

Effect of the infection with *Eimeria acervulina*, *E. maxima* and *E. tenella* on pigment absorption and skin deposition in broiler chickens[#]

Efecto de la infección con *Eimeria acervulina*, *E. maxima* y *E. tenella* en la absorción y el depósito del pigmento en la piel de pollos de engorde

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ABSTRACT. Two experiments were conducted in broiler chickens from 21 to 49 d of age to evaluate the plasmatic level, deposition, and digestibility of xanthophylls (XA) from marigold flower (*Tagetes erecta*) after infection with vaccine strains of *Eimeria* spp. In Experiment 1, 432 birds were assigned to 4 treatments: 1) non-infected control; 2) 8.32×10^4 sporulated *Eimeria* oocysts (SEO)/bird; 3) 17.82×10^4 SEO/bird, and 4) 49.92×10^4 SEO/bird. In Experiment 2, 400 broilers were assigned to 4 treatments: 1) non-infected control; treatments 2 to 4 were challenged with 8.32×10^4 SEO/bird. The birds received 85, 108, 141, and 162 ppm of total dietary XA from d 35 to 49 for treatments 1 to 4, respectively. Both experiments contained 4 replications (2/sex) per treatment. In Experiment 1, the SEO dose was associated with the reduction in body weight gain (BWG), skin yellowness units (b*), and plasma xanthophylls (PX). In both sexes, b* increased by 1.36/d of XA consumption. For every 10^4 SEO, PX decreased by 0.8 µg/ml/d, and 0.06b*. In Experiment 2, no difference was detected in PX or b* at 49 d between treatments. XA digestibility decreased by 15 units in the infected birds. Results suggest that XA digestion and absorption are similar between males and females. However, females have a greater ability for skin XA deposition. After a mild *Eimeria* spp. infection, it is possible to achieve adequate skin yellowness (SY) levels if the infected birds are treated immediately and receive at least 62 mg of dietary XA/bird for 14 d.

Key words: broiler chicken, *Eimeria*, plasma xanthophyll, skin pigment.

RESUMEN. Se evaluó el nivel plasmático, depósito cutáneo y digestibilidad de xantófilas (XA) de flor de cempasúchil (*Tagetes erecta*) en pollos infectados por vía oral con cepas vacuales de *Eimeria* spp. En el experimento 1 se probaron diferentes dosis infectantes: 1) testigo no infectado; 2) $8,32 \times 10^4$ ooquistes esporulados de *Eimeria* (OEE)/ave; 3) $17,82 \times 10^4$ OEE/ave, y 4) $49,92 \times 10^4$ OEE/ave; en el experimento 2 se utilizó una dosis infectante y diferentes dosis de pigmento en la dieta: 1) testigo no infectado; y los tratamientos 2 a 4 se desafiaron con $8,32 \times 10^4$ OEE/ave, las aves fueron tratadas, y se proporcionaron 85, 108, 141, o 162 ppm de XA totales en la dieta en los grupos 1 a 4, del día 35 al 49 de edad. Cada experimento tuvo 4 réplicas (2/sexo) por tratamiento. En el Experimento 1 la dosis de OEE se asoció con la reducción en la ganancia de peso, unidades de amarilleamiento cutáneo (b*), y nivel de xantófilas plasmáticas (XP). Por cada 10^4 OEE las XP disminuyeron 0,80 mg/ml/d, y 0,06 en b*. En el Experimento 2 la digestibilidad de las XA disminuyó 15 unidades en las aves infectadas y los niveles de XP o b* fueron similares entre tratamientos al final de la prueba. Se sugiere que la digestión y absorción de XA es entre sexos, pero la hembra deposita más XA cutáneas. Después de una infección moderada por *Eimeria* es posible lograr niveles comercializables de amarilleamiento cutáneo (SY) mediante un tratamiento y al menos 62 mg de XA/ave/14 d.

Palabras clave: pollo de engorda, *Eimeria*, xantófilas plasmáticas, pigmento cutáneo.

INTRODUCTION

In China, Spain, Italy, Mexico and Peru, amongst other countries, the consumer demands chicken with yellow skin

pigmentation. Since skin pigmentation is associated with health status and product freshness (Breithaupt 2007, Liu *et al* 2008, Hernandez-Velasco *et al* 2014), there is a large volume of whole chicken or parts commercialized with the skin (Baker and Günther 2004). The inclusion level of dietary pigment for broiler chickens varies depending upon the company, region, and country.

Commercial competition and shorter grow-out periods require the use of high concentrations of dietary xanthophylls (XA), which may represent 8 to 10% of the diet cost. In Mexico, it is very typical to feed 80 ppm or more in the last 3 or 4 weeks of the grow-out (Muñoz-Díaz *et al* 2012), with XA consumption per bird between 240 and 500 mg. These levels usually yield skin pigmentation levels of at least 22 skin yellowness units (b*) in live birds, and greater than 47 b* in cold carcasses, as measured in the lateral apterial pectoral area (Muñoz-Díaz *et al* 2012, Hernandez-Velasco *et al* 2014).

Accepted: 15.10.2015.

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[#]This project was sponsored by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) de la Dirección General de Apoyo al Personal Académico (DGAPA); Proyecto IN203910-3, Universidad Nacional Autónoma de México.

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Amongst dietary carotenoids, in particular the XA (dihydroxycarotenoids) lutein and zeaxanthin are the components of major economic interest in Aztec marigold flower (*Tagetes erecta*) extract that are associated with natural yellow coloration in chicken skin and fat (Hadden *et al* 1999, Kotake-Nara and Nagao 2011). In addition to their pigmenting properties, XA are also antioxidants and provide natural UV protection, thus XA might be helpful to maintain healthy vision and skin. Therefore, inclusion of natural pigments in diets for poultry destined for human consumption may pose additional value to the health-conscious consumer (Baker and Günther 2004, Kijlstra *et al* 2012).

Skin pigmentation depends upon various factors such as: XA dose and consumption time, dietary nutritive quality, bird's sex, bird health status, and slaughter processing conditions, among others (Britton *et al* 1998). From the diseases that influence skin pigmentation, coccidiosis is perhaps one of the most frequent and costly infections that affect the poultry industry globally (Ruff and Fuller 1975, Tyczkowski and Hamilton 1991). The severity of the coccidiosis effect on skin pigmentation will depend on the *Eimeria* strain and virulence, immune status of the bird, and other complicating factors. *E. acervulina* and *E. maxima* produce a significant reduction in plasma carotenoids (Ruff and Fuller 1975, Sharma and Fernando 1975, Ogbuokiri and Edgar 1986, Tyczkowski and Hamilton 1991). These infections are sometimes associated with lower body weight gain (BWG) that is a consequence of poor nutrient absorption due intestinal sloughing and villi shortening (Allen 1987). Plasma xanthophylls (PX) concentrations have proven to be sensitive indicators of the severity of *Eimeria* infections compared to other criteria such as live weight, macroscopic lesion scoring, and the number of oocysts shed in feces (Conway *et al* 1990).

Despite the economic impact of skin pigmentation in broiler chickens across various countries, and the fact that coccidiosis is the main infectious disease that affects it, there are very few studies, if any, that quantify the effect of *Eimeria* spp. infections on broiler skin pigmentation and how it may be influenced by bird's sex. Therefore, the objectives of the current study were to evaluate the effect of *Eimeria* spp infection on XA absorption and deposition and ileal digestibility in broiler chickens, and to assess the recovery in skin pigmentation in broilers exposed to a mild coccidia infection.

MATERIAL AND METHODS

FACILITIES

The experiments were conducted at the Center for Research, Education, and Extension in Poultry Production (CEIEPAV) and the diagnostic laboratory of the Avian Medicine Department (DMZA) of the College of Veterinary

Medicine and Zootechnics (FMVZ), at the National Autonomous University of Mexico (UNAM).

EXPERIMENTAL BIRDS

Two experiments were conducted with sexed Ross 308 broiler chickens. The birds were separated by sex, and raised from 0 to 21 d of age in floor pens in an open-sided house. The experiments were conducted in accordance with the Guidelines of the Institutional Animal Care and Use Committee of the FMVZ, at the UNAM (CICUAE-FMVZ-UNAM). For both experiments, the birds were individually weighed at 21 d of age such that each pen had similar weight and weight distribution.

FEED

Diets were based on sorghum-soybean meal, and were formulated for three feeding phases: starter, 0-10 d; grower, 11-20 d; and finisher, 21-49 d of age. The diets contained meat and bone meal, and were designed to meet the nutrient specifications as recommended by the primary breeder (Aviagen 2007¹). Feed and water were provided *ad libitum* in a mash form. During the starter phase, all birds received 125 ppm of nicarbazine, and 100 ppm of sodium monensin during the grower phase, and only treatment one in the finisher phase in Experiment 1. In the finisher phase, all birds received 85 ppm of dietary XA from Aztec marigold flower.

EXPERIMENTAL DESIGN

Experiment 1. Four hundred and thirty two 21-d-old Ross 308 sexed broiler chickens were randomly assigned to 4 treatments: 1) Non-infected control; 2) 8.32×10^4 sporulated *Eimeria* oocysts (SEO) per bird; 3) 17.82×10^4 SEO per bird; and 4) 49.92×10^4 SEO per bird. Treatments contained 4 replications (2 replications of males and 2 of females) of 27 birds each. All birds received 85 ppm of dietary XA from Aztec marigold flower from 21 to 49 d of age.

Experiment 2. Four hundred 21-d-old Ross 308 sexed broiler chickens were randomly assigned to 4 dietary treatments containing 4 replications (2 replications of males and 2 of females) of 25 birds each. Treatments were designed as follows: 1) Non-infected control + 85 ppm of dietary XA; 2) 8.32×10^4 SEO/bird + additional 23ppm of XA (108 ppm total); 3) 8.32×10^4 SEO/bird + additional 56 ppm of XA (141 ppm total); and 4) 8.32×10^4 SEO/bird + additional 77ppm of dietary XA (162 ppm total). At 28, 29, 38, and 39 d of age, all birds were treated with toltrazuril at 7mg/kg of body weight (BW)

¹ Aviagen, 2007. Broiler nutrition specification Ross 308. Aviagen, Huntsville, Alabama. Web MD. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross_308_Broiler_Nutrition_Spec.pdf. Accessed September 2014.

via drinking water. The infected birds (treatments 2, 3, and 4) received sodium monensin from 36 to 49 d of age. All birds received 85 ppm of XA from 21 to 36 d of age. The infected treatments received higher levels of dietary XA from 36 to 49 d of age, at the levels indicated above.

CHALLENGE

In both experiments, at 21 d of age, the birds from treatments 2, 3, and 4 received an inoculum containing vaccine strains of *Eimeria acervulina* (58%), *E. maxima* (20%), and *E. tenella* (22%) in 1 ml of sterile PBS via oral gavage. The concentration of the inoculum for each experiment was given as described above.

OOCYST COUNTS AND LESION SCORING

Prior to the beginning of the experiment, fecal samples from 5 birds/pen were collected to obtain a baseline measurement of *Eimeria* spp. infection. Coccidia oocysts were quantified by using the McMaster technique as previously described (Long and Rowell 1975). This procedure was performed at 14 d, 21 d, and weekly during the experimental period. Intestinal lesion scoring was conducted in 2 birds/pen on the same days as oocyst counts using the scoring system developed by Johnson and Reid (1970).

PLASMA XANTHOPHYLLS

Starting at 21 d, and then weekly for the duration of the study, 2 ml of blood was collected from the wing veins of 3 birds per pen and mixed with EDTA in a 10:1 proportion. The test tubes were covered with aluminum foil to protect the blood samples from light, and stored in refrigeration for further measurement of plasma XA according to the method reported by Allen (1987).

SKIN YELLOWNESS

Starting at 21 d, and then weekly for the duration of the study, skin yellowness units (b^*) were determined in the right lateral apertal area of 10 birds/pen, using a reflectance colorimeter (Model CR-400, Minolta Corp., Ramsay, NJ.) (Muñoz-Díaz *et al* 2012).

GROWTH PERFORMANCE

All birds were weighed by pen at 21 d and 49 d of age. Feed intake was measured weekly to calculate BWG (g), feed consumption, pigment consumption, and feed conversion ratio (FCR).

APPARENT ILEAL PIGMENT DIGESTIBILITY

In Experiment 2, titanium dioxide was added to the diet at 0.2% and fed to the control and challenged birds

from 28 to 35 d of age. At 35 d of age, 2 birds per pen were euthanized via CO₂ asphyxiation and the ileal digesta was collected and frozen, freeze-dried, homogenised, and split into 2 subsamples for measurement of yellow XA by HPLC and titanium dioxide by spectrophotometry according to the methodology described by Ravindran *et al* (1999). A feed sample from control and challenged groups was collected and analysed for total XA and titanium dioxide.

STATISTICAL ANALYSIS

Growth performance, b^* , and plasma xanthophyll concentration were analyzed by ANOVA for a 4 x 2 factorial arrangement, where the first factor was the coccidia infection at 4 levels, and the second factor was sex at 2 levels. Data for ileal digestibility was analyzed using a 2 x 2 factorial arrangement in which the first factor was the infection level (non-infected or infected), and the second factor was the sex (males or females). Treatment means were compared by Tukey's multiple comparison procedure ($P < 0.05$).

Data for skin and plasma pigmentation was fitted to a multiple regression model in which the independent variables were the time of pigment consumption and the coccidia infection level; sex was used as an indicative variable for which 0 = females, and 1 = males (Neter *et al* 1985). Oocyst fecal count data and the intestinal lesion scores were analyzed using the non-parametric Kruskal-Wallis test. Treatment medians were compared by the Mann-Witney U test (Kuehl 2001).

RESULTS

EXPERIMENT 1

In Experiment 1, from 21 to 49 d of age, BWG in birds from the control group (2,427 g) was significantly greater than in birds from treatment 4 (2,239 g), which was challenged with the highest coccidia dose ($P < 0.05$). The best FCR was observed in the birds from the control treatment (1.96 kg:kg), which was significantly better from that observed in the treatment with the highest coccidia challenge (2.22 kg:kg). As expected, there was a significant effect of sex on growth performance. In males, BWG (2,538 g), feed consumption (5,110g) and pigment consumption (434mg) were significantly greater than in females (2,102 g, 4,589 g, 390 mg, respectively), while FCR was better in males (2.01 kg:kg) when compared to the females (2.18 kg:kg) (table 1).

Between 0 and 28 d posinoculation (21 and 49 d of age), for every d that pigment was consumed, skin yellowness was increased by 1.36 b^* for both males and females. However, the rate of skin pigmentation was greater in female birds (1.94 b^*). For every 10,000 SEO that birds received, skin pigmentation decreased by 0.06 b^* (figure 1). This relationship can be described by the following equation: $b^* = 2.08$

Table 1. Feed consumption, pigment consumption, body weight gain, feed conversion ratio, skin yellowness units (b*), and plasma xanthophylls of broiler chickens at 49 d of age infected with increasing doses of *Eimeria* spp. oocysts at 21 d of age, Experiment 1.

Consumo de alimento, consumo de pigmento, ganancia de peso, índice de conversión, amarilleamiento cutáneo (b*) y xantófilas plasmáticas de pollos de engorda de 49 días de edad, infectados con dosis ascendentes de ooquistes de *Eimeria* spp. al día 21 de edad, Experimento 1.

Treatment/ dose of oocysts	Feed consumption, g/ bird	Pigment consumption, mg/bird	BW gain, g/ bird	FCR, kg:kg	b*	Plasma xanthophylls, µg/ml
Control	4,758 ^a	404 ^a	2,427 ^a	1.96 ^b	17.6 ^a	39.7 ^a
8.32 x 10 ⁴	4,764 ^a	405 ^a	2,293 ^{ab}	2.08 ^{ab}	13.1 ^b	25.5 ^b
17.82 x 10 ⁴	4,945 ^a	420 ^a	2,319 ^{ab}	2.13 ^{ab}	11.9 ^b	19.9 ^b
49.92 x 10 ⁴	4,932 ^a	419 ^a	2,240 ^b	2.22 ^a	13.8 ^b	25.6 ^b
SEM	123	10.4	40.6	0.04	0.72	2.1
SEX						
Males	5,106	434	2,538	2.01	12.8	28.2
Females	4,758	390	2,102	2.18	15.5	27.2
SEM	87	7.4	28.7	0.03	0.51	1.5
			Probability			
Treatment	NS	NS	*	*	*	*
Sex	*	*	*	*	*	NS
Treatment X sex	NS	NS	NS	NS	NS	NS

Means with different superscripts within a same column differ significantly (P < 0.05).

*= (P < 0.05).

Medias con diferentes superíndices en una misma columna difieren significativamente (P < 0,05).

* = (P < 0,05).

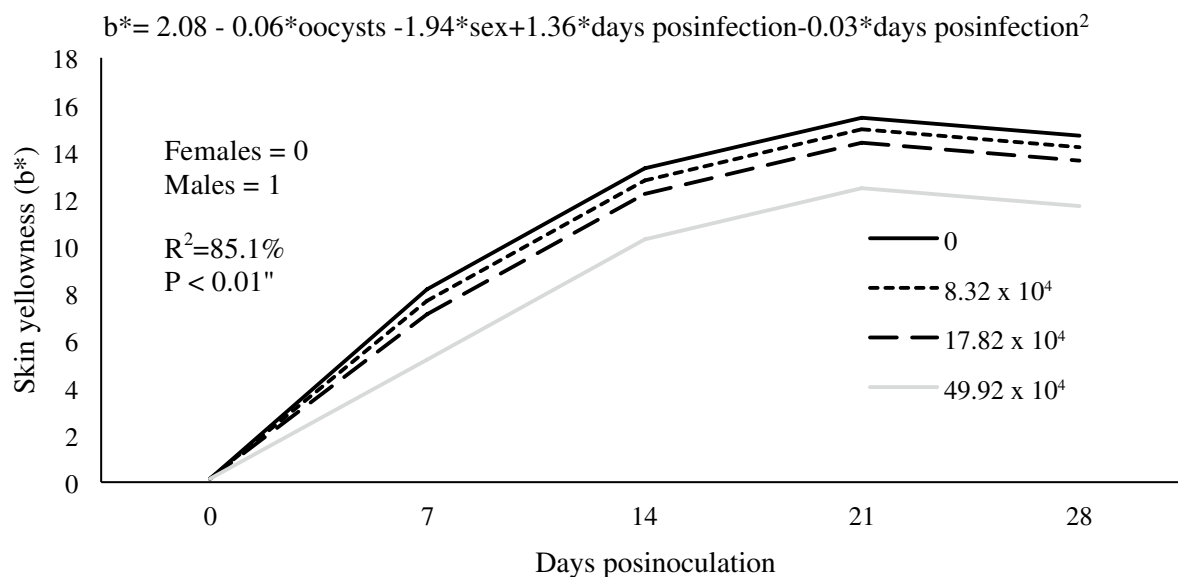


Figure 1. Skin yellowness units (b*) *in vivo* from 0 to 28 d posinoculation (21 to 49 d of age) in broiler chickens infected with *Eimeria* spp. at 21 d of age and fed 85 ppm of dietary xanthophylls from Aztec marigold flower, Experiment 1.

Unidades de amarilleamiento cutáneo (b*) *in vivo* del día 0 al 28 posinoculación (21 a 49 días de edad) en pollos de engorda infectados con *Eimeria* spp. al día 21 de edad y alimentados con 85 ppm de xantófilas de flor de compasúchil, Experimento 1.

- 0.06*oocysts - 1.94*sex + 1.36*d post-infection - 0.03*d post-infection²; R²=85.1%; P < 0.01. Skin yellowness (SY) in live birds at 49 d of age was significantly greater (P < 0.05) in the control group (17.6b*) with respect to treatments 2, 3, and 4 (13.07, 11.9 y 13.8 b*).

was significantly lower than in females (12.8 b* and 15.5 b*, respectively) (P < 0.05) (table 2).

Concentration of PX was reduced by 0.8 µg/ml for every 10,000 SEO given to each bird. (figure 2). The effect of coccidia inoculation on PX concentration can be

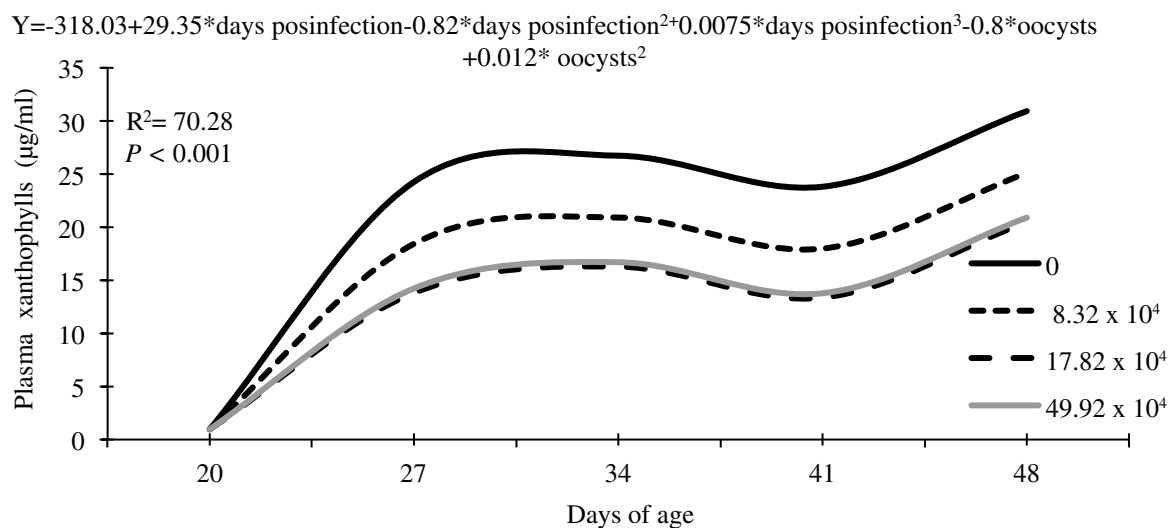
Table 2. Skin yellowness units (b*) and plasma xanthophyll concentration (µg/ml) in broiler chickens at 49 d of age after infection with increasing doses of *Eimeria* spp. oocysts at 21 d of age, Experiment 1.

Unidades de amarilleamiento cutáneo (b*) y concentración de xantófilas en plasma en pollos de engorda de 49 días de edad después de la infección con dosis ascendentes de *Eimeria* spp. al día 21 de edad, Experimento 1.

Treatment / dose of oocysts	Males	Females	Average
b*			
1. Control	16.4±1.02	19.0±1.02	17.6±0.72 ^a
2. 8.32 x 10 ⁴	12.2±1.02	14.0±1.02	13.1±0.72 ^b
3. 17.82 x 10 ⁴	10.9±1.02	12.9±1.02	11.9±0.72 ^b
4. 49.92 x 10 ⁴	11.7±1.02	16.0±1.02	13.8±0.72 ^b
Average	12.8±0.51 ^y	15.5±0.51 ^z	
Plasma xanthophyll concentration (µg/ml)			
1. Control	37.0±3.02	42.9±3.02	39.7±2.1 ^a
2. 8.32 x 10 ⁴	25.4±3.02	25.5±3.02	25.5±2.1 ^b
3. 17.82 x 10 ⁴	20.4±3.02	19.4±3.02	19.9±2.1 ^b
4. 49.92 x 10 ⁴	29.8±3.02	21.3±3.02	25.6±2.1 ^b
Average	28.2±1.5 ^z	27.2±1.5 ^z	

Means ± SE with different superscripts within a same column or arrow differ significantly (P < 0.01).

Medias ± EE con diferentes superíndices en una misma columna o renglón difieren significativamente (P < 0,01).

**Figure 2.** Plasma xanthophylls (µg/ml) in broiler chickens infected with *Eimeria* spp. at 21 d of age and fed 85 ppm of dietary xanthophylls from Aztec marigold flower from 21 to 49 d of age, Experiment 1.

Xantófilas plasmáticas (µg/ml) en pollos de engorda infectados con *Eimeria* spp. al día 21 de edad y alimentados con 85 ppm de xantófilas de flor de compasúchil del día 21 al 49 de edad, Experimento 1.

explained by the following equation: $Y = -318.03 + 29.35 \cdot DI - 0.82 \cdot DI^2 + 0.0075 \cdot DI^3 - 0.8 \cdot oei + 0.012 \cdot SOE^2$; $R^2 = 70.28$; $P < 0.001$, in which Y = PX concentration; DI = d post-infection; SEO = sporulated *Eimeria* spp. oocysts inoculated. Results for PX concentration are presented in table 2. Birds from the unchallenged control treatment had significantly greater PX concentration (39.7 µg/ml) compared to the challenged treatments (25.5, 19.9 and 25.6 µg/ml respectively). Sex had no significant effect on

PX concentration, and there were no interactions between treatments ($P < 0.05$).

The oocyst count at the beginning of the study was negative for all treatments. After 21 d of age, the control treatment remained negative for oocysts. At 7 d post-inoculation, birds from treatment 4 had significantly greater oocyst counts medians (16.14×10^4 oocysts/g) with respect to treatments 2 or 3 (2.42×10^4 and 4.42×10^4 oocysts/g, respectively). At 14 d post-inoculation, there was a significant

reduction in oocyst count in treatment 4 when compared to the previous week (3.45×10^4 oocysts/g). In this treatment, the oocyst count was lower than in treatment 3 (6.98×10^4 oocysts/g), but not different from treatment 2 (2.88×10^4 oocysts/g). At 21 d and 28 d post-inoculation, the oocyst counts were lower for treatment 2 (1.08×10^4 oocysts/g) with respect to treatment 4 (3.96×10^4 oocysts/g), but not different from treatment 3 (2.31×10^4 oocysts/g) ($P < 0.05$). The median of intestinal lesion scores in treatment 1 was zero, whereas for treatments 2, 3, or 4, the median lesion score was 1+ or less, and no statistical difference was found between these treatments ($P > 0.05$), (data not shown).

EXPERIMENT 2

Growth performance was not significantly different between treatments at 49 d of age. However, males had significantly higher feed consumption (5,243 g), BWG (2,565 g), and better FCR (2.05 kg:kg) than females (4,856 g, 2,310 g, and 2.1 kg:kg, respectively) ($P < 0.05$). Pigment consumption was significantly greater in the males, and it differed across treatments according to the level of dietary XA. Birds from treatments 2, 3, and 4 received higher concentration of dietary XA from 35 to 49 d of age, and their XA consumption was 62, 178, and 223 mg per bird greater than that observed in the birds from the control treatment (table 3).

SY was not different between treatments at 49 d of age (figure 3). Female birds had significantly greater skin yellowness (23.72b*) than male birds (21.47b*). PX concentration was not different between treatments at the end

of the study (figure 4), and no significant effect of bird's sex was detected ($P > 0.05$).

Apparent ileal XA digestibility was significantly reduced in the birds challenged with coccidia (53.4% in males and 44.2% in females) in comparison with the control group (66.2% in males and 61.3% in females). No effect of bird's sex was detected ($P > 0.05$).

DISCUSSION

The reduction in BWG and increase in FCR observed in the birds challenged with the highest coccidia dose is in agreement with previous research findings. Infection with *E. acervulina* and *E. maxima* detrimentally affected growth performance 2 to 3 weeks after inoculation (Hein 1968, Preston-Mafham and Sykes 1970). This effect is related to the damage caused by the replication of the parasite in the intestine (Allen and Danforth 1984) that leads to poor digestion and absorption of nutrients (Ruff and Fuller 1975). As expected, the birds that received lower coccidia challenge doses showed intermediate values for BWG and FCR, due to the degree of damage associated with the lower dose of infective oocysts (Stephens *et al* 1967). In 9-d-old birds infected with 10^5 *E. acervulina* oocysts, 6.7×10^3 *E. maxima* oocysts, and 10^4 *E. tenella* oocysts, there was a significant reduction in BW at 5 d post-inoculation (Conway *et al* 1993).

The rate of XA absorption is affected by coccidia infections and it might not recover to normal levels for up to 20 d post-infection. Absorption of other nutrients like glucose might not recover to normal rates before 14

Table 3. Feed consumption, pigment consumption, body weight (BW) gain, and feed conversion ratio of broiler chickens fed increasing levels of dietary xanthophylls from 21 to 49 d of age and infected with *Eimeria* spp. oocysts at 21 d of age, Experiment 2.

Consumo de alimento, consumo de pigmento, ganancia de peso e índice de conversión, de pollos de engorda alimentados con niveles ascendentes de xantófilas del día 21 a 49 de edad, e infectados con ooquistes de *Eimeria* spp. al día 21 de edad, Experimento 2.

Treatment (ppm of xanthophylls)	Feed consumption, g/bird	BW gain, g/bird	Pigment consumption, mg/bird	FCR, kg:kg
85	5,044	2,499	428 ^a	2.023 ^a
108	5,003	2,390	490 ^b	2.097 ^a
141	5,128	2,404	606 ^c	2.133 ^a
162	5,024	2,456	651 ^d	2.055 ^a
SEM	65.4	80.0	5.3	0.05
Sex				
Males	5,243	2,565	566	2.052
Females	4,856	2,310	522	2.102
SEM	46.2	56.6	7.6	0.04
Probability				
Treatment	NS	NS	*	NS
Sex	*	*	*	*
Treatment x Sex	NS	NS	NS	NS

Means with different superscripts within a column differ significantly ($P < 0.05$). Medias con diferentes superíndices en una misma columna difieren significativamente ($P < 0,05$).

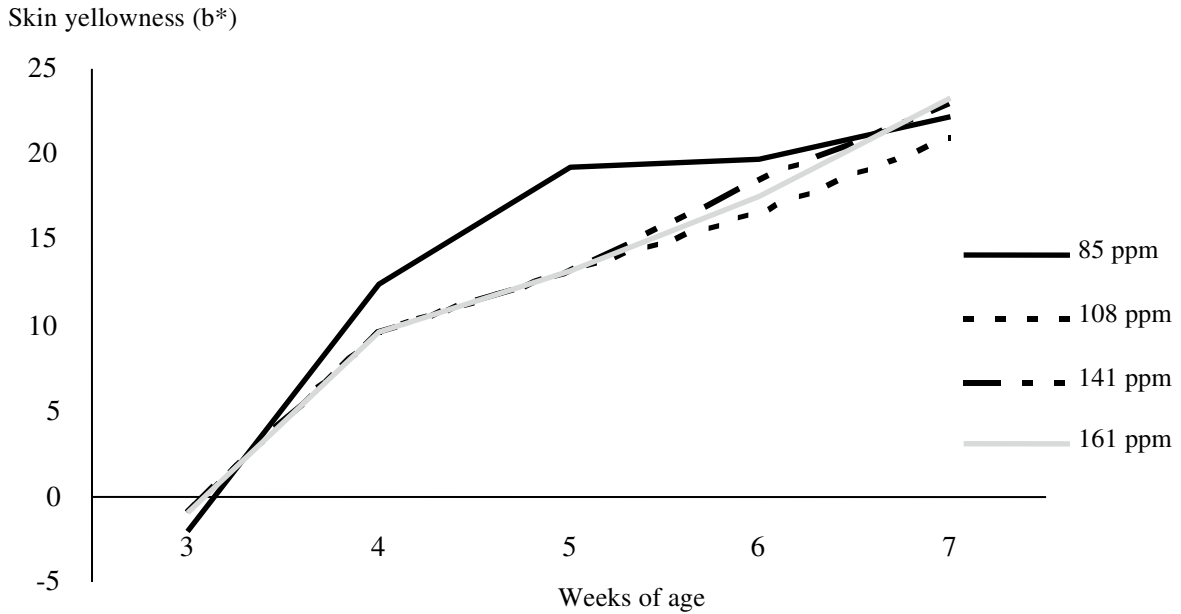


Figure 3. Skin yellowness units (b*) in broiler chickens challenged with 8.32×10^4 oocysts of *Eimeria* spp. and fed increasing doses of xanthophylls, Experiment 2.

Unidades de amarilleamiento cutáneo (b*) en pollos de engorda desafiados con $8,32 \times 10^4$ ooquistes de *Eimeria* spp. y alimentados con dosis ascendentes de xantófilas, Experimento 2.

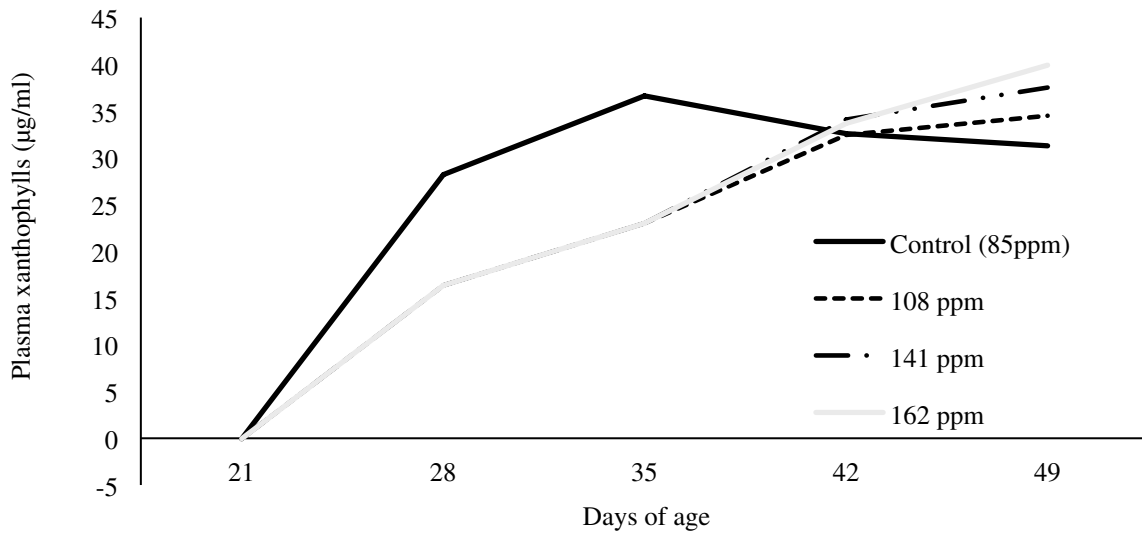


Figure 4. Plasma xanthophylls (µg/ml) in broiler chickens challenged with 8.32×10^4 sporulated *Eimeria* spp. oocysts, treated with a coccidiostat, and fed high doses of xanthophylls, Experiment 2.

Xantófilas plasmáticas (µg/ml) en pollos de engorda desafiados con $8,32 \times 10^4$ ooquistes esporulados de *Eimeria* spp. tratados con un coccidiostato, y alimentados con dosis ascendentes de xantófilas, Experimento 2.

d post-infection (Ruff and Wilkins 1980), thus birds may recover their BWG before they regain skin pigmentation after a coccidia infection. This could explain why birds from treatments 2 and 3 had better BWG than treatment 4. Nevertheless, none of the birds from the infected treatments achieved the same skin pigmentation observed in the non-challenged birds.

In Experiment 1, the reduction in skin pigmentation in birds challenged with coccidia was due to the reduction in the absorption of pigment, mainly caused by *E. acervulina* and *E. maxima*. Both *Eimeria* species produce villi sloughing and shortening in the intestinal regions where most of the pigment absorption occurs. On the other hand, *E. tenella* may induce a mild but continuous decrease in

plasma carotenoids due to the blood loss associated with the damage in the cecal epithelium (McDougald and Fitz-Coy 2008). At the end of the study, SY was similar in all treatments, and the only difference was due to bird's sex. The female birds had significantly higher skin pigmentation, even though the males showed greater pigment consumption. This finding is in agreement with the reports by Sirri *et al* (2010) and Muñoz-Díaz *et al* (2012) and it is attributed to higher deposition of subcutaneous fat in the females, which is related to genetic and hormonal factors (Le Bihan-Duval *et al* 1998, Rance *et al* 2002).

In both experiments there was a reduction in PX concentration at 8 and 13 d post-infection, which has been reported previously (Juárez *et al* 2002). This might be due to the greater quantities of merozoites and gametocytes in the intestinal mucosa during this post-infection period (McDougald and Fitz-Coy 2008) that detrimentally influence XA absorption. The reduction in plasma carotenoids in birds infected with *Eimeria* spp. might also be related with a decline in the synthesis of Apo-I and Apo-III proteins by the enterocyte. These proteins have a major role in the transport of pigment to the liver and other tissues (Allen 1987). Furthermore, *E. acervulina* reduced the intestinal pH, which negatively affects pigment absorption (Kouwenhoven and van der Host 1969).

The highest PX concentration was found at 29 d of pigment consumption in all treatments. This is in agreement with Tepox (2013), who measured the highest PX concentrations after 28 d of consumption of dietary XA concentrations between 65 and 200ppm.

In Experiment 1, the number of oocysts shed through feces in the birds challenged with the highest infective dose (49.92×10^4 SOE) peaked at 7 d post-inoculation, and declined rapidly afterwards. A similar trend was observed in the intestinal lesions (gross white plaques) caused by *E. acervulina* for the same treatment. Such a reduction in oocyst shedding might be due to faster development of immunity in response to a more severe challenge. Therefore, the severity of intestinal lesions and oocyst shedding diminished rapidly. On the other hand, the birds challenged with lower oocyst doses required longer time to develop immunity, thus prolonging oocyst shedding. This could explain why the birds challenged with the intermediate oocyst doses had the highest oocyst shedding counts at 21 d post-infection.

In Experiment 1, there was no significant difference in the intestinal lesion scores, which were compatible with a subclinical infection according to the scale described by Johnson and Reid (1970). Although intestinal lesion scores have been used over the years to assess the degree of damage by coccidia infections both experimentally and under field situations, there seems to be no correlation between the degree of infection or the number of oocysts inoculated or shed through feces (Conway *et al* 1990, Kogut and Powell 1993).

Mild or subclinical *Eimeria* spp. infections are not easily detectable since they do not induce clear negative effects

on growth performance. In countries where pigmented chicken is produced, subclinical coccidia infections are suspected only based on substandard skin pigmentation. Various studies have demonstrated that levels of plasma carotenoids and skin pigmentation are excellent response variables to evaluate coccidia infections due to the sensitivity of these measurements to the disease (Ruff and Fuller 1975, Sharma and Fernando 1975). In this study these parameters were confirmed to be very good indicators of physiological intestinal integrity (Conway *et al* 1993).

Broiler chickens can reach adequate skin pigmentation levels if higher concentrations of dietary XA are fed for 14 d prior to slaughter age (Muñoz-Díaz *et al* 2012). In Experiment 2, even though the birds were infected with *Eimeria* spp., they were able to recover the expected SY after being treated with an anticoccidial drug, and fed with at least 62 mg of XA/bird from 35 to 49 d of age. It is possible that this effect might have been influenced by the high enterocyte turnover rate. During mild coccidia infections (1×10^5 *E. acervulina* oocysts per bird), the acute phase (4-8 d post-infection) is characterized by a sharp drop in absorption and deposition of pigment, and a recovery phase from 9 to 14 d post-infection (Sharma and Fernando 1975). Regeneration of intestinal epithelium occurs rapidly during mild coccidia infections and can be completed by 10 d post-infection (McDougald and Fitz-Coy 2008). Our results suggest that if the coccidia infection is controlled, and the birds received prompt anticoccidial treatment, absorption capacity and skin deposition of pigment may be fully recovered (Mathis *et al* 2004). In addition, feeding very high concentrations of dietary XA sometimes does not result in a significant increase of SY but results in a waste of XA.

No difference was detected in apparent ileal pigment digestibility between males and females, which is in agreement with previous research reported elsewhere (Tepox 2013). The results of the current study suggest that pigment is absorbed in a similar rate in males and females. However, pigment deposition seems to be more efficient in females. In the study reported herein, pigment digestibility was reduced by 15 percent in birds challenged with coccidia with respect to unchallenged birds. Although digestibility of carbohydrates, protein, and amino acids in chickens has been vastly evaluated (Yutste *et al* 1991, Ravindran *et al* 1999, Lemme *et al* 2004), to the author's knowledge, pigment digestibility has not been reported before, especially as influenced by coccidia infections.

Pigmentation measurements could be helpful tools to determine the virulence of *Eimeria* species and strains. It would be necessary to conduct further studies to evaluate the effect of other *Eimeria* strains on these measurements to determine their limitations or benefits as compared to other criteria commonly used to determine the pathological effects of *Eimeria* infections such as oocyst counts and lesion scoring.

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