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Effect of Pigments with Different Origins on Pigmentation and Performance of Broilers

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Abstract.- Broiler skin color plays a key role in consumer demand for food products in China and other Asian countries. Here the effects of natural lutein and two synthetic pigments, canthaxanthin (authorized) and orange-II (unauthorized), on the performance and pigmentation of broilers were evaluated. Histological changes in the pectoral muscles of broilers fed diets supplemented with various pigments were also examined. One-day-old chicks were randomly assigned into 10 groups according to dietary treatment and fed basal diets plus natural lutein,canthaxanthin and orange-II (25, 50 and 100 mg/kg) or control, respectively, over 7 weeks. There were no significant treatment effects on broiler performance and no interactions between treatment and gender found for any parameter ($p > 0.05$). Color analysis with a Roche Color Fan showed that pigmentation of chicken breast, vent and shank skin was significantly improved ($p<0.0001$) in canthaxanthin-fed chicks (25, 50 and 100 mg/kg), with higher pigmentation than the natural lutein and orange-II groups. More severe fibrosis was observed in broiler pectoral muscles in chicks fed the orange-II diet compared to other treatments and the control group by week 7 ($p < 0.05$). These findings argue against the use of unauthorized pigments in broiler diets.

Keywords: Pigments, broiler performance, skin pigmentation, histology of broiler tissue, lutein, canthaxanthin, orange-II

INTRODUCTION

In many countries, pigmentation is one of the most important characteristics of broiler chickens that determines consumer acceptance and perceived quality of broilers prior to buying or consumption (Castaneda *et al*., 2005; Ouart *et al*., 1988). The colors of the skin, meat and egg yolk play an important role in consumer demand for the food products (Fletcher, 1999). Broiler chickens with a yellow skin color have been shown to be considered desirable by consumers and therefore in some parts of China, pigments have been used in broilers for nearly 40 years. Healthy birds ingest pigments from their basal diet, which are transported in the blood and deposited in the subcutaneous fat tissues and skin. This process is impaired in birds afflicted with diseases, especially intestinal infections and parasitic infestations Tyczkowski *et al.* (1991). The majority of consumers prefer golden and red chickens, because this implies that the bird is reasonably free of health problems (Sunde, 1992). Chickens with less desirable coloring have a lower market value and these products are purchased less often by consumers.

More than 750 carotenoids have been identified in nature Britton *et al*. (2004) and they play important roles in the coloration of many plants, invertebrates, fishes, amphibians, reptiles and birds (Goodwin, 1984). In addition, pigments are used as additives to enhance the color of broiler and aquaculture animal products. These compounds are not naturally synthesized by chickens and instead are mostly derived from diet (Breithaupt, 2007). The carotenoid pigmentation in poultry is also involved in growth metabolism and fertility (Scheldt, 1998). Several carotenoids function as a predecessor for the synthesis of vitamin A (Surai and Speake, 1998), whereas others have protective mechanisms in the body and actas physiological antioxidants (Burton, 1989), thus enhancing the immune system (Bendich, 1989; and Blanch, 1999). However, several studies have demonstrated the

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function of lutein and canthaxanthin in the pigmentation of egg yolks and broilers (Hencken, 1992; Saylor, 1986). Canthaxanthin can significantly enhance the scale of pigmentation in broilers when used in diets containing yellow carotenoids (Marusich and Bauernfeind, 1981). The most extensively used source of yellow pigments is the flower petal of marigolds (*Tagetes erecta*), which contain up to 2,000 mg/kg carotenoids (Tyczkowski and Hamilton, 1986).

Most of the natural carotenoids that are related to poultry pigmentation occur in the free form, but lutein from marigold petals occur mostly as diesters of palmitic and myristic acids (Hencken, 1992). Feed carotenoids are absorbed in their free form and thus esterified hydroxy carotenoids must be saponified before they are absorbed. Feed carotenoids occur in a natural complex and approximately 60-90% are in *trans*-form and 10-30% are in *cis*-form. When evaluated *in vitro*, the transisomer is a more effective pigment than the cisisomer because of a redder hue and better stability (Hencken, 1992). However, a few studies in eggs have revealed that the cis:trans profile in yolk is quite stable, independent of the *cis*:*trans* profile of feed carotenoids, which would imply that there is no benefit of using *trans*-carotenoids *in vivo* (Hencken, 1992). Digested carotenoids are absorbed in different parts of the intestine, whereas the absorption of lutein occurs in the duodenum and jejunum, while canthaxanthin is absorbed in the small intestine and transported into the liver (Tyczkowski and Hamilton, 1986). Following absorption, the carotenoids are rapidly deposited in broiler adipose tissues, breast, shank, skin and toe-web..

The typical corn and soybean-based commercial poultry diets do not supply the necessary amount and type of xanthophylls required to produce the deep yellow and orange yellow skin color preferred by consumers (Saha *et al*., 1999). Therefore, in order to achieve the desire color, poultry and feed producers usually combine a yellow carotenoid in the diet by adding natural lutein (apo-ester, lutein, or zeaxanthin), a synthetic red one (canthaxanthin) and orange-II, which is a banned dye belonging to a class of organic compounds known as azo-dyes (Mollah *et al*., 2004). Dyes are important chemical compounds found in foods, pharmaceuticals, cosmetics, textiles and leather industries (Hildenbrand *et al.*, 1999) and can be associated with toxicity, mutagenicity and carcinogenicity (Tan *et al*., 1999). The azo-dye orange-II, also known as acid orange 7 or 1-(4'-sulfophenylazo)-2-naphthol, contains a sulfonic group as a substituent and is thus considered a sulfonated azo-dye. Studies have shown that these dyes pose a potential risk to human health and are even carcinogenic (Boeninger, 1980). Many countries now regulate the use of azo-dyes in food products and the use of these dyes in food is strongly prohibited because of the health concerns related to their intake. Azo-dyes are widely used in the dyeing industries and approximately 50-70% of the azo-dyes are currently available in the market (Bauer *et al.*, 2001). Pigment orange-II is an organic substance that has been used worldwide as an orange dye in inks, paper, paint coatings and plastics. Pigment orange-II is not naturally produced in the environment and exists as a solid particle, which is not soluble in water and is not volatile. In addition, this pigment is likely to accumulate in tissues high in lipid content due to the solubility of known analogues. In China, the use of pigments has gradually changed from natural colorants to synthetic ones. This has led certain irresponsible poultry traders and producers to buy banned dye from local markets at low cost and mix the dye with broiler diets to improve the yellow color of broiler skin. However, several studies suggest that orange-II is a suspected human carcinogen (Stylidi *et al.*, 2003) and therefore unauthorized synthetic colorants added to broiler diets would be unacceptable at any level.

The primary aim of this study was to investigate the effects of natural lutein, synthetic canthaxanthin (approved) and orange-II (banned dye) on broiler performance and pigmentation. In addition, histomorphological changes in pectoral muscles from broilers fed diets containing various pigments were examined.

MATERIALS AND METHODS

Experimental birds, housing and management

All of the experimental designs involving

animals were approved by the Animal Ethics Committee of the Institute of Quality Standard and Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences (Permission number: 2012-08-1045). Arbor Acres1-day-old broiler chicks (n=300 males and 300 females) were obtained from Huado Breeding Co. Ltd. (Beijing, P. R. China). The chicks were raised in a room with forced ventilation, automated heating and a programmable fluorescent light system. The room contained a total of 60 pens (60×40×130cm; length×height×width) and each pen was equipped with a single adjustable height tube feeder and adjustable waterers connected to the central water line. Approximately 10 chickens were allotted in each pen according to the dietary treatment; each treatment had three replicates. All birds had free access to water and were assigned a specific experimental diet *ad libitum* throughout the experiment. The lighting program was 23 h of light during the first 4 d, 20 h of light until day 10 and 18 h thereafter. The initial room temperature was 32–34°C, which was gradually decreased to 20–22°C and then maintained until the end of the experiment. Maximum and minimum temperatures and light intensity were checked twice daily and confirmed to meet the recommendations in the Arbor Acre (AA) production guide. Disease prevention practices were based on the vaccination schedules of the breeder farms. The health status of birds was monitored twice daily to remove decreased birds and to indentify possible health concerns. Vent-pecking and other abnormal behaviors were not observed in any group. The experiment lasted for 7 weeks.

Experimental diet,feeding programs and treatments

Soybean meal, wheat and maize-based experimental diets during the starter period (0–21d) contained 2,990 kcal/kg of metabolize energy (me) and 21.63% crude protein (CP) and 3,120 kcal/kg of ME and 18.58% CP for the finisher period (21–49d). Experimental diets met the National Research Council recommendations to cover all nutrient requirements of broilers (National Research Council, 1994). In the present study, 20% maize was used in feed formulation to reduce the natural pigment in the diet.The compositions of the basal experimental diets are shown in Table I. The

Table I.- Dietary ingredients and composition of starter and finisher diets used in this study.

Ingredient, %	Basal diets	
	Starter (1–21 d)	**Finisher (22–49 d)**
Wheat	36.2	46
Soybean meal	34.15	23.59
Maize	20	20
Calcium phosphate	1.95	1.75
Limestone	1	0.85
NaCl	0.3	0.3
Lysine	0.15	0.28
Methionine	0.2	0.18
Premix	1	1
Soya bean oil	5	6
Xylanase	0.05	0.05
Calculated nutrient values		
ME (Kcal/Kg)	2990	3120
CP	21.63	18.58
Calcium	0.99	0.87
Available P	0.47	0.43
Lysine	1.19	1.03
Methionine	0.52	0.47
Methionine+Cystine	0.71	0.67

Broiler 1% premix supplied the following amount of vitamins and trace elements of nutritional requirements (per kg of feed):Mn 90 mg; Zn 50 mg; Fe 90 mg; Cu 10 mg; I 0.4 mg; Se 0.2 mg; VA 5000IU; VD3 500 IU; VE 10 IU; VK 0.5 mg; VB1 1.5 mg; VB2 6.0 mg; panthothenic acid 12mg; niacin 35 mg; VB6 6.0mg; folic acid 0.8 mg; VB12 0.01 mg; biotin 0.18 mg.

experiment was conducted according to a randomized complete block factorial design (3 × 3 × 2 × 4; replication × treatment × sex × level). The experimental design was structured to study the effects of 3 dietary treatments at varying levels:T-1 basal diet plus natural lutein (25, 50 and 100 mg/kg), T-2 basal diet plus canthaxanthin (25, 50 and 100 mg/kg) and T-3 basal diet plus orange-II (25, 50 and 100 mg/kg). A control group of birds was used that received a diet without pigments. Natural lutein and canthaxanthin were provided by Guangzhou leader Bio Technology Co., Ltd, China and orange-II was purchased from a local market in Beijing, China.The chicks were randomly allocated to 3 dietary treatment groups with 3 replicates each. All treatments were mixed in feed for 10-15 minutes using a Y-type mixer and then tested during the growing period from day 1 until the end of the experiment on day 49. All feed was prepared at the integrating feed processing plant at the Animal

Sciences Institute (ASI).

Dried dietary ingredients were analyzed in duplicate for dry matter (DM) and Ash using the Association of Official Analytical Chemists (AOAC, 2000) procedures. Ash concentration was determined after 8 h of oxidation at 550°C in a muffle furnace. Total nitrogen (N) was determined using an auto micro-Kjeldahl N analyzer (model KDY-9830, Tongrunyuan Electromechanical Technology Co., Ltd, China). Calcium (Ca) and phosphorus (P) concentrations were determined using the atomic absorption spectrometric method (AOAC, 2000).

Growth performance and color evaluation

The chicks were weighed at the beginning of the experiment and cumulative body weight gain, feed consumption and feed conversion (FCR) was calculated at weekly intervals on a per pen basis. The amount of feed provided to each pen was recorded and any uneaten feed was weighed daily. Skin color was visually assessed using the Roche Color Fan (RCF), in which evaluation was performed using agraduated visual aid color fan with a minimum and maximum value range from 1–15 RCF (Vuilleumier, 1969). Skin color can be determined objectively using mechanical equipment (for example, MiniScan XE Hunter Lab, Minolta colorimeter CR 300) or subjectively using the RCF. Both systems are well correlated, but because the RCF is more economical and readily available, it has become the preferred method in most parts of the world.The breast, vent and shank skin color was measured in live broiler chickens (3-7wks) after slaughtering and chilling the carcasses.

Histological analysis

At experiment termination (week 7), 90 broiler chickens were selected randomly from each replicate of each treatment. The selected birds were euthanized by cervical dislocation and the skin was removed and pectoral muscle samples were collected. Samples were fixed in formaldehyde, dehydrated and then embedded in paraffin. Thin serial sections (5µm) were cut and mounted on a slide, stained with hematoxylin and eosin (H&E) in an autostainer using saturated ethanolic picric acid as a post-dewax treatment and manually mounted with coverslips (Romeis, 1968). Tissues were examined with a light microscope and attached digital camera (Olympus BX-51, Olympus Optical Company, Tokyo, Japan) to determine histopathological changes in the pectoral muscles (Dubowitz and Brooke, 1973). The severity of fibrosis was scored subjectively as follows: 0 = no visible fibrosis (control), 1 = occasional visible fibrosis, 2 = mild fibrosis and 3 = severe fibrosis. As a result, all muscle fibers were examined in the cross sections of pectoral muscles.

Statistical analysis

The data obtained for the performance of average daily body weight gain were analyzed for interactions between sex, treatment and level. If interactions were not observed, then the data were combined. Statistical analysis of data was conducted by analysis of variance (ANOVA) using the method in SAS software (SAS, 2010) as a completely randomized design. Significant differences between treatment and level means were separated by Duncan's new multiple-range test with a 5% level of probability.

RESULTS

Growth performance

Data for the effects of treatments and levels are presented in Table II. In general, there were no significant differences found in any level ($p > 0.05$). No significant correlations between sex and dietary treatments were found and thus data from males and females were pooled. However, after 4-5 and 6-7wks, a significant difference in the average daily gain was observed between the dietary treatments ($p < 0.05$). No significant differences in average daily gain were observed at 1-3 wk. However, at 4-5 wks, birds fed the 100 mg/kg orange-II diet had a significantly decreased average daily gain compared to all other groups.This may have been due to the unusual flavor and odor of orange-II in the diet. At 6-7 wks, birds fed the 100 mg/kg orange-II had a significantly increased final average daily gain compared to other treatments ($p < 0.05$). At 6-7 wks, birds fed the 25 mg/kg natural lutein diet had a significantly increased average daily gain compared to other treatments and the control group ($p < 0.05$).

Table II.- Performance of average daily gain in broilers fed diets containing various types and levels of pigments.

Bird age (wk)	Treatment	Control		25 mg/kg		50 mg/kg		100 mg/kg	
		n	ADG±STD (g/dl)	n	ADG±STD (g/dl)	n	ADG±STD (g/dl)	n	ADG±STD (g/dl)
1-3	Natural Lutein	18	25.402±9.90 ^{ba}	18	26.130±9.28 ^{ca}	18	28.584±10.6 ^{da}	18	27.139±10.96 ^{da}
	Canthaxanthin	18	25.402±9.90 ^{ba}	18	25.549±10.22 ^{ca}	18	27.425±10.91 ^{da}	18	26.193±10.16 ^{da}
	Orange-II	18	25.402±9.90 ^{ba}	18	24.337±9.38 ^{ca}	18	23.850±9.055 ^{da}	18	25.947±8.74 ^{da}
4-5	Natural Lutein	12	59.858±19.15 ^{aa}	12	58.712±24.82 ^{ba}	12	58.060±11.74 ^{ca}	12	63.314±20.5 ^{bca}
	Canthaxanthin	12	59.858±19.15 ^{aa}	12	60.276±12.11 ^{ba}	12	64.463±18.42 ^{bca}	12	67.329±17.78 ^{bca}
	Orange-II	12	59.858±19.15 ^{aa}	12	65.279±23.78 ^{ba}	12	65.556±27.36 ^{bca}	12	54.233±20.03 ^{ca}
6-7	Natural Lutein	12	73.278±22.46 ^{aa}	12	83.427±40.71 ^{aa}	12	79.493±23.91 ^{aba}	12	80.389±36.53 ^{aba}
	Canthaxanthin	12	73.278±22.46 ^{aa}	12	72.522±22.33 ^{aba}	12	76.426±25.94 ^{aba}	12	89.017±39.08 ^{aa}
	Orange-II	12	73.278±22.46 ^{aa}	12	69.293±24.38 ^{aba}	12	86.225±34.94 ^{aa}	12	94.339±31.77 ^{aa}

ADG, average daily gain; STD, standard deviation. (a-d):indicates ANOVA for treatments. *(a)*: ANOVA for levels.
The data represent means from 6 replicates (*i.e.*, pens) per treatment and per week. Means with a different superscript letter in a column within weeks and treatments are significantly different ($p<0.05$). Data were pooled for males and females. No interactions between treatment and gender were found for any parameter.

Effect of natural and synthetic pigments on pigmentation

The breast, vent and shank pigmentation measurements were performed using the Color Fan Scale (RCF). After two weeks of the experimental diet, pigmentation effects were not obvious in the skin of the breast, vent and shank. After two weeks, the effect of the pigments resulted in a deeper yellow color compared to the previous weeks. The results of the broiler color attribute score data are presented in (Fig.1 A-E). Breast,vent and shank color was significantly different among the treatments. Lower concentrations of natural lutein and orange-II (25 and 50 mg/kg) showed lower scores from 3-7 wks compared to the respective groups fed with the highest level (100 mg/kg). Moreover, birds fed 25, 50, or 100 mg/kg canthaxanthin showed a deeper pigmentation score throughout the experimental period compared to other treatments (Fig. 2 A-B). One possible reason for this finding is that synthetic pigments may be absorbed better. Therefore, lower and higher levels of canthaxanthin are recommended to promote better skin color that is demanded by consumers in the market.

Histology of broiler muscles

Histomorphological data were observed in broiler muscles for the individual treatments and levels (Table III). There were no significant differences found at any level ($p >0.05$). The structure of muscles in the control group was normal and no fibrosis was observed (Fig. 3A-D). However, a greater variation of the broiler muscle fiber score was found in response to different treatments. The group receiving orange-II (100 mg/kg) showed significant differences ($p <0.05$) compared to the other treatments. The histomorphological results reveal significant changes ($p<0.05$) in broiler muscles due to the frequent use of orange-II pigments in broilers diets.

Table III.- Day 49 histomorphological changes observed in broiler pectoral muscles in response to various treatments and levels of pigments.

Treatment	n	Level (mg/kg)			
		0	25	50	100
Natural Lutein	3	0^{ab}	0.33^{ba}	0.33^{ba}	0.83^{ba}
Canthaxanthin	3	0^{ab}	0.67^{baab}	1.17^{aa}	0.5^{bab}
Orange-II	3	0^{ab}	1.83^{aa}	2^{aa}	3^{aa}

(a,b) showing ANOVA for treatments; (*a,b*) showing ANOVA for levels
Means with a different superscript letter in a column within different treatments and levels are significantly different($p<0.05$).

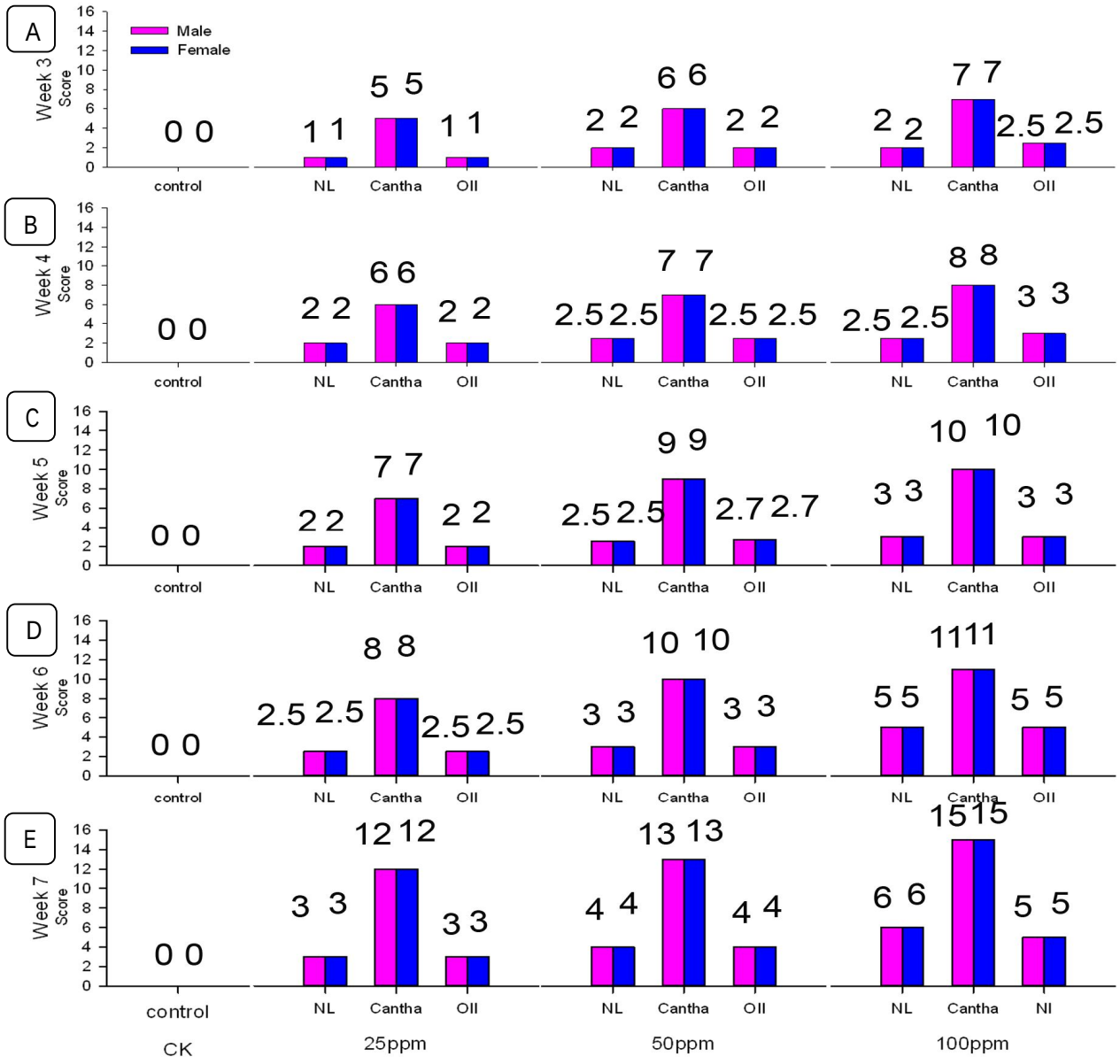

Fig. 1. Color attribute scores found in breast, vent, and shank skin pigmentation of broilers in response to various levels of natural lutein, canthaxanthin, or orange-II at **(A)** 3 weeks, **(B)** 4 weeks, **(C)** 5 weeks, **(D)** 6 weeks, or **(E)** 7 weeks. No interactions between treatments and gender were found. CK, control; NL, natural lutein; Cantha, canthaxanthin; OII=orange-II.

DISCUSSION

This study has demonstrated that broiler performance with regard to average daily gain is not affected by the inclusion of natural lutein, canthaxanthin and orange-II in the broiler diet (Table II). Similar results were observed in the study by Fletcher (1999), which found that broiler performance was not adversely affected when birds were fed with different sources of xanthophylls, such as an Alocacia leaf meal, saponified carotenoid mixture, Carophyll red®, Carophyll yellow®, or

A **B**

Fig. 2. Effect of dietary treatments **(A)** on shank skin **(B)** on breast and vent skin of broilers. CK, control; NL, natural lutein; Cantha, canthaxanthin; OII, orange-II; numbers indicate the level of treatment: 25mg/kg, 50mg/kg, and 100mg/kg.

yellow corn. The addition of carotenoid Phaffiarhodozyma yeast into the diet for broiler chickens had no significant effects on performance compared to diets without supplementation (Akiba *et al*., 2001). Other studies have also found that dietary inclusion of chemically isomerized marigold, conventional marigold and canthaxanthin had no significant effects on broiler chicken pigmentation and performance (Perez-Vendrell *et al*., 2001). The present study is also in agreement with the results of a previous study that compared the effect of different levels of extracted pigment from Dietzia natronolimnaea biomass, which provided a source of canthaxanthin, to synthetic canthaxanthin on egg yolk pigmentation and layer hen performance (4, 8, or 16 mg/kg) (Esfahani-Mashhour *et al*., 2009). Broiler feed supplemented with 25, 50, 75 and 100 mg/kg of vitamin E, which is another antioxidant, had no significant effect ($p>0.05$) on body weight and viability during a 30 wk experimental period (Hossain *et al*., 1998).This current study indicated that the performance of birds was not affected by the inclusion of different amounts of the tested pigments in diets and thus is in agreement with the findings of previous studies.

In this study, changes in breast,vent and shank skin pigmentation were measured using the Roche Color Fan (RCF) starting at 3 wks and ending at 7 wks (Fig.1 A-E). However, at 4,5,6 and 7 wks, significant differencesin pigmentation scores were found between the treatments groups ($p >0.0001$). Birds from the canthaxanthin group (25, 50 and 100 mg/kg) had the most pigmentation throughout the experiments and the control group had the least, as expected. Both were significantly different in breast, vent and shank from birds fed natural lutein (25, 50 and 100 mg/kg) and orange-II (Fig. 2A-B). However, the highest yellow pigmentation score was observed withnatural lutein at 100 mg/kg, while orange-II acheived the same

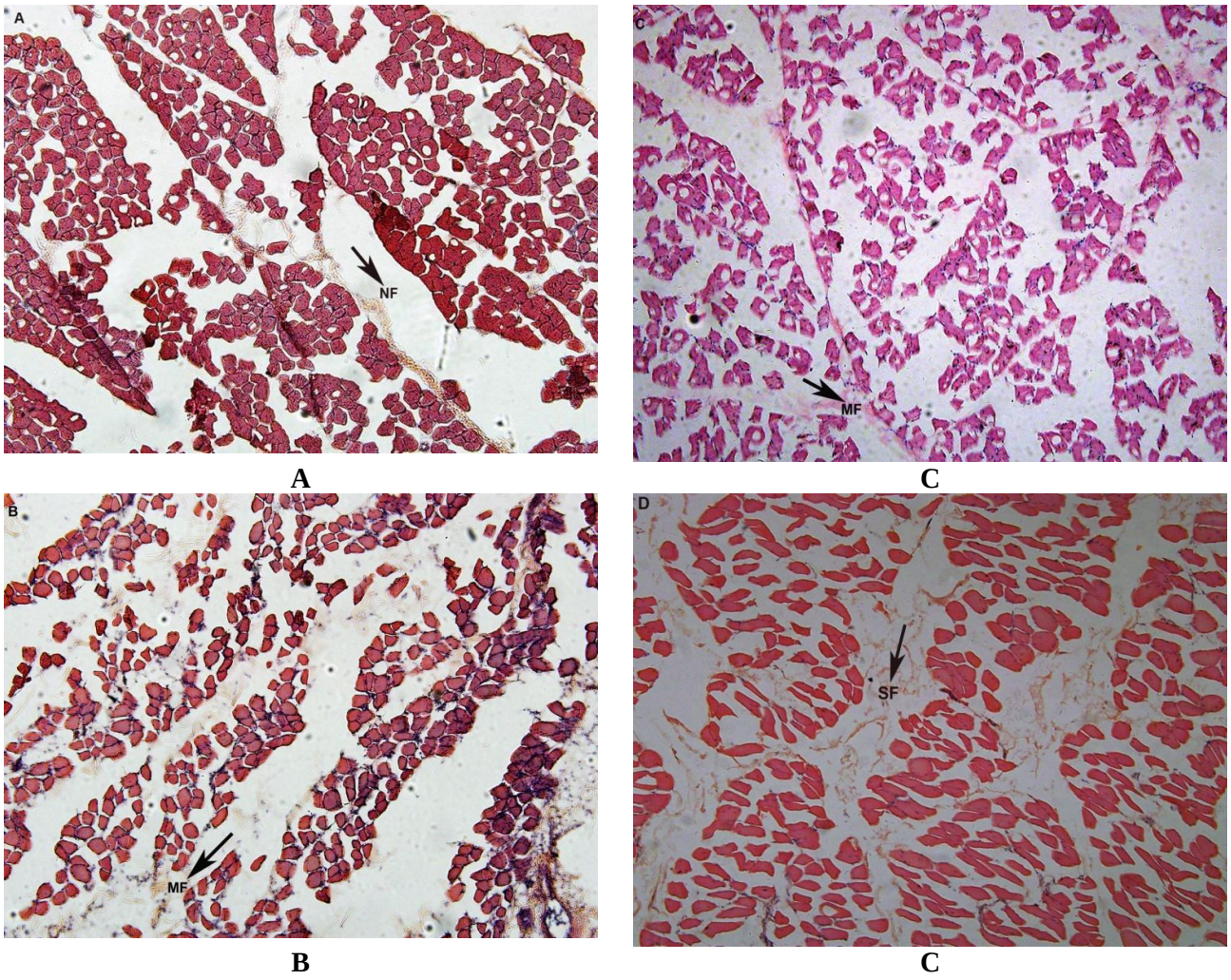

Fig.. 3. Histological structure of broiler pectoral muscles in a transverse view in response to different treatments of pigments (1-49 d). **A**, Broilers fed diets without pigment, no fibers were observed, hematoxylin and eosin (H&E) 100×; **B**, Broilers fed diets containing canthaxanthin, mild fibers observed, H&E, 200×; **C**, Broilers fed diets containing natural lutein, mild fibers were observed H&E, 100×; **D**, Broilers fed diets containing orange-II, severe fibers were observed, H&E, 200×. F, no fibers; MF, mild fibers and SF, severe fibers.

skin color at all three concentrations tested. Thus, the effect of orange-II was not different from natural lutein at the same concentration, which could be why poultry traders purchase and use orange-II from local markets rather than the more expensive natural lutein. This allows poultry producers to meet the consumer demand for natural lutein pigmentation at a much cheaper price, despite the known carcinogenic potential of orange-II in humans.

By 7 wks, low and high levels of canthaxanthin (25, 50 and 100 mg/kg) as well as high levels of natural lutein and orange-II (100 mg/kg) generated results that would meet consumer demand and commercial industry marketing goals. The mean values of pigmentation scores found in this study are similar to those reported in previous studies (Bilgili *et al*., 1998; Fletcher, 2002). However, the results of this study were not in agreement with another group (Branellec, 1985) who reported that zeaxanthin and canthaxanthin are mainly deposited in broiler fat, whereas lutein has better pigmenting properties in the skin.

Nevertheless, no study to date has shown an effect of orange-II on broiler performance and pigmentation.

Changes in the histomorphology of pectoral muscles of broilers fed the various diets and orange-II significantly increased fibrosis in broilers compared to the other diets and control (Table III). The histological results in broiler pectoral muscles at 7 wk (Fig. 3 A-D) showed that the muscles from bird fed orange-II contained more fibrosis, whereas in the birds fed canthaxanthin and natural lutein, only mild fibrosis was observed. It is well known that histological characteristics of muscles depend on good nutrition. The role of nutrition in broiler chicken growth is a complex subject of major feed restriction in quantity and quality leads to an increase in the number of muscle fibers, which compromises the quality of meat (Rehfeldt *et al.*, 2004). Moreover, the size and number of muscle fibers are factors that influence muscle mass and meat quality (Berri, 2000). A similar conclusion was also found in turkeys, where birds selected for rapid growth of connective tissue showed a loss of muscle fiber integrity throughout the study (Sosnicki and Wilson, 1991). On the other hand, meat from broiler chickens with smaller fibers would be better adapted to further processing compared to those with larger fibers (Sosnicki and Wilson 1991). Similarly, another study found that breast muscles with large fibers have compromised taste after cooking compared to breast muscle with small fibers (Duclos *et al*., 2007). These results confirmed that orange-II supplementation has a negative effect on muscles and the observed histological changes regarding pectoral muscles also provided useful information.

CONCLUSIONS

In conclusion, the results of the present study indicate that broiler performance is not affected by the treatments assessed in this study. The best average weight gain was seen in groups supplemented with highest level of natural and synthetic pigments (100 mg/kg). Canthaxanthin showed better degrees of pigmentation in different parts of the chicken. This study also suggests that authorized synthetic pigments are absorbed better than natural pigments. In addition, the histological results indicate that inclusion of orange-II in the diets of broilers causes deterioration in the quality of the meat. Therefore, the frequent use of unauthorized synthetic pigments should be avoided in poultry diets and instead high quality antioxidant pigments should be used. However, broiler performance could be affected by both natural and synthetic pigments present in broiler diets at high concentrations (*i.e.*, > 300 mg/kg). Further research is required to accurately determine the effects of unauthorized synthetic pigments in broiler meat, skin and feed through GC-MS, FAB-MS and ESI-MS methods on a large scale.

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