

Effect of addition annatto seed powder (*Bixa orellana*) and astaxanthin to the diet in carcass traits, microflora, and oxidation indices after meat storage for broiler (Ross-308)

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Abstract.

This experiment was conducted in the farm of Al-Anwar Poultry Company in Babil Governorate, for a period 35 days from 15/2/2022 - 22/3/2022, during which the effect of adding powder annatto seeds (*Bixa orellana*) and astaxanthin to the diet in quality traits, microorganisms and oxidative indices after storage, using 225 unsexed one-day-old (Ross-308) chicks, The chicks were randomly divided 15 cages with 5 treatments (45 chicks per treatment) and each treatment contains 3 replicates for each replicate 15 chicks, and the experimental treatments were as follows: without addition, Annatto seed powder 0.5 & 1 g / kg feed, astaxanthin 50 & 100 mg / kg feed for T1, T2, T3, T4, and T5 respectively. As for the following study results: significant superiority ($p \leq 0.01$) for T3 and T5 treatments in carcass percentage, gizzard, and spleen relative weight, and for T4 treatment in the liver, and bursa of fabricius, meanwhile significant improvement ($p \leq 0.01$) for T3, T4, and T5 in the chest, as well as for T2, T3, and T5 in thigh, significant superiority ($p \leq 0.01$) for T2, and T1 in neck and back respectively. A significant increase ($p \leq 0.01$) for all treatments addition in ileum Lactobacillus count as well as a significant decrease ($p \leq 0.01$) for all treatments addition in ileum E. coli count. A significant decrease for all treatments addition in FFA%, PV, and TBA.

Key Word: annatto seeds, astaxanthin, Broiler Chickens , Quality traits

Introduction

The use of antibiotics and medicines in poultry feed harms on the health of birds and human consumers, as strains of pathological microorganisms have emerged that are resistant to antibiotics, in addition to their effect on the immune system of birds and consumers alike [21 , 39] This was an incentive for the researchers to turn to the use of alternatives to reduce these constraints by

using natural antioxidants in poultry diets, which proved their ability to improve the productive characteristics of poultry birds by inhibiting the growth of pathological microbiota and improving the effectiveness of beneficial microbiota in the intestine [13 , 30 , 33]. Annatto seeds (*Bixa orellana* L) known as achiote, is a type of tree plant from tropical regions in America, from which a pigment containing a high percentage of carotene is

extracted consisting mainly of bixin and amounts of isopixin and norbixin [32], and most breeders prefer to use these seeds as they have an antimicrobial and antioxidant role and phenolic compounds and vitamin E content [40 , 23 , 4 , 18]. Astaxanthin extracted from *Haematococcus pluvialis* is one of the natural carotenoids and a safe natural antioxidant that has obtained a permit from the European Food and Safety Authority (EFSA) and the Food and Allergy Committee (NDA) regarding the safety and validity of the product as a food supplement for humans and animals added to poultry diets [14 , 34] , The importance of Astaxanthin is that it deals with an unlimited number of free radicals generated as a result of oxidative stress, inhibiting their action and protecting protein, lipids and cell membranes from oxidative processes by extending across the cell membrane (double-layer) compared to other antioxidants whose effect is either on specific sites Inside or outside the cell membrane [3] .

Materials and methods

This study was conducted in the fields of Al-Anwar Poultry Company in Babil Governorate for 35 days from 15/2/2022 until 22/3/2022, 225 chicks (Ross-308) were used one day old unsexed and the chicks were reared in ground cages (pens) with 15 cages of 1.5×1 m² dimensions were randomly divided with 5 treatments (45 chicks per treatment) and each

treatment contained 3 replications for each replicate of 15 chicks , and the experimental treatments were as follows: T1 control treatment without addition, T2 and T3 adding Annatto seed powder (0.5 & 1 g / kg feed) , T4 and T5 adding astaxanthin (50 & 100 mg / kg feed), and all supplies were prepared And the appropriate environmental conditions required for raising the broiler chickens used in the experiment, and special programs were used to vaccinate the birds as recommended in the breeding of chickens. chicks were fed on a starter diet (protein ratio 23.04% and energy amount 3021.45 kcal/kg feed) from the age of one day until the third week of the birds' life, after that it was replaced by a growth diet (protein ratio 20.06 and the amount of energy 3194.92 kcal /kg feed) until the end of the fifth week, and table (1) shows the composition of my diet Starter and growth used in feeding the chicks throughout the duration of the experiment and the calculated chemical composition, and it was measured carcass Percentage and weight of carcass and internal edible and non-edible viscera based on the method [1], and the calculation of the number of microorganisms in the ileum based on the method [19], and indicators of oxidative for meat after storing the meat for a while 60 days by freezing at -18°C TBA according to method [38] as for PV and FFA according to method [11], use Statistical Analysis System – SAS [31] in analyzing the data to study the

effect of different treatments on the studied traits according to a complete random design (CRD), and the significant differences

between the averages were compared with Duncan's [10] multinomial test.

Table 1: Percentages of the components of the diet used in the study and its chemical composition

Ingredients	starter diet % 1-21 days	growth diet % 22-35 days
yellow corn	30	40
wheat	28.25	24
soybean meal (48% protein)	31.75	24.8
protein concentrate	5	5
sunflower oil	2.9	4.4
limestone	0.9	0.6
Dicalcium PhosphateDCP	0.7	0.9
Premix	0.2	0.2
salt	0.3	0.1
Total	100	100
Crude protein (%)	23.04	20.06
Metabolic energy (kilocalories/kg feed)	3021.45	3194.92
lysine %	1.27	1.07
methionine %	0.41	0.38
cysteine%	0.35	0.30
Methionine + cysteine %	0.82	0.78
available phosphorous %	0.41	0.43
c/p Energy Ratio : Protein %	131.14	159.77

*Protein center type Brocon-5 Special W: Chinese origin, each kg of which contains:: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorus, 3.25% lysine, 3.90% methionine + cysteine, 2.2% sodium, 2100 kilocalories / kg represented energy, 20000 IU vitamin A, 40,000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6, 300 mg B12, 10 mg folic acid, 100 mcg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT)

**The chemical analysis of the diet was calculated according to NRC [25].

Results

carcass traits

carcass Percentage and internal viscera

Table 2 results indicate to the effect of the treatments on the dressing percentage and internal viscera, concerning the carcass percentage (without the internal viscera) we note that there is a highly significant superiority ($p \leq 0.01$) for T3 and T5 compared to the treatments T1, T2 and T4, as for the liver we see a significant superiority ($p \leq 0.05$) for T4 compared to treatment T2, As for the

heart that are no significant differences for all treatments of the experiment. As for the gizzard we see a highly significant superiority ($p \leq 0.01$) for treatments T2 and T5 compared to treatments T1 and T4, as well as treatments T3 and T4 excelled compared to treatment T1. As for the spleen we see a significant superiority ($p \leq 0.05$) for the treatment T5 compared to the treatment T4, and for the bursa of fabricius we notice a significant superiority ($p \leq 0.05$) for treatment T4 compared to treatment T2.

Table 2: Effect of adding Annatto seed powder (*Bixa orellana*) and astaxanthin to the diet of broiler chickens in the carcass percentage of offal and internal viscera.

Treatments	mean \pm standard error					
	carcass percentage %	liver	heart	gizzard	spleen	bursa of fabricius
T1	72.78 \pm 0.14b	2.47 \pm 0.12ab	0.530 \pm 0.03	1.88 \pm 0.01c	0.145 \pm 0.01ab	0.145 \pm 0.02ab
T2	72.70 \pm 0.01b	2.36 \pm 0.08b	0.555 \pm 0.02	1.99 \pm 0.01a	0.170 \pm 0.02ab	0.085 \pm 0.01b
T3	75.01 \pm 0.89a	2.55 \pm 0.09ab	0.475 \pm 0.04	1.97 \pm 0.01ab	0.150 \pm 0.02ab	0.105 \pm 0.02ab
T4	72.80 \pm 0.02b	2.96 \pm 0.26a	0.560 \pm 0.09	1.94 \pm 0.01b	0.110 \pm 0.02b	0.160 \pm 0.01a
T5	75.76 \pm 0.12a	2.67 \pm 0.01ab	0.530 \pm 0.05	2.01 \pm 0.01a	0.195 \pm 0.02a	0.150 \pm 0.02ab
significant	**	*	N.S	**	*	*

Means with different letters indicate a significant difference in probability level.
* (P<0.05), ** (P<0.01), NS: not significant.

Carcass

Table 3 results indicate to the effect of the treatments on the percentage of carcass, with regard to chest we see a highly significant superiority ($p \leq 0.01$) for treatments T3, T4 and T5 compared to treatments T1 and T2. As for the thigh we also notice a highly significant superiority ($p \leq 0.01$) for the treatments T2, T3 and T5 compared to the treatments T1 and T4, and in the neck we notice a significant

superiority ($p \leq 0.05$) for treatment T2 compared to treatments T3 and T5, as for the wing the table shows that there are no significant differences for all the experimental treatments, and for the back we see a highly significant superiority ($p \leq 0.01$) for treatment T1 compared to treatments T2, T3, T4 and T5, as well as treatment T4 excelled compared to treatment T2 and T5, and treatment T2 and T3 excelled compared to treatment T5.

Table 3: Effect of adding Annatto (*Bixa orellana*) seed powder and astaxanthin to the broiler diet in the percentage of carcass.

Treatments	mean \pm standard error				
	chest	thigh	neck	wing	back
T1	38.04 \pm 0.02b	26.00 \pm 0.13b	5.85 \pm 0.42ab	10.00 \pm 0.10	19.10 \pm 0.33a
T2	37.72 \pm 0.19b	29.76 \pm 0.77a	6.59 \pm 0.17a	9.81 \pm 0.03	15.32 \pm 0.63c
T3	39.07 \pm 0.42a	29.50 \pm 0.61a	5.39 \pm 0.27b	9.07 \pm 0.86	16.20 \pm 0.06bc
T4	39.44 \pm 0.19a	27.29 \pm 0.78b	5.92 \pm 0.22ab	9.25 \pm 0.48	17.28 \pm 0.50b
T5	39.52 \pm 0.11a	30.82 \pm 0.06a	5.45 \pm 0.13b	10.31 \pm 0.26	12.68 \pm 0.04d
significant	**	**	*	N.S	**

Means with different letters indicate a significant difference in probability level.
* (P<0.05), ** (P<0.01), NS: not significant.

Ileum microflora count

Table 4 results indicate to the effect of the treatments on the numbers of microorganisms in the ileum, which include both Lactobacillus bacteria and E-Coli bacteria, the preparation of Lactobacillus bacteria we notice a highly significant superiority ($p \leq 0.01$) for treatment T3 compared to treatments T1, T2, T4 and T5, as well as treatments T4 and T5 excelled

compared to treatments T1 and T2, and treatment T2 excelled compared to treatment T1, about the number of E-Coli bacteria we see a highly significant improvement ($p \leq 0.01$) for treatment T5 compared to treatment T1, T2, T3 and T4, as well as treatment improvement T2, T3 and T4 compared to treatment T1.

Table 4 : Effect of adding Annatto seed powder (*Bixa orellana*) and astaxanthin to broiler diet in the microflora count in the ileum (Lactobacillus and E-Coli).

Treatments	mean \pm standard error	
	Lactobacillus	E-Coli
T1	4.03 \pm 0.03 d	4.47 \pm 0.03 a
T2	4.27 \pm 0.02 c	4.28 \pm 0.02 b
T3	4.71 \pm 0.02 a	4.27 \pm 0.07 b
T4	4.61 \pm 0.01 b	4.25 \pm 0.05 b
T5	4.55 \pm 0.01 b	3.95 \pm 0.05 c
significant	**	**
Means with different letters indicate a significant difference in probability level. **(P<0.01).		

Indicators of oxidative in meat

Table 5 results indicate to the effect of the treatments on the values of thiobarbituric acid (TBA), the peroxide value (PV) and the percentage of free fatty acid (FFA), with regard to the values of thiobarbituric acid (TBA) we see a highly significant improvement ($p \leq 0.01$) for treatment T5 compared to treatment T1, T2 and T4, and treatment T3 improved compared to treatment

T1 and T2, and treatment T2 and T4 improved compared to treatment T1. As for the peroxide value (PV) we notice a significant improvement ($p \leq 0.05$) for treatments T2, T3, T4 and T5 compared to treatment T1. As for the percentage of free fatty acids (FFA) we see a highly significant improvement ($p \leq 0.01$) for treatment T3 compared to treatment T1 and T2, as well as treatment improvement T2, T4 and T5 compared to treatment T1.

Table 5: Effect of adding annatto seed powder (*Bixa orellana*) and astaxanthin to broiler diet in the values of thiobarbutyric acid (TBA), peroxide number (PV) and free fatty acids (FFA) .

Treatments	mean \pm standard error		
	TBA	PV	FFA%
T1	0.076 \pm 0.001 a	5.14 \pm 0.02 a	0.640 \pm 0.02 a
T2	0.049 \pm 0.001 b	4.02 \pm 0.02 b	0.510 \pm 0.01 b
T3	0.042 \pm 0.001 cd	3.70 \pm 0.005 b	0.405 \pm 0.005 c
T4	0.045 \pm 0.001 bc	3.84 \pm 0.04 b	0.460 \pm 0.01 bc
T5	0.0405 \pm 0.002 d	4.01 \pm 0.51 b	0.415 \pm 0.05 bc
significant	**	*	**
Means with different letters indicate a significant difference in probability level.*($P < 0.05$). **($P < 0.01$).			

Discussion

The increase in the dressing percentage may be due to the benefit of annatto addition transactions (T3) and astaxanthin (T5) lead to higher body weight and weight gain, as there is a direct relationship between final body weight, carcass weight, and dressing percentage [1 , 20],The improvement may be due to the role of astaxanthin, whose concentration increases in the body and its transmission to the blood system, and its work to inhibit the action of free radicals that destroy biologically related molecules such as DNA, carbohydrates, fats, and proteins through its work to prevent the breakdown of these molecules because the cell walls of these molecules are rich in fatty acids unsaturated, which is susceptible to oxidation, which leads to its damage, and the presence of a large percentage of astaxanthin in the intestinal cavity of the body of the bird without absorption and its effects on changes that have a positive effect in protecting the microbial balance in favor of beneficial bacteria inside the gastrointestinal tract, which enhances the production of a number of amino acids Which participate in building the fleshy muscles and as a result lead to an increase in the average body weight of the bird [41].The reason for the increase in the weight of the glandular

fascia and spleen may be attributed to the treatments of astaxanthin through the deposition in the tissues of the bird's body, it stimulates beneficial bacteria to make the food medium acidic, and this leads to an increase in the concentration of the hormone thyroxine, which has an important role in the metabolism process, and in increasing the weight of the Fabricia gland and lymphatic organs, in addition to its role as an effective antioxidant that maintains the structure of the cell membrane without causing damage to it [15 , 16 , 36] . The superiority of the proportions of the main and secondary carcasses in favor of the treatments of adding annatto and astaxanthin may be a natural result of the fact that there is a direct relationship between the average body weight and the percentages of the main carcasses [1], and the superiority of the proportions of the major and minor carcasses in favor of the treatments for adding astaxanthin may be due to the role of astaxanthin in the availability of beneficial intestinal flora that secretes several of digestive enzymes such as protease, lipase and amylase, which increase the efficiency of utilization of food proteins in the formation of body muscle proteins and then Increased body weight and thus increased the relative weight of carcasses [37 , 36].

The improvement in the treatments of adding Annatto seeds is due to its antimicrobial activity against Gram-positive and Gram-negative bacteria such as Bacillus, Pseudomonas aeruginosa and Escherichia coli [17], in addition to its work in maintaining the microbial balance in favor of beneficial bacteria and thus this is reflected in the improvement of the health of birds [35 , 2], and the role of annatto seeds against harmful bacteria comes Through the mechanism of action of seeds that change the permeability of the cellular membrane of microbial cells and deform it, and thus cause a change in cell functions and as a result lead to its death [40]. As for the improvement in the treatments of adding astaxanthin, it is due to the positive role of astaxanthin in promoting the growth of the microbial community in the intestine and increasing the numbers of beneficial bacteria such as Lactobacillus bacteria and through their activity through adhesion to the receptors on the epithelial cells that make up the intestinal wall and thus the exclusion of pathological bacteria such as E.coil and salmonella bacteria from the body. With waste, this is what is called the antagonistic activity of beneficial microorganisms, thus making the microbial balance in the intestine in favor of beneficial bacteria [9].

The reason for the low indicators of oxidation of meat may be because the effective contents of annatto seeds reduce and prevent the deterioration and oxidation of fats and expose them to rancidity, which results from the interaction of free oxygen atoms with phospholipids of cell membranes forming peroxides, in addition to the seeds containing beta-carotene, which the body can convert into vitaminA, which enhances antioxidant activity and immune response [24 , 27], in addition to the fact that annatto seeds contain phenolic compounds that inhibit the formation of hydroperoxide by blocking the peroxide radical, thus preventing the oxidation of triglycerides [22 , 6 , 8]. While the low TBA values are attributed to the treatment of adding astaxanthin to bird diets due to the high effectiveness of astaxanthin in its work on breaking the chain of oxidation reactions in

the presence of the methyl group along the polyene chain that gives the hydrogen atom to free radicals or fatty acid, and thus through this process it works to capture and remove free radicals And that astaxanthin works to prevent the oxidation process and lipid peroxidation from occurring through its containment of two unsymmetrical carbon atoms on the site (3, 3) in addition to a hydroxyl group (HO) at both ends of the astaxanthin molecule, which interacts with fatty acids, thus preventing the separation of hydrogen atoms from the bonding sites. Double polyunsaturated fatty acids [28 , 43 , 12 , 5] .While the low values of the reason for the decrease in the peroxide number values of the astaxanthin addition treatments is due to its high ability as an effective antioxidant in quenching free radicals and limiting the production of hydroxyl radicals (HO), because the increase in free radicals leads to interaction with hydroxyl radicals to produce the active lipid radical, which in turn reacts with oxygen to produce a peroxy radical lipid [29 , 7 , 26]. And the decrease in the value of free fatty acids in favor of the treatment of adding astaxanthin, because it has a polyene chain that represents a double-bond system responsible for the antioxidant activity in carotenoids, and then comes the role of the terminal ring that removes free radicals and suppresses the oxygen atom from the inner and outer parts of the cell membrane and ends the chain of oxidation reactions [42 , 3].

conclusions

-The addition of Annatto seed powder and astaxanthin showed significant superiority in the qualitative traits of broiler chickens.

-The addition of annatto seeds powder and astaxanthin showed a significant improvement in the oxidation indicators of meat, in addition to a significant superiority in the numbers of bacteria (Lactobacillus) and a significant improvement in the numbers of bacteria (E-Coli) in broiler chickens

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