05$), (**: $p<0.01$). ns: not significant ($p>0.05$).

CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin. L*: lightness, a*: redness, b*: yellowness. WHC: water holding capacity

chemical structure and has potential antioxidant activity (Sangeetha *et al.,* 2008). In the present study, fucoxanthin significantly improved CAT and SOD activities and the GSH levels in the liver, breast and drumstick tissues. Furthermore, fucoxanthin significantly decreased MDA levels in all three tissues. These results are consistent with a previous study in rats, where dietary fucoxanthin inclusion increased plasma and liver CAT and SOD activities (Sangeetha *et al.,* 2008). In another study conducted by Wang *et al.* (2017) in broiler chickens, the addition of lutein and zeaxanthin-containing marigold extract to the feed at a concentration of 0.60% not only significantly increased SOD activity and GSH levels in the liver and SOD activity in the thigh meat, but also decreased MDA levels in these two tissues. Furthermore, Sasaki *et al.* (2010) reported that a daily dose of 10 mg of fucoxanthin per bird significantly decreased the plasma concentrations of thiobarbituric acid-reactive substances (TBARS) in broiler chickens. Amarked effect of fucoxanthin on antioxidant metabolism was observed in the present study not only in the liver tissue, characterized by a high level of metabolic activity, but also in the metabolism of the breast and drumstick tissues.

Microbial count is a significant criterion that defines the quality of meat. While some microorganisms are naturally found in meat, most contaminate meat during processing. It is known that the number of microorganisms in meat increases as storage time is extended (Imik *et al.,* 2012b). In the present study, it was determined that fucoxanthin significantly reduced the *Staphylococcus* spp. and *TMAB* counts in the breast and drumstick meat on the 5$^{th}$ and 6$^{th}$ days of storage. It was observed that the effects of fucoxanthin on bacteria were variable and limited (Tables 4 and 5). Although a previous study reported that marine algae have antimicrobial effects (Cox *et al.,* 2014), to the authors' knowledge, this is the only study on the antimicrobial effect of fucoxanthin on meat. In view of the available literature reports and based on the results obtained in the present study, it is suggested that both the growth and increase in number of the microorganisms found in the initial flora of meat during the storage period can be limited to a certain extent by the use of natural additives (Bórnez *et al.,* 2009; Imik *et al.,* 2012b).

Meat color has a strong influence on consumer preference. The globin, porphyrin ring and iron ion in

**Table 7 –** Effects of dietary fucoxanthin supplementation and storage period on colour parameters, water holding capacity and pH in chicken drumstick meat.

| Days | Groups | L* | a* | b* | WHC | pH |
|---|---|---|---|---|---|---|
| 1 | CONT | 51.00±2.85 | 6.51±0.25 | 6.51±0.61$^{b}$ | 0.987±0.002 | 6.11±0.10 |
| | FUCO1 | 58.90±5.00 | 6.49±1.29 | 11.77±0.95$^{a}$ | 0.992±0.001 | 6.05±0.05 |
| | FUCO2 | 47.98±1.62 | 6.84±1.08 | 8.43±0.89$^{b}$ | 0.986±0.004 | 6.27±0.01 |
| | *p*-value | ns | ns | ** | ns | ns |
| 2 | CONT | 54.73±3.99 | 6.98±0.40 | 6.89±0.83$^{ab}$ | 0.992±0.002 | 6.03±0.00 |
| | FUCO1 | 50.01±2.05 | 6.76±0.71 | 4.71±1.18$^{b}$ | 0.991±0.001 | 6.36±0.16 |
| | FUCO2 | 49.52±0.61 | 7.04±0.73 | 8.65±0.53$^{a}$ | 0.989±0.000 | 6.17±0.01 |
| | *p*-value | ns | ns | * | ns | ns |
| 3 | CONT | 56.09±0.20 | 5.66±0.50 | 6.33±2.66 | 0.989±0.002 | 6.11±0.20 |
| | FUCO1 | 52.04±1.93 | 6.76±1.11 | 6.85±1.43 | 0.985±0.002 | 6.12±0.10 |
| | FUCO2 | 48.70±1.25 | 8.35±0.94 | 9.30±0.68 | 0.988±0.001 | 6.35±0.01 |
| | *p*-value | ns | ns | ns | ns | ns |
| 4 | CONT | 50.06±1.87 | 6.98±1.17 | 6.93±1.37 | 0.989±0.000 | 6.21±0.13 |
| | FUCO1 | 49.67±2.35 | 8.16±1.88 | 9.64±0.78 | 0.990±0.000 | 6.23±0.08 |
| | FUCO2 | 47.39±1.00 | 6.62±0.26 | 7.54±1.24 | 0.991±0.000 | 6.25±0.04 |
| | *p*-value | ns | ns | ns | ns | ns |
| 5 | CONT | 51.16±1.85 | 6.74±0.70 | 6.34±0.94 | 0.987±0.001$_{b}$ | 6.43±0.36 |
| | FUCO1 | 53.43±0.73 | 7.06±0.63 | 10.49±1.75 | 0.987±0.001$^{b}$ | 6.10±0.13 |
| | FUCO2 | 51.51±0.88 | 6.71±0.81 | 9.92±0.55 | 0.995±0.000$^{a}$ | 6.37±0.06 |
| | *p*-value | ns | ns | ns | * | ns |
| 6 | CONT | 50.89±1.41 | 7.77±0.79 | 5.04±0.63 | 0.994±0.003 | 6.26±0.22 |
| | FUCO1 | 52.13±1.42 | 4.89±1.11 | 8.66±1.82 | 0.992±0.001 | 6.56±0.21 |
| | FUCO2 | 49.46±0.86 | 5.31±0.82 | 9.24±0.57 | 0.990±0.000 | 6.31±0.01 |
| | *p*-value | ns | ns | ns | ns | ns |

All values are given as mean ± SEM, (n=10). a-d: Means in the same column with different superscripts differ (*: $p<0.05$), (**: $p<0.01$). ns: not significant ($p>0.05$).

CONT, basal diet; FUCO1, basal diet+100 mg/kg diet of Fucoxanthin; FUCO2, basal diet+200 mg/kg diet of Fucoxanthin. L*: lightness, a*: redness, b*: yellowness. WHC: water holding capacity

the myoglobin and hemoglobin pigments, which give meat its specific color, are known to be very sensitive to oxidation (Barbut, 2016; Mancini & Hunt, 2005). Lipid peroxidation during storage not only adversely affects meat quality (color and taste), but also constitutes a health risk in the event of the consumption of meat that has undergone lipid peroxidation (Esterbauer *et al.,* 1991; Rhee, 1988). In the present study, it was determined that although fucoxanthin supplementation had no effect on the L* and a* values (color parameters) of the breast and drumstick meat, it significantly reduced the adverse effects of storage on the b*value (Tables 6, 7). Sasaki *et al.* (2010) reported that a daily oral dose of 10 mg of fucoxanthin administered to broiler chickens did not affect either meat *TBARS* concentration or L* and a* values, but significantly increased b* value. It has also been reported that the addition of 200 mg/kg of fucoxanthin to chicken breast meat, before or after cooking, increased a* and b* values and decreased the L* value on 1 and 6 days of storage when the meat was stored at +4°C (Sasaki *et al.,* 2008). Furthermore, it has been determined that, while the addition of marigold extract to broiler feed at percentages of 0.075%, 0.15%, 0.30% and 0.60%did not affect the L* value, the a* value was increased only by the addition of 0.60% of marigold extract, and the b* value was increased by the addition of 0.15%, 0.30% and 0.60% of marigold extract (Wang *et al.,* 2017). In a study with broiler chickens, Perenlei *et al.* (2014) determined that the addition of astaxanthin-rich yeast *(Phaffiarhodozyma)* to the feed at concentrations of 10 and 20 mg/kg did not have any effect on the L* value of breast meat at 0and 48 h, but its supplementation at a concentration of 20 mg/kg significantly increased both a* and b* values.

Meat water activity describes the ability of muscle tissue to retain moisture and directly influences meat palatability in terms of taste, tenderness and juiciness. The maintenance of meat $a_w$ at a minimal level is a major influence on *post-mortem* metabolism and meat quality (Isengard, 2001; Zhou *et al.,* 2010). In the present study, fucoxanthin had no effect on the $a_w$ of the breast or drumstick meat (Tables 6 and 7). To the authors' knowledge, there are no reports to date on the effect of fucoxanthin on the $a_w$ of meat. On the other hand, marigold extract, which contains lutein and zeaxanthin of the various carotenoid types, has been reported to decrease chicken meat water holding

capacity (Wang *et al.,* 2017), where as astaxanthin-rich yeast *(Phaffiarhodozyma)* has not shown such effect (Perenlei *et al.,* 2014).

The conversion of muscle glycogen into lactic acid and the reduction of meat pH value both play an important role in the *post-mortem* metabolism of meat. In the present study, fucoxanthin did not affect the pH values of the breast or drumstick meat of broiler chickens (on 1, 2, 3, 4, 5 and 6 days of storage) (Tables 6, 7). These results are consistent with the study of Perenlei *et al.* (2014), who supplemented a broiler feed with 10 and20 mg/kg of astaxanthin-rich yeast *(Phaffiarhodozyma)* and did not observe any effect on the pH value of the breast meat at 0 and 48 h post-slaughter. Furthermore, Wang *et al.*(2017)reported that marigold extract had no effect on the pH value of chicken thigh meat at 45 min and 24 h post-slaughter.

## CONCLUSION

The results of the present study show that the dietary addition of fucoxanthin did not affect the growth performance of broilers. However, it reduced *Staphylococcus* spp. counts in the meat obtained and had variable effects on other microorganisms in meat. The effects of fucoxanthin on meat color parameters were also variable. In addition, fucoxanthin dietary supplementation enhanced CAT and SOD activities in the liver, breast and drumstick tissues, and the reduction of lipid peroxidation levels demonstrated that fucoxanthin is a natural antioxidant capable of regulating the antioxidant metabolism of chicken meat.

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