Effect of Adding Different Levels of Astaxanthin Extracted From an Algae Haematococcus Pluvialis to The Diet on Some Immunological Characteristics of Broilers Reared Under Natural and Elevated Environmental Conditions

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Abstract

This experiment was conducted at the poultry farm / Department of Animal Production / College of Agriculture / Al-Qasim Green University And for two experiments, The first for the period from 27/4/2019 to 7/6/2019 and the second from 1/7/2019 to 4/8/2019 for the second experiment to see the effect of adding different levels of astaxanthin to the broiler diet on some immune characteristics of broilers raised under environmental conditions Natural and elevated. Use 240 unsexed birds of one day age ROSS 308 strain, distributed randomly into five treatments by 48 birds/treatment and the birds of each treatment were divided into three replicates (16 birds/ replicate). The chicks were fed on three diets that included the initiator, growth and final 23, 21.5 and 19.44% crude protein respectively, and the representative energy was 3000.5, 3100.7 and 3199.25 kcal/kg feed, respectively, in addition to the astaxanthin powder at levels 0, 10, 20, 30, 40 mg/kg of feed for T1, T2, T3, T4 and T5 treatments, respectively. The results of the first trial showed a significant superiority (P<0.05) for treatment T2 in the relative weight of the fabrichia gland and for the fabrichia index, and significant superiority for treatment T5 and T3 in the size standard of antibodies directed against Newcastle disease, while treatment T2 and T3 outperformed the size criterion of antibodies directed against camboro disease compared With the control treatment T1, and the second trial, the additional factors T2, T3, T4 and T5 achieved significant superiority (P<0.01) in all the immunological characteristics studied by treatment T1. It is concluded from this study that the addition of astaxanthin to the broiler meat diet led to an improvement in the immune characteristics of broilers raised under normal and elevated environmental temperatures.

Keywords: Poultry meat, Heat stress, Astaxanthin.

1. Introduction

The poultry industry has witnessed widespread interest by researchers and breeders interested in developing this sector as a result of the rapid growth and high efficiency in food conversion [1]. Poultry meat and its products are considered one of the most important sources of animal protein with high nutritional value and a health food ingredient for the people of the world. It has biological importance in building the body and maintaining human health [2], but this development has been accompanied by some basic problems such as stress, which is one of the stresses that cause great economic losses in the poultry industry [3], which causes a decrease in product performance. The immune system and the deterioration of the meat quality of broilers as a result of the weak digestive system’s ability to digest and absorb nutrients, the peripheral vessels expand and the blood flow decreases, The secretion of the hormone Corticosterone responsible for damage to the intestinal mucosa increases, causing the production of inflammatory factors that activate in the intestinal epithelium that cause an increase in the permeability of the mucous membrane to enter the antigens Pathogenic and thus negatively affected health [4-6], and the disruption of the microbial balance of the bird's gastrointestinal tract is reflected in the increase of harmful microorganisms at the expense of the beneficial ones, which negatively affects the vitality, performance and immunity of broilers [7,8]. As a result, recent scientific research and studies have tended towards finding alternatives and practical solutions that reduce these obstacles by using safe natural additives in poultry diets such as carotenoids that are deposited within the body tissues and enhance the productive and immune performance [9,10]. The extracted Astaxanthin was
considered From the alga *Haematococcus pluvialis* is a safe natural substance and effective antioxidant according to the authorization issued by the European Food and Safety Authority (EFSA) and the Food and Allergy Committee (NDA) to use it as a food supplement for humans and animals [11], as its importance is due to its extension across the cell membrane (dual Layer) compared to other antioxidants whose effect is either in specific locations inside or outside the cell membrane [12,13]. It deals with an unspecified number of free radicals generated as a result of oxidative stress, inhibiting their action and protecting protein, fat and cell membranes from processes Oxidation [14,15] and disposal of hydrogen peroxide, which causes damage to all cell components such as lipids, proteins, and nucleic acids [16,17]Which improves the functions of the immune system [18,19], and in view of the immunological and therapeutic roles of Astaxanthin as a natural antioxidant and the presence of limited studies on the use of this substance in poultry diets, this study aimed to use Astaxanthin in poultry meat and to determine the optimal level of reduction From oxidative damage and maintaining the immune performance of broilers.

2. Materials and Methods

This experiment was conducted at the poultry farm / Department of Animal Production / College of Agriculture / Al-Qasim Green University and for two experiments, the first from 27/4/2019 to 7/6/2019 and the second from 1/7/2019 to 4/8/2019 to study the effect of adding Astaxanthin. In different proportions 0, 10, 20, 30, 40 mg / kg of feed to feed in the immune performance of broilers. In this experiment, 240 unsexed birds were used with the ROSS 308 equipped from a private hatchery. The birds were randomly distributed at one day age and with a starting weight of 41 and 38 g/chick for the two experiments respectively for five treatments. Each treatment contained three replicates, at a rate of 16 chicks / replicate, as follows: The first treatment (T1): a control treatment devoid of any addition. The second treatment (T2): Add 10 mg Astaxanthin/kg feed. Third treatment (T3): Add 20 mg Astaxanthin/kg feed. Fourth treatment (T4): Add 30 mg Astaxanthin/kg feed. Fifth treatment (T5): Add 40 mg Astaxanthin/ kg feed. The Astaxanthin powder used in the study was obtained from AstaPure® and comes naturally from *Haematococcus pluvialis*. The chicks were raised in cages with dimensions of 2 x 1.5 m and all the appropriate conditions were created for the first experiment, while a special program for heat stress was used for the second experiment, starting from the first week of the birds' life, and temperature readings were taken at 12:00, 2:00, 4:00, 6:00 hours after Back using four thermometers distributed inside the breeding room, where the temperature ranges between 34-36 °C, the birds were vaccinated against infectious bronchitis, Newcastle and Infectious bursal disease and bird flu according to a vaccination program prepared for this purpose. The chicks were fed free feed Ad libitum with fodder Crushed during the period of the experiment, a standardized starter diet was used for the period from 1-21 days of its life. It contained 23% raw protein 3000.5 kcal/kg energy feed represented according to the feeding guide for chicks ROSS 308 to form the energy to protein ratio (C/P Ratio) 130.45, followed by the diet The first and second growth, which contained 21.5% and 19.44% crude protein and represented energy 3100.7 and 3199.25 kilocalories/kg of feed, and with this the energy to protein ratio was equal to 144.218 and 164.57, respectively, which continued until the end of the experiment.

The relative weight of the fabricia pod (the weight of the fabricia pod to the live body weight) was studied after weighing the birds individually, then slaughtering them, and extracting the Fabricia pod after cutting the connecting tissue around the pouch, then weighing it with a sensitive scale of four decimal places and calculating the relative weight of the pouch according to the method of [20] According to the following equation:

\[
\text{Relative weight of fabricia pod} = \left( \frac{\text{pod weight (g)}}{\text{vivo weight (g)}} \right) \times 100
\]

While the Fabricchia index was calculated as indicated by [20] according to the following equation:

\[
\text{Fabricchia index} = \frac{\text{relative weight of bursa in experimental treatment}}{\text{relative weight of bursa in control treatment}}
\]

As for the volumetric standard (Titer) of serum antibodies directed against the fever causing NDV and IBD patients, they were measured at the end of the experiment period as reported by [21] to determine the level of the bird's immune response. Data were analyzed using the ready-made statistical program [22] and the design was applied In complete randomization of data analysis,[23] was used to test the differences between the multi-data factors and compare the significant differences between the averages of the studied traits.

Results and Discussion

Table (1) for the first experiment shows a significant improvement (P<0.05) in both the relative weight of the fabricia pod and the fabricia index in favor of the addition treatment T2 compared to the treatment T1, which was matched by T3, T4, and T5 respectively, and in the volumetric criterion of antibodies directed against disease Newcastle, the T5 addition treatment
recorded a significant increase compared to the rest of the trial factors, and the effect was similar to the two addition factors T3 and T2, which did not significantly differ on treatment T4 and which did not differ significantly from treatment T1. As for the size criterion of antibodies directed against camboro disease, the two addition factors T2 and T3 increased significant compared to treatment T1 that did not differ significantly with T4 and T5, respectively.

Table (2) for the second experiment shows that there is significant superiority (P<0.01) in the relative weight of the fabricia pod and in favor of the addition factors T2, T3, T4 and T5 compared to the control treatment (T1), and in the volumetric standard of antibodies directed against Newcastle disease, the treatment recorded a significant increase in T2. Compared to the rest of the trial parameters, and their similarity in the effect, T3 and T5, which did not differ significantly from treatment T4 and then T1, and in the volumetric criterion of antibodies directed against camboro disease, the addition treatment T3 recorded significant superiority compared to the rest of the trial factors, followed by the effect T2, T4, T5, and then T1, respectively.

The reason for the significantly excelled in the relative weight of the Fabricia follicle and the Fabricia index for treatment T2 in the first experiment compared to treatment T1 may be due to the nature of the experiment conditions that favored its results for the less concentrated treatment of Astaxanthin despite the absence of significant differences between it and the other addition treatments that were mathematically excelled to the control treatment T1. Whereas, the results of the second experiment indicate that the increase in Astaxanthin concentrations was matched by a significant increase in all the studied immune traits due to the different conditions of the experiment in which heat stress was used. The reason for the relatively high weight of the fabricia pod of the groups of birds fed on diets containing Astaxanthin may be due to the apparent role of Astaxanthin in protecting the immune cell membranes from heat stress damage and raising the immune response of birds through the process of phagocytosis and providing them with immunity during the attack of parasites and viruses due to its containment of hydroxyl groups.

Which plays the role of donors of hydrogen and works to break down free radicals and make them ineffective at the beginning of the chain of oxidation processes of fatty acids in cell membranes, thus maintaining the flexibility of those membranes that have an important role in antigen diagnosis, rapid cell response and repair of damaged membranes [14,24,25]. As Macrophages swallow the antigen and then display a part of the antigen on their membrane, this leads to the stimulation of the lymphocytes to produce antibodies, in addition to these cells secreting both interleukins and interferon's, which stimulate the type B lymphocytes to produce antibodies. Also, the accumulation and sedimentation of Astaxanthin inside the tissues of the bird's body in addition factors contributed to the enhancement and proliferation of the production of some organic acids in the intestinal lumen, such as lactic acid from the genera Lactobacillus, Streptococcus and Bifidobacterium, thus increasing the fermentation activity in them, which in turn increases the concentration of short-chain fatty acids, thus reducing the pH. Providing an acidic environment that is not suitable for the growth of pathological bacteria will have an inhibitory and fatal effect, and it is one of the reasons for the good health and immune status of the addition treatment birds and the increase of antibodies directed against Newcastle and Cambro patients [26,27,28]. The improvement in the values of the weight of the follicle and the index of fabricia in this study is an indication of the availability of immunity and raising the immune response of the bird, as the superiority of the fabricia index for the value of the control treatment is evidence of an improvement in the immune response as this gland is responsible for humoral immunity [29], Astaxanthin is a carotenoid that reduces oxidative damage and improves immune system function [30,31,32].

It also plays a role in promoting, activating, and dividing B-lymphocytes. Raising the level of immune performance by increasing the antibody criteria, which is the best guide for determining the resistance in birds against Newcastle disease. This is confirmed by [33] that measuring the level of antibodies in bird serum gives sufficient evidence to determine the bird’s immunity against Newcastle disease with an increase in the natural killer cells (NK). For the production of interleukin-2, and in general all the measures of the immune response in this study indicate an improvement and activity of the immune system and the immunity of the body for transactional birds that have deposited this substance in their tissues for the role of Astaxanthin in maintaining immune cell membranes, inhibiting free radicals and maintaining the flexibility of cell membranes, which have an important role. In diagnosing antigen, rapid cell responses, and repairing damaged membranes. The results of this study are consistent with what [34,35,36,37,38,39] who observed an improvement in immunological parameters when using Astaxanthin in poultry diets.
Table 1. Effect of addition of different levels of Astaxanthin on the immunological characteristics of broilers reared under normal environmental temperatures (mean ± standard error).

| The Studied traits                                      | T1                   | T2                   | T3                   | T4                   | T5                   | Significant level |
|--------------------------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|-------------------|
| The relative weight of the fabricia bod (%)            | ±0.006±0.110 b       | 0.15±0.008 a         | 0.13±0.003 ab        | 0.12±0.011 ab        | 0.14±0.018 ab        | *                 |
| Fabricia Guide                                         | 1.07±0.06 b          | 1.53±0.088 a         | 1.27±0.033 ab        | 1.20±0.115 ab        | 1.43±0.185 ab        | *                 |
| Volumetric standard for antibodies directed against Newcastle disease | 154.1±124.80 c       | 2150±198.52 abc      | 3042±858.87 ab       | 1738±242.03 bc       | 3196±163.77 a       | *                 |
| The volumetric standard for antibodies directed against camboro disease | 7265±2551.1 b        | 13752±1140.56 a      | 13610±558.01 ab      | 9579±218.52 ab       | 10936±381.16 ab     | *                 |

* The different letters within the same row mean that there are significant differences between the averages of the treatments.
** Means the presence of significant differences at (P<0.05) in the ANOVA table.
*** The parameters include the following: - T1 control treatment without addition, T2, T3, T4, T5 and the addition of Astaxanthin at level 10, 20, 30, 40 mg/kg feed, respectively.

Table 2. Effect of adding different levels of Astaxanthin to the suspension on the immunological characteristics of broilers reared under elevated environmental temperatures (mean ± standard error).

| The Studied traits                                      | T1                   | T2                   | T3                   | T4                   | T5                   | Significant level |
|--------------------------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|-------------------|
| The relative weight of the fabricia bod (%)            | 0.07±0.003 b         | 0.13±0.004 a         | 0.12±0.007 a         | 0.13±0.002 a         | 0.12±0.003 a         | **                |
| Fabricia Guide                                         | 0.99±0.047 b         | 1.94±0.066 a         | 1.87±0.109 a         | 1.95±0.040 a         | 1.91±0.058 a         | **                |
| Volumetric standard for antibodies directed against Newcastle disease | 611.66±65.13 c       | 3171.67±34.41 a      | 2836.67±112.49 ab    | 2455±276.63 b        | 2847.67±83.66 ab     | **                |
| The volumetric standard for antibodies directed against camboro disease | ±226.65 c            | ±461.29 a            | ±443.64 a            | ±158.80 a            | ±130.78 a            | **                |

* The different letters within the same row mean that there are significant differences between the averages of the treatments.
** Means the presence of significant differences at (P <0.01) in the ANOVA table.
*** The parameters include the following: - T1 control treatment without addition, T2, T3, T4, T5 and the addition of Astaxanthin at level 10, 20, 30, 40 mg/kg feed, respectively.
References

[1] Marangoni, F. G., Corsello, C., Cricelli, N., Ferrara, A., Ghiselli, L., Lucchin, and A. Poli, (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. Food & nutrition research, 59, 27606.

[2] Donna, M.M.; and O. Donna, (2017). Beneficial Effects of Poultry Meat Consumption on Cardiovascular Health and the Prevention of Childhood Obesity. Med One, 2(4).

[3] Hirakawa, R.; S: Nurjanah, K.; Furukawa, A.; Murai, M.; Kikusato, T.; Nochi, and M. Toyomizu, (2020). Heat stress causes immune abnormalities via massive damage to effect proliferation and differentiation of lymphocytes in broiler chickens. Frontiers in Veterinary Science, 7, 46.

[4] Olfati, A.; A; Mojtabahedin, T.; Sadeghi, M.; Akbari, and F. Martínez-Pastor. (2018). Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress and thermo neutral conditions. Spanish journal of agricultural research, 16: 15.

[5] Zaboli, G.; X; Huang, X.; Feng, and D.U. Ahn, (2019). How can heat stress affect chicken meat quality? - a review. Poultry sci, 98: 1551-1556.

[6] Emami, N.K., U.; Jung, B.; Voy, and S. Dridi, (2021). Radical Response: Effects of Heat-Stress-Induced Oxidative Stress on Lipid Metabolism in the Avian Liver. Antioxidants, 10(1), 35.

[7] Alhenaky, A.; A; Abdelqader, M.; Abujamieh, and A.R. Al-Fatafah, (2017). The effect of heat stress on intestinal integrity and Salmonella invasion in broiler birds. Journal of thermal biology, 70: 9-14.

[8] Shi, D.; L; Bai, Q.; Qu, S.; Zhou, M.; Yang, S.; Guo, and C. Liu, (2019). Impact of gut microbiota structure in heat-stressed broilers. Poultry science, 98: 2405-2413.

[9] Çalıslar, S. (2019). The Important of beta carotene on poultry nutrition. Selcuk Journal of Agriculture and Food Sciences, 33(3), 252-259.

[10] Nabi, F.; Arain, M.A., N.; Rajput, M.; Alagawany, J.; Soomro, M.; Umer, and J. Liu, (2020). Health benefits of carotenoids and potential application in poultry industry: A review. Journal of animal physiology and animal nutrition, 104(6), 1809-1818.

[11] Turck, D.; J; Castenmiller, S.; de Henauw, K.I.; Hirsch-Ernst, J.; Kearney, A.; Macciuk, and K. Pentieva, (2020). Safety of Astaxanthin for its use as a novel food in food supplements. EFSA Journal, 18(2).

[12] Yang, Y.; B; Kim, and J.Y. Lee. (2013). Astaxanthin structure, metabolism, and health benefits. J. Hum. Nutr. Food Sci., 1003:1-1003:11.

[13] Ekpe, L.; Inaku, and V. Ekpe, (2018). Antioxidant effects of Astaxanthin in various diseases-A review. Journal of Molecular Pathophysiology, 7: 1-6.

[14] Ambati, R.R.; P.; Moi, S.; Ravi, and R.G. Aswathanarayana. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar. Drugs, 12,128-152.

[15] AL-Rubeii, A.M. and AL-Ghanimi, G.M. (2020). Studying the effect of adding different concentrations of Astaxanthin and allyl isothiocyanate and their synergistic action in lipid oxidation and some quality characteristics for minced veal meat at cold storage. Diyal Journal of Agricultural Sciences, 2020, Volume 12, Issue 1.

[16] AL-Kanaan, Z.T; Al-Ali, R.M. and M.A. AL-Taj. (2017). Study Activity Antioxidant Astaxanthin dye extracted from the shrimp shell. Thi-Qar University Journal for Agricultural Researches, Volume 6(1) pp: 41-51.

[17] Alsultani, M.J., Abed, H.H., Ghazi, R.A., Mohammed, M.A. , (2020), Electrical Characterization of Thin Films (TiO2: ZnO)1-x(GO)x / FTO Heterojunction. Materials Today: Proceedings, 35:5530-5536.

[18] Yamaahishi E. (2013). Astaxanthin as a medical food. Funct Foods Health Dis; 3: 254-258.

[19] Yamaahishi E. (2015). Let Astaxanthin be thy medicine. Pharmacy Nutrition 3: 115–122.

[20] Shakir, A.A., Salman, E.F., Shakir, A.J., Mohammed, M.A., Abdulridha, W.M., Almayahi, B.A. , (2019), Optical properties of polyvinyl alcohol membrane with n-HAp for bio-medical applications, Pensa Medica Argentina, 105 (11), pp. 836-841.

[21] Mond, G.A. (1997). Development of specific ELISA antibody Monoclonal antibody. Poult. Sci. 76:302-301.

[22] SAS. (2012). Statistical Analysis System, User’s Guide. Statistical. Version 9.1 ed. SAS. Inst. Inc. Cary. N.C. USA.

[23] Duncan, D.B. (1955). Multiple Range and Multiple F-test. Biometrics, Vol. 11, No. 1, pp. 1-42.

[24] Sun, T.; R; Yin, A.D.; Magnuson, S.A.; Tolba, G.; Liu, and Lei, X.G. (2018). "Dose-Dependent Enrichments and Improved Redox Status in Tissues of Broiler Chicks under Heat Stress by Dietary Supplemental Micronagal Astaxanthin. Journal of agricultural and food chemistry, 66: 5521-5530.

[25] Kim, S.H.; and H. Kim, (2018). Inhibitory Effect of Astaxanthin on Oxidative Stress-Induced Mitochondrial Dysfunction-A Mini-Review. Nutrients, 10: 1137.

[26] Yonei, Y.; M; Yagi, M.; Nakamura, L.; Parengkuan, M.; Ogura, T.; Taira, S.; Asano, and H.H. Liu, (2013). Effects of Astaxanthin on intestinal microflora in mice fed a high-fat diet. Anti-Aging Medicine, 10: 77-91.

[27] Salazar, P.C.R.; G; de Valle Polycarpo, J.C.; Dadalt, D.A.P.; Ribeiro, P.M.F.; de Castro Burbarelli, V.C.; Cruz-Polycarpo, and R. Albuquerque, (2018). Lactic and butyric acids, isolated and associated, as alternatives to avilamycin on the immune response of broiler chickens to Newcastle disease. Avian Pathol., 47:194-198.

[28] Konnai, G.; D; Sapcota, G.K.; Saikia, P.; Deka, J.D.; Mahanta, N.; Kalita, B.N.; Saikia, and J.K. Talukdar, (2019). Studies on immune response to Newcastle disease virus in broiler chickens fed with Lactobacillus reuteri PFA16 isolated from the gut of indigenous chickens of Assam, India. Veterinary world, 12: 1251–1255.

[29] Abdul Ahad, A. E. (1996). Diseases and Immunology of Borrelia Anserina spores in chickens. IOP Conf. Series: Earth and Environmental Science 410 (2021) 012003 doi:10.1088/1755-1315/410/1/012003
[31] Ruiz García, L., S., Delgado, P., Ruas-Madiedo, B., Sánchez, and A. Margolles Barros, (2017). Bifidobacterium and their molecular communication with the immune system.

[32] Vieco-Saiz, N., Y., Belguesmia, R., Raspoet, E., Auclair, F., Gancel, I., Kempf, and D. Drider, (2019). Benefits and Inputs From Lactic Acid Bacteria and Their Bacteriocins as Alternatives to Antibiotic Growth Promoters During Food-Animal Production. Frontiers in microbiology, 10, 57.

[33] Grimes, S.E. (2002). A basic laboratory manual for the small-scale production and testing of I-2 Newcastle disease vaccine. RAP publication, 136.

[34] Kim, K.S.; J.H.; Lee, M.S.; Shin, M.S.; Cho, Y.P.; Kim, S.K.; Cho, and Y.J. Kang, (2005). Effect of dietary probiotics supplementation contained with Astaxanthin produced by Phaffia rhodozyma on the productivity and meat quality of ducks. Korean Journal of Poultry Science, 32: 73-80.

[35] Takimoto, T.; K.; Takahashi, and Y. Akiba, (2007). Effect of dietary supplementation of Astaxanthin by Phaffia rhodozyma on lipid peroxidation, drug metabolism and some immunological variables in male broiler chicks fed on diets with or without oxidized fat. Br Poult Sci.;48:90-7.

[36] Lee, C.; B.; Lee, J.; Na, and G. An. (2010). Carotenoid Accumulation and Their Antioxidant Activity in Spent Laying Hens as Affected by Polarity and Feeding Period. Asian-Australasian Journal of Animal Sciences, 23: 799-805.

[37] Ohh, M.H.; S.; Kim, S.C.; Pak, and K.M. Chee, (2016). Effects of Dietary Supplementation with Astaxanthin on Histamine Induced Lesions in the Gizzards of Broiler Chicks. Asian-Australas J Anim Sci. 29:672-8.

[38] Elwan, H.; S.S.; ELNESR, Y.; Abdallah, A.; Hamdy, and A.H. El-Bogdady, (2019). Red yeast (Phaffia rhodozyma) as a source of Astaxanthin and its impacts on productive performance and physiological responses of poultry. World’s Poultry Science Journal. 75. 273-284.

[39] Ao, X.; and H.I. Kim. (2019). Effects of Astaxanthin produced by Phaffia rhodozyma on growth performance, antioxidant activities, and meat quality in Pekin ducks. Poultry sci, 98: 4954-4960.