Effects of dietary combination of 25-OH-D_3 and canthaxanthin on performance, meat yield, bone characteristics and antioxidant status of broilers housed under commercial and experimental conditions

by

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ABSTRACT

The effects of 25-hydroxy vitamin D_3 (25-OH-D_3), canthaxanthin (CX), alone and in combination on broiler performance, meat yield, oxidative status, skin colour and bone characteristics were evaluated in a field trial in Colombia and also under controlled experimental conditions in Canada. The effects of regional and housing conditions on performance, meat yield and bone characteristics were also assessed. In the field trial, one whole broiler cycle of 4,922,130 broilers of both sexes (reared separately) of two commercial broiler strains (A and B) was followed from placement to processing. Birds were fed with a Control diet containing vitamin D_3 at 4,000 IU per kg of complete feed, and a treatment diet (MaxiChick®, MC) containing CX at 6 mg, 2,760 IU of 25-OH-D_3, plus the Control level of vitamin D_3 from 0 to approximately 21 d of age. Additionally, 53% of the males also received 1.0 g per kg of complete feed of marigold extract (MG) from approximately d 22 to processing age.

Strain A had lower FCR and higher weights and yield of carcass, whole breast, breast fillet, and thighs than strain B, but BW and feed intake were similar. MC reduced yield of most of the carcass traits, especially in strain A. Males fed MG had higher feed intake and FCR than males not fed MG. During the fifth week of age, MG increased weight gain and reduced FCR, especially in strain A. During the same period, in males not fed MG, MC reduced weight gain and increased FCR. MG increased weight gain but increased mortality in males fed MC. From d 29 to d 35, MC reduced mortality, but not in males fed MG. Males fed MG had higher breast weight, and weight and yield of carcass, drumstick, and thigh than males not fed MG. MG reduced skin lightness and redness, but increased yellowness. Bone breaking stress was increased and bone breaking strength was nearly increased ($P = 0.0768$) by MC. MC increased bone breaking strength only in strain B

birds. The regional and housing analysis showed that most differences in broiler traits were related to differences in environmental temperature.

In controlled conditions, birds were fed one of seven diets: Control (2,760 IU of vitamin D_3/kg of feed from d 0 to 40); 25D (2,760 IU 25-OH-D_3 /kg of feed from d 0 to 40); CX (Control + 6 mg/kg CX from d 0 to 40); 25DCX (25D diet + 6 mg/kg CX, from d 0 to 40); 25D-Early (25D diet from 0 to 19 d; Control diet thereafter); CX-Early (CX diet from 0 to 19 d; Control diet thereafter); 25DCX-Early (25DCX diet from 0 to 19 d; Control diet thereafter). Diets containing CX increased BW and reduced FCR at d 11 and had a tendency to increase Pectoralis major weights ($P < 0.1$) at 19 and 39 d. CX increased redness and yellowness of shank and breast skin, and breast muscle; especially when fed during the full grow-out period. The presence of CX reduced malondialdehyde concentrations of liver samples at 11 and 19 d. At d 19, an increased trabecular bone cross sectional area at 30% of total femur length from the proximal epiphysis evidenced a synergy between 25-OH-D_3 and CX. At d 39, 25D-Early and 25DCX-Early increased bone breaking strength relative to the other treatments. It was concluded that broiler productivity was strongly strain-dependent. The increased productivity at early ages in the treatments containing CX and in birds fed MG was likely due to an increased antioxidant status. The increased bone quality in birds fed MC, 25D-Early and 25DCX-Early was likely due to the inclusion of 25-OH-D_3 and it confirms the higher biopotency relative to vitamin D_3 at early ages. Both active compounds in MC may positively influence livability and bone formation through different metabolic pathways. Therefore, dietary MC has the potential to increase profitability by increasing the number of saleable birds at processing age, but also may increase bird welfare.

DEDICATION

To Clara. Her support, encouragement, quiet patience and endless love were undeniably the bedrock upon which this dream was built. Her tolerance during my bad moments and long nights is the biggest testimony of her unyielding devotion and love for me.

To Fabio, my father. He always taught me to never give up and showed me that smiling is possible despite of the biggest suffering.

To María del Carmen, my mother. Because during my whole life she always has taught me the power of faith and love.

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$1,25\text{-}(OH)_2D_3$ – Calcitriol; 1,25 dihydroxycholecalciferol

25D – Treatment providing 69 μg/kg $25\text{-}OH\text{-}D_3$ from d 0 to 40

25DCX – Treatment providing 25D diet + 6 mg/kg CX, from d 0 to 40

25D-Early – Treatment providing 25D diet from 0 to 19 d; Control diet thereafter

25DCX-Early – Treatment providing 25DCX diet from 0 to 19 d; Control diet thereafter

$25\text{-}OH\text{-}D_3$ – Calcidiol; 25 hydroxycholecalciferol

α – Alpha

A - Area

a* - Redness

ß – Beta

b* - Yellowness

BBS - Bone breaking strength

BMC - Bone mineral content

BMD – Bone mineral density

BW – Body weight

^oC – Centigrade

CF - Concrete floor

CIE - Commission Internationale de l'Eclairage

CIGR - International Commission of Agricultural Engineering (Commission Internationale du Génie Rural)

cm - centimeter

CR - Coffee region

CSA - Bone cross sectional area

CX - Canthaxanthin

CX-Early – Treatment providing CX diet from 0 to 19 d; Control diet thereafter

CV – Coefficient of variation

CYP24A1 - 24-hydroxylase

CYP27A - 25-hydroxylase

CYP27B1 - 1α-hydroxylase

d - Day

DBP - Vitamin D_3 binding proteins

DF - Dirt floor

DNA - Deoxyribonucleic acid

EFSA - European Food Safety Authority

FASEB - Federation of American Societies for Experimental Biology

FCR – Feed conversion ratio

FL - First level of a two-storey house

g – gram

GR – Geographical region

HIS - Heat stress index

HT – House type

IBD - Infectious bursal disease

IU – International units

KgF – Kilogram-force

kN – kilo Newton

L* - Lightness

L:D – Hours light:hours darkness in the photoperiod

m - meter

m^2 – Square meter

MC – MaxiChick®

MDA - Malondialdehyde

MG - Marigold extract

µ - micro

µg – micro gram

mg - milligram

µL – microliter

mL – milliliter

µM – micromoles

mM – millimoles

mm – millimetre

mv – millivolts

n – Number of observations

nmol – nanomoles

NRC - National Research Council

OF - Open-sided with fans house

OL – House of one level or single-storey house

OS - Open-sided house

π - Pi number

PA – Processing age

PLoS – Public Library of Science

PUFA - Polyunsaturated fatty acids

r – Correlation coefficient

r^2 – Square radius

R^2 – Coefficient of determination

RH – Relative humidity

RNA – Ribonucleic acid

s - Seconds

SL - Second level of a two-storey house

TBARS- Thiobarbituric acid reactive substances

TD - Tibial dyschondroplasia

TVF - Tunnel-ventilated house with foggers

TVP - Tunnel-ventilated house with cooling pad

VR - Valle region

VDR - Vitamin D_3 membrane receptors

Vitamin D_3 - Cholecalciferol

WA - Water absorption

wk – Week

1. LITERATURE REVIEW

1.1 INTRODUCTION

The human nutritional demand for high quality protein has promoted research in genetic, nutrition and management in different sectors of the agriculture industry. Broiler chickens have increased greatly in terms of growth rate, feed efficiency and meat yield compared to previous decades (Havenstein et al., 2003; Schmidt et al., 2009). However, these important changes have created additional pressure on physiological systems of the bird, and some undesirable traits have emerged due to such rapid growth including cardiovascular diseases (Tona et al., 2005), skeletal disorders (Shim et al., 2012), impaired immune function (Cheema et al., 2003), and reproductive complications (De Beer and Coon, 2007). Skeletal disorders are still an important concern for the chicken meat industry worldwide, and are responsible for losses in production as well as poor welfare (Knowles et al., 2008; Sun et al., 2013). Additionally, they are a common cause of condemnations in slaughtering plants (Lupo, et al., 2008).

The role of vitamin D_3 in calcium and phosphorus metabolism, and in the prevention of skeletal development problems are well documented (Zhang et al., 1997; Fritts and Waldroup, 2003; Sun et al., 2013). In the poultry and swine industries, vitamin D_3 has traditionally been provided by dietary supplementation, due to insufficient exposure to direct sun light which is needed for endogenous production in the skin (Soares et al., 1995; Coffey et al., 2012). One of the metabolites of vitamin D_3 is 25-hydroxycholecalciferol (25-OH-D_3). Various studies have shown that supplementation of this metabolite increases broiler performance (Brito et al., 2010), meat yield (Yarger et al., 1995), immune function (Chou et al., 2009), and bone strength (Fritts and Waldroup, 2003) in comparison with other sources of vitamin D_3 at equal level of supplementation.

Carotenoids are pigments that have been used in poultry as feed additives, mainly for skin (Castañeda et al., 2005) and egg (Cho et al., 2013) pigmentation. These compounds are naturally occurring or synthetically manufactured pigments with antioxidant properties (Mercadante and Egeland, 2004; Lü et al., 2010). Canthaxanthin (CX) is a carotenoid which can reduce lipid peroxidation and enhance serum total antioxidant capacity (Surai et al., 2003; Zhang et al., 2011; Rocha et al., 2013). Recently, the combination of 25-OH-D_3 and CX has been shown to increase productivity, hatchability and antioxidant status in broiler breeders and its offspring (Rosa et al., 2010a; 2010b).

1.1.1 Rationale for the study

The rationale for carrying out this research project lies in the documented findings in the literature that 25-OH-D_3 and CX positively influence broiler performance through different metabolic pathways. Therefore, it is interesting to evaluate if may exist an additive effect between these two feed additives on performance, livability, and meat yield of broiler chickens. This is the first research where the combination of 25-OH-D_3 and CX is evaluated in broilers. This research also pretend to offer updated information for scientists and poultry producers about the combination of vitamin D_3 and CX and its benefits in performance and profitability. At the end of this process, recommendations and new areas of research will be proposed in order to corroborate our findings or explore new areas of investigation.

The next review explores the mechanisms and effects of 25-OH-D_3 and CX, and how these two feed additives can influence broiler performance, livability and carcass characteristics.

1.2 VITAMIN D

Vitamin D_3 is a pro-hormone that is converted to the active hormone 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] after two metabolic steps (Soares et al., 1995; Norman, 2008). The most well-

known roles of vitamin D_3 are regulation of plasma calcium and phosphorus levels through enhancing intestinal absorption, minimizing renal losses and stimulating bone resorption (Soares et al., 1995; Atencio et al., 2005; Khan et al., 2010). However, vitamin D_3 also exerts a range of effects in various other metabolic processes in birds including bone mineralization (Rama-Rao et al., 2006; 2009), shell formation (Keshavarz et al., 2003; Käppeli et al., 2011), muscle metabolism (Boland, et al., 1985), immune response (Fritts et al., 2004), and reproduction (Coto et al., 2010).

1.2.1 Vitamin D and Metabolites

Vitamin D is an aggregation of compounds with antirachictic activity and a molecular structure similar to steroid hormones (estradiol, cortisol, and aldosterone) whose common structural ring is the cyclopentanoperhydrophenanthrene (Atencio et al., 2005; Norman, 2008). There are approximately 30 molecules in the vitamin D group, but the two most significant forms are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Typically, vitamin D_3 is obtained from the diet or by conversion of 7-dehydrocholesterol into vitamin D_3 in the skin as response to ultraviolet radiation (Soares, et al., 1995; Edwards, 2003). However, as a chicken's body is covered by feathers and little to no direct sunlight reaches the inside of commercial poultry houses, endogenous production of vitamin D_3 is very limited (Atencio et al., 2005). Nonetheless, broilers can also obtain vitamin D_3 from the diet (Edwards, 2003), and hens can pass on a supply of vitamin D to chicks via the yolk sac (Atencio et al., 2005). The biological activity of vitamin D_2 is only 10% of vitamin D_3 activity in birds (Norman, 1987), has no anti-rachitic properties, and vitamin D binding proteins (DBP) do not bind it effectively (Soares et al., 1995; Bar, 2008). Hence, dietary supplementation of vitamin D in poultry is usually done with the synthetic crystalline form of vitamin D_3 or with vitamin D_3 metabolites (Atencio et al., 2005).

The optimum level of inclusion of vitamin D_3 in poultry has been under investigation for several decades. When used at higher levels than the National Research Council (NRC; 1994) recommendation of 200 IU/kg of feed, vitamin D_3 increased growth (Fritts and Waldroup, 2003; 2005), bone mineralization, mineral retention, and reduced tibial dyschondroplasia and rickets (Whitehead et al., 2004; Rama-Rao et al., 2006; 2009; Khan et al., 2010). In broilers, when vitamin D_3 was increased from 300 to 1,200 IU/kg of feed, body weight gain, feed intake, tibia density were increased, and leg abnormalities were reduced (Rama-Rao et al., 2006; 2009). Additionally, increasing dietary level of vitamin D_3 from 200 to 3,600 IU/kg in broiler diets has the potential to reduce the level of inclusion of calcium and phosphorus in the diet by up to 50 and 25 mg/kg respectively without detriment to performance (Rama-Rao et al., 2006). Moreover, broiler performance, bone mineralization, relative weights of lymphoid organs (bursa, thymus and spleen), serum calcium and phosphorus concentration are increased when vitamin D_3 is increased from 200 to 3,500 IU/kg of feed (Khan et al., 2010). Taking this data in to account, commercial poultry diets commonly have an inclusion of vitamin D_3 in a range between 2,000 to 5,000 IU/kg of feed (Fritts and Waldroup, 2003).

1.2.2 Absorption and metabolism of vitamin D_3 and its metabolites

Similar to other fat soluble vitamins, vitamin D_3 is absorbed via diffusion in the intestinal tract, principally in the duodenum and upper jejunum (Bar et al., 1980; Borel, 2003). As with other highly lipophilic food micro-constituents, absorption occurs as micelles form and is facilitated by the presence of fat and bile salts (Garrett and Young, 1975). Micelles containing vitamin D_3 remain in suspension in the intestinal lumen until they are absorbed by the enterocytes. The micelles then enter the portal system as large lipoproteins (portomicrons) and are transported via the blood to the liver while bound to a specific plasma DBP (Norman, 1987; Elaroussi et al., 1994).

In the liver, the first hydroxylation of vitamin D_3 occurs when an OH group is added in position 25 by 25-hydroxylase (CYP27A), generating the metabolite 25-OH-D_3 or calcidiol (Shanmugasundaram and Selvaraj, 2012), the most common circulating form of vitamin D_3 (Haussler and Rasmussen, 1972). Thus, 25-OH-D_3 is the best marker to evaluate vitamin D_3 status in poultry (Soares et al., 1995; Bar et al., 2003). The 25-OH-D_3 leaves the liver bound to DBP for transport to the kidneys (Soares et al., 1995). In the kidney, cubilin and megalin are proteins which assist the endocytosis of 25-OH-D_3 into the proximal tubule cells (Nykjaer et al., 1999). At that point, 1α-hydroxylase (CYP27B) causes a second hydroxylation in position 1 forming l,25-$(OH)_2$-D_3 or calcitriol which is the active form of vitamin D_3 (Shanmugasundaram and Selvaraj, 2012). In chickens, 1α-hydroxylase is specific for 25-OH-D_3 and is not able to hydroxylate cholecalciferol (Shanmugasundaram and Selvaraj, 2012). The cytochrome P-450 also contains the enzyme 24-hydroxylase (CYP24) which converts l,25-$(OH)_2$-D_3 to its excretion products: calcitroic acid and 1α,25-$(OH)_2D_3$-26,23-lactone (Prosser and Jones, 2004). This metabolic pathway is illustrated in Figure 1.1, and forms the basis of the vitamin D_3 endocrine system.

In renal cells, 25-OH-D_3 can also be converted to 24,25-$(OH)_2$-D_3 by 24 hydroxylase which is the first stage of vitamin D_3 elimination (De Matos, 2008). There is evidence that 24,25-$(OH)_2$-D_3 participates in bone development (Seo et al., 1997) and sexual maturation in hens (Norman et al., 1983). During hypocalcaemia, hydroxylation at position 1 is promoted; but when calcemia is normal or high, hydroxylation at position 24 is up-regulated while 1α-hydroxylase activity is reduced (De Matos, 2008).

In the bloodstream, l,25-$(OH)_2$-D_3 is carried primarily by DBP (Prosser and Jones, 2004), and interacts with vitamin D membrane receptors (VDR) located several organs and tissues such as intestines (Nemere et al., 1994), muscle cells, bone, kidney, parathyroid gland, pancreas,

pituitary, chorioallantoic membrane and the egg shell gland (Norman, 1987; Elaroussi et al., 1994). The affinity of DBP for 25-OH-D_3 and 24,25-$(OH)_2$-D_3 is similar and is approximately 10 times higher than the affinity for 1,25-$(OH)_2$-D_3, which is an advantage for dietary supplementation of 25-OH-D_3 (Soares et al., 1995; Teegarden et al., 2000).

1.2.3 Vitamin D_3 metabolites in poultry

In poultry nutrition, 25-OH-D_3 and l,25-$(OH)_2$-D_3 are the most studied metabolites of vitamin D_3. However, due to some advantages when compared to vitamin D_3, 25-OH-D_3 is used routinely in numerous commercial poultry nutrition programs (Atencio et al., 2005). In poultry, 25-OH-D_3 has the highest absorption rate and considerably lower daily excretion rate among the metabolites of vitamin D_3 (Chou et al., 2009). The overall absorption of 25-OH-D_3 was greater (74.9%) relative to vitamin D_3 (66.5%) at the same level of inclusion, which is likely related to the greater polarity of 25-OH-D_3 (Bar et al., 1980). Among lipophilic feed constituents such as vitamin D_3 and carotenoids, the more polar molecules are less dependent on bile salts for absorption (Compston et al., 1981, Borel, 2003), which facilitates intestinal absorption (Haussler and Rasmussen, 1972). The endocytosis of 25-OH-D_3 in the intestine is more efficient relative to vitamin D_3, due to the higher affinity of this metabolite to the DBP (Soares et al., 1995; Teegarden et al., 2000), and for the presence of specific receptors for 25-OH-D_3 in the intestinal epithelia (Nemere et al., 1994; Phadnis and Nemere, 2003). In addition, 25-OH-D_3 has a higher retention compared to vitamin D_3 (93% and 80% respectively) in chicks when included at the same dietary level (Bar et al., 1980), and its biological activity is 2.0 to 2.5 times higher than vitamin D_3 per molecule (Soares et al., 1978). However, this higher biopotency is more noticeable when 25-OH-D_3 and vitamin D_3 are compared at levels lower than typically used in the poultry industry (125 to

500 IU/kg of feed), but not at higher dietary levels (1,000 to 4,000 IU/kg of feed; Fritts and Waldroup, 2003).

During the first 10 d of age, the hepatic production of 25-OH-D_3 in chickens is low (Saunders-Blades, 2008); moreover, at those ages the vitamin D_3 absorption is lower because the digestive enzymatic system is not fully developed and the digestive tract is still immature (Noy and Sklan, 1995). Liver function and the corresponding hydroxylation activity may be reduced by stressful conditions (Thaxton and Puvadolpirod, 2000), mycotoxicosis (Yarru et al., 2009), and bacterial infection (Peighambari et al., 2000). These limitations may be overcome by dietary inclusion of 25-OH-D_3 since this metabolite is not hydroxylated in the liver (Käppeli et al., 2011), hence vitamin D_3 status can be supported and increased by feeding 25-OH-D_3. Currently 25-OH-D_3 is commercially manufactured from cholecalciferol and marketed under the commercial name of HyD® by DSM Nutritional Products, Basel, Switzerland (Käppeli et al., 2011).

Other vitamin D_3 metabolites are commercially available for poultry, including 1-α-hydroxycholecalciferol (1α-OH-D_3, alpha-calcidiol), a synthetic analogue to 1,25-$(OH)_2$-D_3, which is converted in the liver to active vitamin D_3 without requiring kidney hydroxylation (Han et al., 2012). However, because its transformation is not dependent on plasma calcium levels and birds start to show signs of toxicity at 3 times the level commonly used in the broiler industry (5 µg/kg), alpha-calcidiol has a higher risk of toxicity than other vitamin D_3 metabolites (Pesti and Shivaprasad, 2010). Another commercially available metabolite of vitamin D_3 is extracted from the plant *Solanum glaucophyllum* and contains large amounts of 1,25-$(OH)_2$-D_3, as a glycoside molecule [1,25-$(OH)_2$-D_3-glycoside]. This plant extract can increase 1,25-$(OH)_2$-D_3 plasma levels faster than vitamin D_3 or 25-OH-D_3, but has a shorter half-life in the body, just 6 to 8 hours (Rovegno et al., 2012). This metabolite does not need to be hydroxylated in the liver or in the

kidney, therefore bypasses the regulatory control of the synthesis of 1,25-$(OH)_2$-D_3. At high doses this glycoside can lead to hypervitaminosis and toxicity in rabbits (Zanuzzi et al., 2012). In broilers, dietary 25-OH-D_3 did not show renal signs of toxicity or a reduced BW at up to 10 times of a dietary inclusion of 69 µg/kg; however, this metabolite is 5 to 10 times more toxic than vitamin D_3 (Yarger et al., 1995). Conventionally, the equivalence of 25-OH-D_3 with vitamin D_3 activity has been calculated based on the conversion of 0.025 µg of cholecalciferol to 1 IU; therefore the manufacturer's recommended level of 69 µg/kg is equivalent to 2,760 IU of vitamin D_3 activity per kg of feed (NRC, 1994; Fritts and Waldroup, 2003).

1.2.4 Dietary supplementation of 25-OH-D_3 to broilers

Dietary inclusion of 25-OH-D_3, as a partial replacement or sole source of dietary vitamin D_3 activity, can increase BW and feed efficiency, especially when included throughout the grow-out period (Mireles et al., 1996; Fritts and Waldroup, 2003). In 10 trials comparing dietary 25-OH-D_3 and vitamin D_3 at the same basal level (2,760 IU/kg), BW at processing (from 46 to 52 d) increased by an average of 42 g and FCR was reduced by 0.026 kg/kg (Yarger et al., 1995). Various reports indicate that the combination of 25-OH-D_3 and vitamin D_3 in broiler rations results in increased weight gain compared to when either source is used independently and at the same basal level (Papešová et al., 2008; Brito et al., 2010; Michalczuk et al., 2010), which can be related to the more efficient intestinal absorption of 25-OH-D_3, its greater biopotency, and that it by-passes hydroxylation in the liver. In broilers, the characteristics of the small intestine can be affected by supplemental 25-OH-D_3. At 7 d, the small intestine weight and crypt depth were reduced in broilers fed 25-OH-D_3 (2,760 IU/kg and 1,380 IU/kg of feed in starter and grower diets respectively) relative to birds fed vitamin D_3 (3,000 IU/kg). A decreased small intestine weight and crypt depth

can reduce the energy demand and enterocyte turnover, helping the bird to conserve more energy and nutrients for growth and other physiological needs (Moghaddam et al., 2012).

There are few studies regarding the effects of 25-OH-D_3 on processing yield and the results are not conclusive. However, in 10 trials, Yarger et al. (1995) reported an average increment of 0.53% in breast yield in birds fed 2,760 IU/kg 25-OH-D_3 compared to birds fed with vitamin D_3 at the equivalent level of inclusion. Similarly, Brito et al., (2010) reported a mean increment of 0.9% in carcass yield in favor of the supplementation of 25-OH-D_3 during each of the three periods (1-21 d, 22-38 d, and 39-45 d) compared to vitamin D_3 at equivalent levels of inclusion. Also in that study, the highest carcass yields were obtained with dietary inclusion of 25-OH-D_3 of 1,500, 1,200, and 750 IU/kg at the three periods of inclusion, respectively. More recent evidence indicates that dietary 25-OH-D_3 in broilers increases meat yield in comparison to broilers fed vitamin D_3 at the same level of inclusion (5,520 IU/kg), particularly when fed throughout the grow-out period (Vignale et al., 2013). Notwithstanding, the mechanisms of increment in carcass and breast yield have not been clearly established yet. Michalczuk et al. (2010) found a higher water chill retention by muscle fibers in broilers fed with a combination of 25-OH-D_3 (2,760 IU/kg) and vitamin D_3 (1,260 IU/kg) in comparison to broilers fed dietary vitamin D_3 (4,000 IU), which could increase meat yield indirectly.

Chicken meat quality also seems to be influenced by 25-OH-D_3. In broilers, dietary combination of 25-OH-D_3 and vitamin D_3 (at total of 4,000 IU/kg of vitamin D_3 activity) increased monounsaturated fatty acid content and reduced the level of polyunsaturated fatty acids in fat of chicken carcass; in addition, thiobarbituric acid reactive substances (TBARS) were reduced in the abdominal fat and fat of leg muscle samples (Michalczuk et al., 2010). The mechanism of these findings have not been not established yet; however, a reduced level of polyunsaturated fatty acids

in chicken meat can retard the rate of fat oxidation during storage, since polyunsaturated fatty acids are more susceptible of oxidative deterioration (Cortinas et al., 2005).

Bone characteristics were influenced by 25-OH-D_3 dietary inclusion. Dietary 25-OH-D_3 inclusion decreased severity of tibial dyschondroplasia (TD) and increased bone ash. A reduction in the incidence of TD from 64% to 10% in 3-wk-old broilers was seen when vitamin D_3 (3,000 IU/kg) was replaced with the same level of 25-OH-D_3 (Rennie and Whithead, 1996). Similarly, at 21 and 42 d a greater bone ash content and lower TD incidence was found in broilers fed increasing levels of 25-OH-D_3 (from 125 to 4,000 IU/kg) compared to birds fed vitamin D_3 at equivalent levels of inclusion (Fritts and Waldroup; 2003). Therefore, 25-OH-D_3 enhanced mineral utilization which led to a greater bone quality, and this effect was more noticeable when the dietary calcium and phosphorus level were reduced in broiler diets (Ledwaba and Roberson, 2003; Angel et al., 2006). Bone breaking strength and ash content were highly correlated in broilers ($r = 0.98$; Rowland et al., 1967). Bone breaking strength was increased at 41d in broilers fed 25-OH-D_3 throughout the grow-out period or at early ages (0 to 28d) in comparison with birds fed vitamin D_3 at same level of activity (2,760 IU/kg; Saunders-Blades, 2008).

Dietary 25-OH-D_3 has not shown a consistent effect on mortality (Yarger et al., 1995; Fritts and Waldroup, 2003; 2005; Papešová et al., 2008; Brito, 2010). However, supplementation of 25-OH-D_3 influences immune response in chicks. An increase in *Salmonella*-specific antibodies was found in chicks fed 25-OH-D_3 (2,760 IU/kg from 0 to 21 d, and 1,380 IU/kg from 22 to 39 d) in comparison to birds fed vitamin D_3 at 3,000 IU/kg in response to an experimental infection with *Salmonella typhimurium*; in addition, phagocytosis activity was higher in birds fed 25-OH-D_3 (45%) than in those fed vitamin D_3 (35%; Chou et al., 2009).

1.3 OXIDANTS AND ANTIOXIDANTS

Oxidants are highly reactive atoms or molecules with one or more unpaired electrons in the external orbitals that make them unstable (Fang et al., 2002; Lü et al., 2010). The unpaired electrons form a magnetic field that attracts other molecules or atoms around them, causing oxidation of those compounds (Lü et al., 2010). Oxidation occurs through removal of a hydrogen atom, abstraction of an electron or the addition of oxygen (Buettner, 1993; Lü et al., 2010). When oxidants are present at excessive levels they can induce tissue damage (Iqbal et al., 2002), oxidize cell components such as lipids (Gutteridge, 1995; Smet et al., 2008; Tavárez et al., 2011), protein (Iqbal et al., 2004), DNA (Voljč et al., 2011), and reduce broiler performance (Mujahid et al., 2005).

Reactive species of oxygen, nitrogen, and chlorine are oxidants produced during metabolism (Rastogi et al., 2010), and are also physiological mediators and signaling molecules (Lander, 1997; Lee et al., 2011). These reactive species are classified in two groups: free radicals such as superoxide, hydroxyl radical, peroxyl, hydroperoxyl, nitric oxide; or non-radical such as hydrogen peroxide, ozone, singlet oxygen, hypochlorous acid, nitrous acid, nitryl chloride (Gutteridge, 1995). These molecules are controlled in the organism by antioxidants which are compounds capable of donating electrons to oxidants, diminishing their reactivity (Huang, 2005). By doing this, antioxidants become more reactive, but are more stable than radicals and do not cause cellular damage (Lü et al., 2010).

Antioxidants are classified as synthetic or natural. Synthetic antioxidants including butylated hydroxyanisole, butylated hydroxytoluene (Surak et al., 1977), tertbutyl hydroxiquinone, dodecyl, propyl and octyl gallate (Tavárez et al., 2011), and ethoxyquin (Cabel et al., 1988) have been used as feed preservatives in poultry production. Natural antioxidants include enzymatic and non-

enzymatic antioxidants (Zhang et al., 2014). Among non-enzymatic antioxidants are dietary components like vitamins C and E (Puthpongsiriporn et al., 2001), carotenoids (Rajput et al., 2013), polyphenols and flavonoids (Brenes et al., 2008), and plasma molecules such as uric acid (Cohen et al., 2007). Catalase, superoxide dismutase, and glutathione peroxidase are some of the enzymatic antioxidants (Gaál et al., 1995). It has been proposed that antioxidant status in broilers can be increased by including dietary antioxidants (Jensen et al., 1998). However, to be effective, they must be absorbed from the diet in reasonable amounts and accumulated in the target tissues (Surai, 2003).

The higher proportion of oxidants relative to antioxidants at a cellular or tissue level is defined as oxidative stress (Voljč et al., 2011). In poultry, oxidative stress may occur as a consequence of contamination of feed with fungal toxins (Frankič et al., 2006), high environmental temperatures (Lin et al., 2000; Mujahid et al., 2005), high stocking densities (Beloor et al., 2010), or due to several pathological conditions, such as coccidiosis (Georgieva et al., 2006). Furthermore, broilers are exposed to many stressors at market age, such as catching, crating (Akşit et al., 2006), and transport that have the potential to increase ROS production leading to lipid peroxidation (Zhang et al., 2010). Additionally, poultry diets often include a large proportion of dietary polyunsaturated fatty acids (PUFA) which are highly susceptible to oxidation (Lauridsen et al., 1997). The level of PUFA in eggs and meat are increased at higher dietary levels of inclusion; but these poultry products become more susceptible to oxidative deterioration, thus reducing shelf time (Cortinas et al., 2005; Narciso-Gaytán et al., 2010).

1.3.1 Carotenoids: an overview

Carotenoids are the biggest group of pigments in nature. Approximately 750 pigments belonging to this family have been isolated and characterized (Mercadante and Egeland, 2004).

Carotenoids are antioxidants that are able to quench singlet molecular oxygen and porphyrin triplet energies, and are potent free radical scavengers (Lü et al., 2010, Zhang et al., 2014). They are organic molecules synthesized by plants and photosynthetic microorganisms such as some algae and bacteria (Fraser and Bramley, 2004). Provitamin A activity is the best established function of carotenoids, but not all carotenoids are vitamin A precursors (Surai et al., 2001). Carotenoids that can be converted to vitamin A must be contain at least one ß-ionone ring such as ß-carotene, α-carotene and ß-cryptoxanthin (Fraser and Bramley, 2004). As poultry diets are normally supplemented with preformed vitamin A, the contribution of the feed-derived carotenoids to vitamin A nutrition is not crucial. Animals and humans are unable to synthesize carotenoids, but are able metabolize them once they are consumed (Ishida and Bartley, 2005). There are two carotenoid subgroups; the first group is carotenes (hydrocarbon carotenoids, including lycopene, ß-carotene, α-carotene) with no oxygen in their structures; these compounds are apolar carotenoids. The other group is called xanthophylls or oxycarotenoids such as lutein, P-cryptoxanthin, canthaxanthin, and astaxanthin which are polar and contain oxygen in the ring which facilitates absorption and deposition (Furr and Clark, 1997; Surai et al., 2001; Ishida and Bartley, 2005).

The carotenoid structure is characterized by a system of conjugated double bonds between the carbon atoms where electrons are delocalized over the length of the chain, so they require less energy to reach an excited state (Bendich and Olson, 1989). The free radical-quenching ability of carotenoids is related to these double bonds; as the number of double bonds increases, the greater the singlet oxygen quenching ability because this configuration permits the electrons to interact easily with other ions (El-Agamey et al., 2004; Surai, 2012). Carotenoids with nine or more conjugated double bonds, like canthaxanthin (Figure 1.2) are the most potent carotenoid

antioxidants (Bendich and Olson, 1989). Accordingly, the efficacy in quenching singlet oxygen among carotenoids has been established as being high for lycopene, astaxanthin and canthaxanthin, intermediate for β-carotene and bixin, and low for lutein, and crocin (Fontana et al., 2000. In birds, carotenoids have the capacity to interact synergistically with other dietary antioxidants like vitamin E, causing a greater antioxidant status relative to individual inclusion of vitamin E. (Jensen et al., 1998; Surai et al., 2003).

Carotenoids are soluble in organic solvents and have limited solubility in water (Borel et al., 1996). Therefore, the absorption of carotenoids is similar to other fat soluble compounds (refer to vitamin D_3 absorption previously described in this chapter). In the liver, carotenoids are incorporated into lipoproteins, and are delivered to peripheral organs depending on the presence of a carotenoid specific binding protein (Tyczkowski and Hamilton, 1986; Surai and Speak, 1998; Na et al., 2004). Carotenoid concentration in plasma is related to the concentration in the feed ingested (Koutsos et al., 2003; Ishida and Bartley, 2005). In birds, carotenoids are deposited in liver, yolk, thymus, bursa, subcutaneous adipose layer, breast, shank and toe-web skin (Pérez-Vendrell et al., 2001; Koutsos et al., 2003; Karadas et al., 2005). Notwithstanding, in poultry carotenoid absorption and accumulation is considered low. Hencken (1992) reported that in broilers about 94% and 83% of astaxanthin and zeaxanthin supplied in the diet were excreted respectively and 0.4% of astaxanthin and 1.7% of zeaxanthin were deposited in the skin. For deposition in yolk by laying hens, this author reported a deposition of 30 to 45% for dietary canthaxanthin, 14% for astaxanthin, and 25% for zeaxanthin.

1.3.2 Canthaxanthin in poultry

Canthaxanthin is a diketo-β-carotene with no pro-vitamin A activity (Surai et al., 2001), and is also classified as an oxycarotenoid (xanthophyll; Zhang et al., 2011). In nature, it can be found

in the mushroom *Cantharellus cinnebari*, in some fish and crustaceans of red colour, and in the flamingo's feathers (Fontana et al., 2000); however, CX can be also synthetically produced and stabilized (EFSA, 2010).

It has been recognized that CX is a potent antioxidant (Surai et al., 2001). A lower lipid peroxidation in rat liver microsomal membranes was detected when CX was added to those tissues as compared to the Control group with no carotenoids (Palozza and Krinsky, 1992). Similarly, a lower lipid oxidation over time was found in liver homogenates from canthaxanthin-fed chicks than homogenates prepared from control-fed chicks (Mayne and Parker, 1989). These findings appear to be related to the ability of CX to quench singlet molecular oxygen, and scavenge other free radicals (Mortensen et al., 1997; Böhm et al., 2012).

The evidence indicates that CX is deposited in yolk and increases hatchability and chick quality, and reduces embryo mortality through a greater antioxidant status (Robert et al., 2007; Zhang et al., 2011; Rosa et al., 2012). The effect on livability can be found not only during the embryonic period but also post-hatch. Cumulative mortality to d 21 was lower in chicks from breeders that received 6 mg of dietary CX per kg of feed than chicks from breeders with no CX supplementation (0 and 4% mortality, respectively); in addition, the group with lower mortality had a higher antioxidant capacity (Zhang et al., 2011). Dietary CX at 0.01 or 0.02% in broiler diets reduced creatine kinase activity in broilers exposed to 5 ppm of aflatoxin in the feed in comparison to the control group without dietary CX (Okotie-Eboh et al.; 1997). Since creatine kinase activity indicates tissue damage (Hocking et al., 1996), a reduction of this enzyme may suggest a reduction in the pathological effects of aflatoxicosis in broilers fed CX.

1.3.3 Skin pigmentation in broilers

Skin pigmentation is an important factor in consumer acceptance of broiler products in many countries, and has become an economically important factor in production and marketing of poultry products (Tyczkowski and Hamilton, 1986; Pérez-Vendrell et al., 2001). Fletcher (2002) mentioned that there are regional differences in consumer preferences for fresh whole broilers according to skin colour. These preferences are based on cultural traditions, conventional and regional ingredient supplies, local feeding practices, genetic stock (there is a different ability of some breeds to deposit carotenoid pigments in the skin), and availability of carotenoids for feed inclusion (Williams, 1992; Fletcher, 2002). In some poultry markets like Mexico and Colombia, customers are willing to pay premium prices for pigmented whole or cut-up chicken; additionally some of them associate the broiler yellow skin colour with organic practices (Williams, 1992; Martínez-Peña et al., 2004; Castañeda et al., 2005).

Different sources of pigments are used in poultry feed. Dietary carotenoid pigments are deposited in the skin and fat (Pérez-Vendrell et al., 2001; Koutsos et al., 2003). Among the natural sources of carotenoids are yellow corn, corn gluten meal, and dehydrated alfalfa meal, concentrates of oxycarotenoids such as algae meals, marigold (MG, *Tagetes erecta*) meals, and okra (*Abelmoschus esculentus*; Ouart et al., 1988; Liu et al., 2008; Muñoz-Díaz et al., 2012). Among the synthetic pigments, the most frequently used in poultry are canthaxanthin, capsanthin and apo-ester (Perez-Vendrell et al., 2001; Castañeda et al., 2005; Liu et al., 2008). To meet the consumer demand and achieve the expected skin colour of chickens, broiler producers may combine a yellow carotenoid such as apo-ester, lutein, or zeaxanthin with a red one such as CX, citranaxanthin, capsanthin, or capsorubin (Pérez-Vendrell et al., 2001; Sirri et al., 2010). Normally, the duration of feeding and level of dietary carotenoids are based on the target level of skin pigmentation, the

most effective combination of red and yellow pigments, and the cost of the pigment inclusion (Marion et al., 1985). In broilers, skin pigmentation depends on the amount of xanthophylls consumed that is result of the daily feed intake, the length of the feeding period and the carotenoid concentration (Bartov and Bornstein, 1969). A higher skin pigmentation is seen when carotenoids are included during the last 2.5 to 3 weeks of the broiler production cycle (Pérez-Vendrell et al., 2001; Castañeda et al., 2005; Muñoz-Díaz et al., 2012). In this period, the broiler feed intake is greater and consequently more pigment is deposited; besides, there is a linear relationship between skin yellowness and the ME/kg, and also with dietary pigment level (Muñoz-Díaz et al., 2012). In contrast, skin pigmentation is reduced by scalding temperatures over 53^{o}C because epidermis removal is increased during plucking (Heath and Thomas, 1973; Petracci and Fletcher, 2002); and also during coccidiosis due to reduce carotenoid absorption (Marusich et al., 1972).

Traditionally the degree of skin pigmentation is measured using the CIE colour system (Commission Internationale de l'Eclairage), which considers colour as a three-dimensional characteristic of appearance: lightness (L*) which grades the presence or absence of light (black=0, white=100), redness (a*) which goes from -60 (green) to +60 (red), and yellowness (b*) which goes from -60 (blue) to +60 (yellow); this method is based on the Hunter scale (Janky et al., 1986; Sirri et al., 2010).

Boiler performance and meat yield appear not to be influenced by changes in skin pigmentation through dietary carotenoid supplementation (Pérez-Vendrell et al., 2001; Martínez-Peña et al., 2004; Castañeda et al., 2005; Muñoz-Díaz et al., 2012; Tunio et al., 2013). However, dietary lutein has the potential to increase BW of broilers through a higher antioxidant status (Rajput et al., 2012). Shanmugasundaram and Selvaraj (2011) found that 50 mg of lutein per kg of diet reduced TBARS in liver samples of 50-d-old turkey poults during an acute phase

inflammatory response following lipopolysaccharide injection compared with the control group (0 mg of lutein), and BW gain was not reduced post-injection in the birds fed lutein while it was reduced in birds not fed lutein.

1.3.4 Dietary combination of 25-OH-D_3 and CX in poultry

MaxiChick® (MC) is a novel feed additive (DSM Nutritional Products, (DSM Nutritional Products, Basel, Switzerland), that combines 6 mg CX and 69 µg 25-OH-D_3 per kg of complete feed when included at the recommended level. This combination has been tested mainly in broiler breeders. In comparison to hens with no MC supplementation, hatchability and fertility were increased by 2.9% and 2.2% respectively, embryo mortality was reduced from 5.46% to 3.46%, and a decreased TBARS was detected in egg yolks of fertile eggs from broiler breeder hens fed MC (Rosa et al., 2010b). Similarly, Cho et al. (2013a) found that early embryonic mortality decreased with hen age when broiler breeder hens were supplemented with MC or 6 mg CX per kg of feed, but was not affected in the negative control group nor the group supplemented only with 69 µg/kg 25-OH-D_3. Sperm concentration, motility and vigor were increased in males fed MC in comparison to males that received the control diet (Rosa et al., 2010a). Taking into account that avian sperm is susceptible to lipid peroxidation (Cerolini et al., 2006; Partyka et al., 2012), these effects can be related to an antioxidant effect of CX on avian sperm. Phagocytic capacity was increased in chicks from hens fed MC (Cho et al., 2013b). This effect may be associated with the ability of 25-OH-D_3 to increase phagocytic capacity (Chou et al., 2009), and of CX to increase antioxidant capacity in broiler chickens (Zhang et al., 2011). Therefore, maternal supplementation of MC has the potential to increase chick livability through a higher phagocytic capacity and a greater antioxidant status. Until now there are no reports about the effect of the dietary combination of CX and 25-OH-D_3 on performance, livability and meat yield of broiler chickens.

1.4 POULTRY HOUSING CONDITIONS

Housing conditions affect broiler performance, health and welfare. Around the world broilers are reared in a diversity of production systems which varies according to environmental conditions, bird type, processing age and investment cost (Tirawattanawanich et al., 2011). The purpose of a broiler house is to provide a suitable environment that allows birds to express their genetic potential for growing (Reece and Lott, 1982). Broiler houses have changed in recent decades in terms of design and equipment with the purpose to increase meat production per unit of area, alleviate heat stress, increase energy efficiency, improve brooding conditions, and reduce welfare issues (Fouad et al., 2008; Liang et al., 2013).

Efficient ventilation is crucial for an optimal thermoregulation and performance in poultry (Yahav et al., 2008). Heat stress increases mortality and decreases weight gain in broilers (Olanrewaju et al., 2010). Tunnel-ventilated poultry houses are replacing conventional houses due to advantages in terms of higher BW and reduced feed conversion in comparison to conventional cross-ventilated houses (Lacy and Czarick, 1992). Lott et al. (1998) found that broilers reared in a tunnel-ventilated house with an air velocity of 2.08 m/s gained significantly more weight from 4 to 6 wk of age than chickens reared in conventional ventilated house (0.25 m/s). Additionally they did not report panting in broilers in the tunnel-ventilated house, which was observed in the broilers in the conventional ventilated house. In broilers, panting can cause dehydration and BW loss because they use more metabolizable energy, and also water loss increases (Dozier et al., 2005; Yahav et al., 2008). Air velocity is an environmental parameter that affects performance in poultry. At 7 weeks, broilers placed at 35^{o}C and 60% RH increased BW and feed efficiency when air speed ranged between 1.5 and 2 m/s in comparison to birds placed in a house with an air speed of 0.5 m/s (Yahav et al., 2001). Dozier et al. (2005) found that an airspeed velocity of 3 m/s is

advantageous to broiler growth and FCR from 21 to 49 d of age when broilers are exposed to high temperatures (35ºC) in comparison to an airspeed of 0 and 2 m/s. Artificial ventilation systems with misting cool down the bird environment, reduce heat stress, and have the potential to increase carcass quality (Ryder et al., 2004). Ventilation increases the sensible heat loss in the bird (the transfer of heat from the bird by the passing of air over the bird), which enhances thermo-tolerance at high environmental temperatures (Feddes et al., 2003; Yahav et al., 2008). As a result, tunnel ventilation is becoming more common in broiler operations; however, in developing countries an obstacle is the high investment cost, and for that reason conventional houses are still in use (Tirawattanawanich et al., 2011). There are no published reports about a comparison between housing systems artificially ventilated, and naturally-ventilated open houses.

The concrete floor in poultry houses has been traditionally recommended because it allows for better disinfection, facilitates management and increases bird comfort (Abreu et al., 2011). However, soil floors in some countries have been used to reduce building cost. There are not many reports in the literature comparing floor types; notwithstanding, Abreu et al. (2011) compared productivity of broilers housed on concrete floors, and hard-packed dirt floors, and determined that broiler live performance was not influenced by floor type, but total mortality and sudden death were higher in broilers raised on a hard-packed dirt floor than on a concrete floor.

Environmental conditions differ between geographical regions due to differences in altitude, winds, humidity, seasonality, and vegetation. Therefore, poultry performance and livability can be affected depending where poultry operations are located. At high altitude, the low partial pressure of oxygen causes hypoxemia in broilers leading to ascites (Arce-Menocal et al., 2009). Moreover, low ambient temperature (below 21ºC the first three weeks of age) can also

increase the ascites incidence because of the necessity to enhance heat production leading to an increment of oxygen requirements of the chick (Özkan et al., 2010).

1.5 CONCLUSION

Today broilers have the capacity to increase their BW from 40 g up to 2 kg (50-fold) in less than 40 days (Tona et al., 2010; Sakomura et al., 2011), and deposit muscle faster than unselected birds (Schmidt et al., 2009). This situation has been implicated in skeletal disorders (Shim et al., 2012), impaired immune function (Cheema et al., 2003), and reproduction issues (De Beer and Coon, 2007). Some of these problems may be alleviated using feed additives such as vitamins D_3 (Chou et al., 2009; Coto et al., 2010; Nääs et al., 2012; Sun et al., 2013) and carotenoids (Zhang et al., 2011; Rosa et al., 2012).

Dietary inclusion of 25-OH-D_3 has the potential to increase broiler performance, feed efficiency (Brito et al., 2010), livability (Chou et al., 2009) and meat yield (Brito et al., 2010; Vignale et al., 2013). Moreover, CX reduces oxidative stress (Rosa et al., 2012) and tissue damage (Okotie-Eboh et al., 1997) in poultry, which may increase performance and livability. Currently, the combination of 25-OH-D_3 and CX is commercially available for use in poultry diets under the trade name MaxiChick® (DSM Nutritional Products, (DSM Nutritional Products, Basel, Switzerland). Therefore, the aim of the current study was to investigate the effect of the dietary inclusion of the combination of 25-OH-D_3 and CX on broiler performance (Chapter 2), meat yield, skin colour, antioxidant status, and bone characteristics (Chapter 3 and Chapter 5).

Furthermore, environmental conditions and management practices have an important role in broiler production. For instance, stocking density (Beloor et al., 2010), environmental temperature (Lin et al., 2000), altitude (Özkan et al., 2010), floor type (Abreu et al., 2011) and air speed (Dozier et al., 2005) can reduce or increase broiler performance. Data from field trials can be difficult to

analyze because many variables can influence the results. However, field trials have been a way to evaluate some feed additives under conditions of commercial broiler production (Timmerman et al., 2006); therefore, a secondary objective of this investigation was to analyze the influence of floor type, floor level, geographical region and environmental control type on broiler performance, meat yield, internal organ yield, and bone strength (Chapter 4).

This research was assessed in two parts. The first part of this investigation was conducted in a field trial involving 372 poultry houses located in two different geographical regions in Colombia (Chapter 2, Chapter 3, and Chapter 4). The second part of this study was set in a broiler trial under controlled environmental conditions at the Poultry Research Centre of the University of Alberta (Chapter 5).

The objectives were achieved in this thesis by testing the following hypotheses:

1. It was hypothesized that the dietary combination of 25-OH-D_3 and CX would increase performance and livability of broilers under field conditions in Colombia.

This hypothesis was addressed in Chapter 2, by evaluating the performance of broilers including body weight, feed conversion ratio, feed consumption and livability on a weekly basis and at processing age.

2. It was hypothesized that the dietary combination of 25-OH-D_3 and CX would increase meat yield, bone strength, and skin colour of broilers under commercial conditions in Colombia.

This hypothesis was addressed in Chapter 3, in which the objectives of the study were to examine the effects of dietary combination of 25-OH-D_3 and CX on broiler processing characteristics by measuring carcass part weights and yields, skin colour, and bone strength of broiler chickens at processing.

3. It was hypothesized that placing broiler chickens on concrete floor, or at ground level, or at lower altitude, or under controlled environmental conditions would increase broiler performance, meat yield, and bone strength of broilers in Colombia.

This hypothesis was addressed in Chapter 4, where the objective of the study was to analyze the effect of floor type, floor level, geographical region and environmental control type on performance of broiler chickens by evaluating body weight, feed conversion ratio, feed consumption, livability, meat yield, internal organs relative weights, skin colour and bone strength.

4. It was hypothesized that dietary combination of 25-OH-D_3 and CX would increase broiler performance, meat yield, antioxidant status, skin colour and bone strength of broilers under experimental conditions in Canada, and that there is a synergy effect between them.

This hypothesis was addressed in Chapter 5, where the objective of the research was to evaluate the effect of dietary combination of 25-OH-D_3 and CX on performance of broilers and processing characteristics, including BW, weight gain, feed intake, FCR, skin colour, and carcass parts weights and yield. Additionally, the effect of dietary combination of 25-OH-D_3 and CX on bone characteristics by measuring bone strength, bone mineral density and bone cross sectional areas (using quantitative computed tomography); and antioxidant status by measuring TBARS in liver and breast of broilers were evaluated.

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1.7 FIGURES

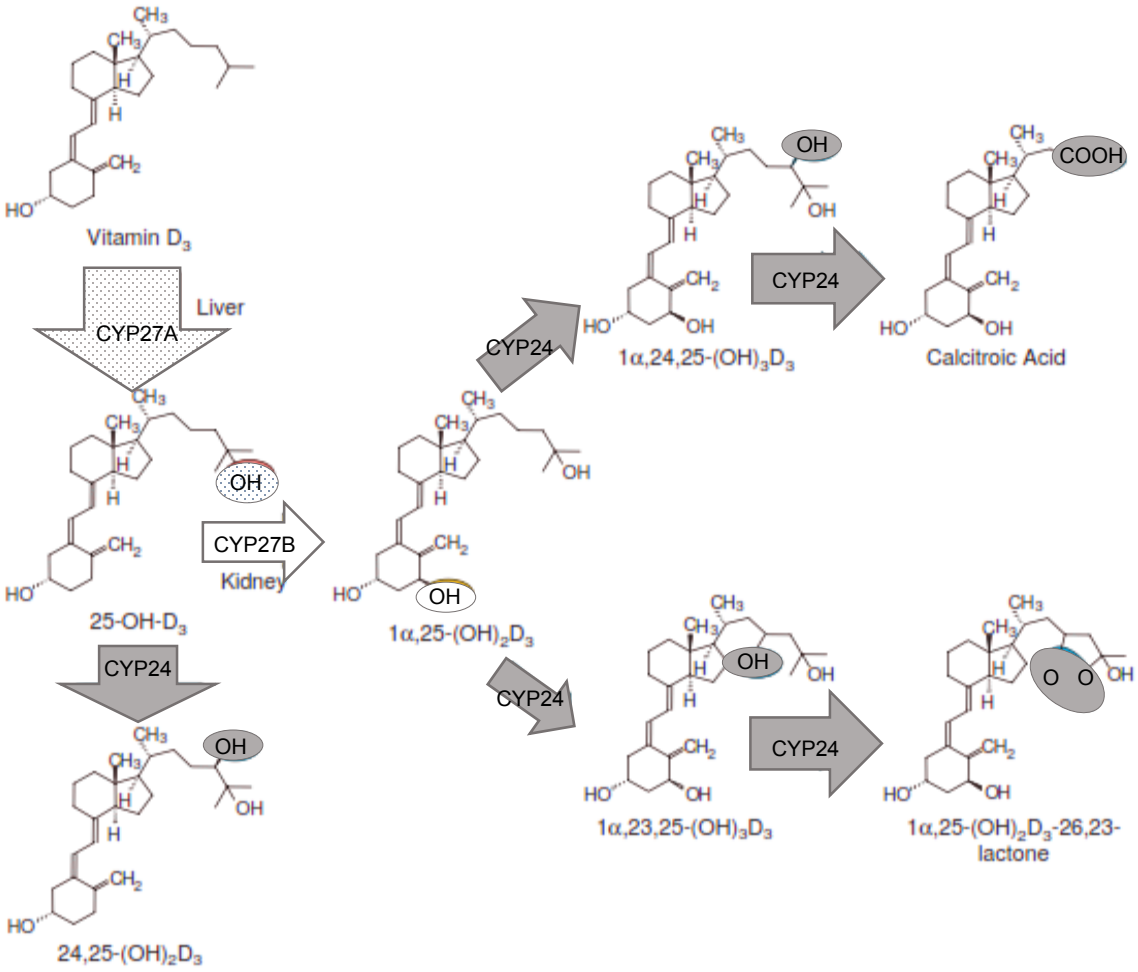

Figure 1.1. Activation and inactivation of vitamin D_3.
Conversion from vitamin D to lα,25-$(OH)_2$-D_3 involving the 25-hydroxylase (dotted arrow) and the 1α-hydroxylase (white arrow). The carbon-24 oxidation pathway from 1α,25-$(OH)_2D_3$ to calcitroic acid and the lactone pathway from 1α,25-$(OH)_2D_3$ to the 1α,25-$(OH)_2$D-26,23-lactone, involving the 24-hydroxylase (grey arrow). Modified from: Prosser and Jones, 2004.

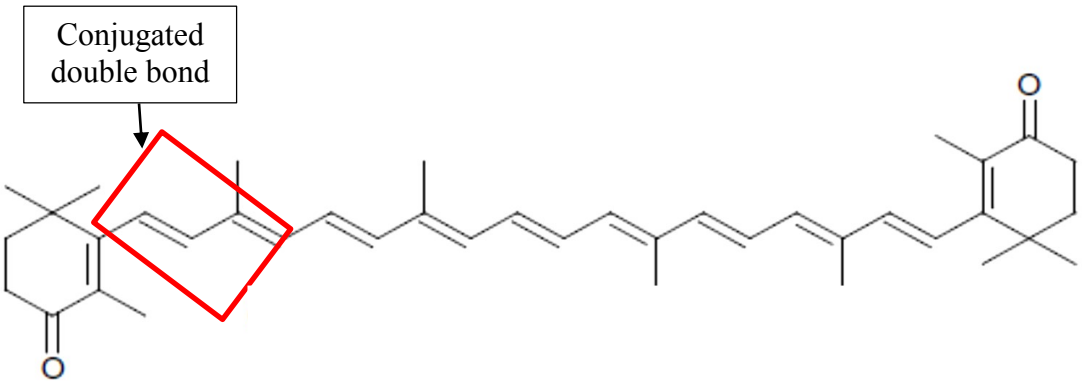

Figure 1.2. Canthaxanthin structure.
Conjugated double bonds in a molecule, mean that the single and double bonds alternate.
From: http://en.wikipedia.org/wiki/Canthaxanthin#mediaviewer/File:Canthaxanthin.svg

2. EFFECT OF DIETARY COMBINATION OF 25-OH-D_3 AND CANTHAXANTHIN ON PERFORMANCE OF BROILERS IN COMMERCIAL CONDITIONS OF PRODUCTION IN COLOMBIA

ABSTRACT

The effects of 25-hydroxy vitamin D_3 (25-OH-D_3) and canthaxanthin (CX) on broiler performance were evaluated in a field trial in Colombia. One whole broiler cycle was followed, involving 4,922,130 broilers of both sexes (reared separately) of two commercial broiler strains (A and B) from placement to processing in each of 372 houses. The Control diet contained a basal level of vitamin D_3 of 4,000 IU per kg of complete feed. The treatment diet (MC) contained CX at 6 mg, 2,760 IU of 25-OH-D_3, and the basal level of vitamin D_3 per kg of complete feed from 0 to approximately 21 d. From approximately d 22 to processing age, 53% of the males also received 1.0 g of marigold extract (MG) per kg of feed with a total xanthophyll content of 12 to 15 mg/kg of feed. Breeder source, geographical region, farm, and housing type were included as random variables in the data analysis. Processing age and heat stress index were included as covariates. BW, feed intake, FCR and mortality were assessed weekly to d 35 and at processing at approximately 43 d. Strain A required 35 g less feed/ kg of gain than strain B, but BW and feed intake were similar. Feed intake and FCR were higher in males fed MG relative to males not fed MG. From 29 to d 35, MG increased weight gain and reduced FCR in strain A relative to strain B. During the same period, MC reduced weight gain and increased FCR in males not fed MG, but MG increased weight gain and increased mortality in males fed MC. From 29 to d 35, dietary MC reduced mortality relative to birds not fed MC, but had no effect in males fed MG. The high vitamin D_3 activity (6,760 IU/kg of feed) did not result in signs of toxicity, but also did not increase broiler

performance. The increased weight gain caused by dietary MG might be related to a higher antioxidant status; however, feed efficiency and livability were reduced.

Key words: Colombia, broiler strain, 25-hydroxy vitamin D_3, canthaxanthin, performance.

2.1 INTRODUCTION

Vitamin D_3 is absorbed in the upper intestinal tract and in the liver is converted into 25-OH-D_3 by the action of 25-hydroxylase; after a second hydroxylation in the kidney, this metabolite is transformed by 1α-hydroxylase into 1,25-$(OH)_2$-D_3 (calcitriol) that is the active form of vitamin D_3 (Bar et al., 1980; Soares et al., 1995). Dietary 25-OH-D_3 inclusion is advantageous for poultry, since its intestinal absorption was more efficient (Nemere et al., 1994; Phadnis and Nemere, 2003), and less dependent on bile salt secretion than vitamin D_3 (Compston et al., 1981). Moreover, 25-OH-D_3 had a higher retention rate (Bar et al., 1980), lower daily excretion rate (Chou et al., 2009), and higher biological activity per molecule than vitamin D_3 (Soares et al., 1978). Dietary inclusion of 25-OH-D_3 as a partial replacement or as a sole source of dietary vitamin D_3 activity increased broiler performance (Yarger et al., 1995a; Fritts and Waldroup, 2003), and this effect was more pronounced when 25-OH-D_3 was supplemented the first weeks of life (Świątkiewicz et al., 2006; Saunders-Blades, 2008) or in combination with vitamin D_3 (Papešová et al., 2008; Brito et al., 2010; Michalczuk et al., 2010). Immune response was positively influenced by dietary 25-OH-D_3 because it increased humoral immune response and phagocytosis activity in broilers (Chou et al., 2009).

The carotenoid canthaxanthin (CX) is a potent free radical scavenger (Zhang et al., 2011), which reduces lipid peroxidation and increases the serum total antioxidant capacity in poultry (Surai et al., 2003; Rosa et al., 2012; Rocha et al., 2013). Dietary CX increased hatchability and chick quality, and reduced embryo mortality through a greater antioxidant status (Robert et al.,

2007; Surai et al., 2003; Rosa et al., 2012). Moreover, CX reduced tissue damage after experimental aflatoxicosis (Okotie-Eboh et al., 1997), and increased antioxidant capacity of newly-hatched chicks (Zhang et al., 2011). There is no previous evidence that CX supplementation increases broiler performance, however lutein (an oxycarotenoid) supplementation in broilers increased BW at processing (Rajput et al., 2012).

MaxiChick® (MC) is new feed additive which provides 6 mg CX and 69 µg 25-OH-D_3 per kg of complete feed (DSM Nutritional Products, Basel, Switzerland). This additive has been used mainly in broiler breeders (Rosa et al., 2010a; 2010b). Embryo mortality of hatching eggs from breeders supplemented with MC was reduced by 36.6% relative to the Control (Rosa et al., 2010b). An increased sperm quality in males fed MC was seen relative to males with no MC (Rosa et al., 2010a). There are no reports about the effect of MC on broiler performance. Therefore, it is logical to suggest that an additive effect on broiler performance and livability might result with the combination of these two nutrients since they have different metabolic pathways, and effects on poultry production. The objectives of the current study were to investigate the effects of dietary MC during the first three weeks of age on broiler performance and livability under commercial conditions in Colombia. It was hypothesized that dietary MC would enhance broiler production traits and livability as compared to a control diet with no MC.

2.2 MATERIALS AND METHODS

2.2.1 Experimental design

One whole broiler cycle in a commercial broiler integration in Colombia (Avidesa, MacPollo de Occidente) was evaluated including 4,922,130 broilers of both sexes (reared separately) that were placed in 58 farms (n = 372 houses). The experiment was set up as a 2 x 2 x 2 x 2 incomplete factorial arrangement of treatments. The treatments corresponded to two commercial broiler

strains (A, B), two sexes, two dietary MC levels (0 or 250 g/tonne), and two dietary marigold extract levels (MG; 0 or 1.0 kg /tonne). The Control diet contained a basal level of vitamin D_3 of 4,000 IU per kg of complete feed. Dietary MC provided 69 μg of 25-OH-D_3 (equivalent to 2,760 IU of vitamin D_3 activity), 6 mg of CX, and the basal level of vitamin D_3 per kg of complete feed in the pre-starter and starter diets from 0 to approximately 21 d. Dietary MG, with a total xanthophyll content ranging from 12 to 15 mg/kg of feed, was fed in 102 male houses (53% of male houses) in the grower and finisher diets following Colombian regional market needs for yellow-skinned chickens. The treatments were randomly assigned to each farm and each house (Figure 2.1 and Figure 2.2), with some logistic constraints imposed by the broiler integration in regards to number of birds to be housed, pigmented, and processed. Birds of strain A were housed in 153 houses (41.1% of the total), and strain B was present in 219 houses (58.9% of the total).

2.2.2 Animals and Housing

2.2.2.1 Breeder flock

The chicks were obtained from 13 different broiler breeder farms belonging to this company. The broiler breeder flock age range was from 27 to 65 weeks. Every broiler flock was formed with chicks from a maximum of four different broiler breeder flocks of similar ages. Most of the eggs (98%) were incubated in the Avidesa's hatchery, the rest of eggs were incubated in a commercial hatchery.

2.2.2.2 Regional effects.

Farms were located in two different geographic regions in Colombia that cover a wide area with different climatic conditions according to its altitude, topography, and season during the year (dry or wet). The Coffee region (CR), which is situated in the departments of Quindío, Caldas, Risaralda and the north-western part of Valle del Cauca, contained 191 houses (51.3%). The Valle

region (VR) is situated mainly in the department of Valle del Cauca, included 181 houses (48.7%). The VR farms were located between 900 to 1,000 meters above sea level whereas the CR farms were between 1,200 to 1,500 meters above sea level. The annual rainfall in VR is considered lower (1,000 to 1,500 mm) in comparison to CR (2,000 mm: Bernal et al., 1989).

2.2.2.3 Housing type.

Chick placements occurred from February 6 to April 6, 2012. The range of flock size placed in a house was 1,500 to 41,084 with an average of 13,267 broiler chickens, depending on poultry house size and logistics of the company. The number of houses per farm ranged from 3 to 16; the majority of houses were 40 m wide and 100 m long. Stocking density in the trial ranged from 9.2 to 19.8 bird/m^2 for females (average 13.2 bird/m^2), and from 7.9 to 16.6 bird/m^2 for males (average 11.6 bird/m^2). To avoid heat stress, this broiler integration determines stocking based on sex of the bird, geographic region, characteristics of each house, and processing age. Most of the houses were similar in structure; but there were some variations among them in house type or environmental controls, floor type, house floor level and equipment. All birds were placed on rice hull litter. The floor of 177 (47.6%) of the houses was concrete, and the rest of the houses (n = 195; 52.4%) had dirt floor. A total of 274 houses (74% of the total) were single-storey; 53 houses were two-storey. In the two-storey houses, both levels had concrete floors. Birds were placed in the first level of all 53 two-storey houses and in the second level of 45 houses, for a total of 98 houses in the two-storey houses (26% of the grand total).

2.2.2.3.1 Environmental control systems or house types.

Most of the broiler houses (n = 290) were open-sided with manual curtains and two rows of fans (1 hp fixed 1.20 m from floor) along the building situated every 4 to 5 meters, which created

a positive air pressure. The others houses were open-sided with manually operated curtains (n = 43), tunnel-ventilated with cooling pads (n = 32), and tunnel-ventilated with foggers (n = 7).

2.2.2.3.2 Heat stress index.

Due to geographic location, housing and external environment, the temperature varied among the houses. Environmental temperature was registered (maximum and minimum) on a daily basis during the whole trial in each house with mercury thermometers that were installed in the middle of each house at bird level. Then, from d 21 to the end of the cycle, a heat stress index (HSI) was calculated for each house according to this formula (Ryder at al., 2004):

$$\text{HSI} = \frac{\sum_{t=21}^{t=42}(T^{\cdot} - T')}{\text{PA} - 21} \text{ (units: } ^{o}\text{C x d)}$$

where HSI was heat stress index, t was time (d), T˙ was the maximum temperature (ºC) recorded in the period t to t+1, T' was the recommended temperature (ºC; Aviagen 2009; Cobb-Vantress, 2012) for the period t to t+1, and PA was processing age (d).

2.2.3 Management

All birds were reared and cared for according to standard protocols established by Avidesa MacPollo in its Quality Manual Handbook that includes daily observations, temperature monitoring, and feeder and water disappearance. Birds were provided free access to water and feed, and had illumination of 23L:1D during the first two days and then the light hours were gradually reduced up to 12L:12D of natural daylight at d 14. From d 35 to processing, the light hours were increased to 16L:8D; incandescent lamps were used to supplement natural daylight. Each house was initially provided with a propane-fired brooders until 14 d of age, plastic feeder trays, and supplemental drinkers. For the first 10 d after placement, one-half of each house was

used for brooding, after which the birds were given access to the entire house. Ambient house temperatures were maintained at approximately 29°C during the first two weeks, following Avidesa's protocols for brooding.

Manual tube-type or automatic pan-type feeders replaced feeder trays from the end of the first wk to the end of the production cycle. Water was provided in each house with either bell drinkers or nipple drinkers.

All chicks were vaccinated at the hatchery against Marek's disease, infectious bursal disease (IBD), Newcastle disease, and infectious bronchitis with commercial vaccines according to standard Avidesa practice. An IBD booster vaccination was administered through the drinking water on day 10 or 11. Processing age varied among houses because this company establishes a different BW objective for males and females according to sales demand and logistics. The average age at processing was 40.8 days for females with a range of 37 to 47 days, and 45.0 days for males with a range of 40 to 48 days.

2.2.3.1 Feeding management.

Feeding phases consisted of a crumble pre-starter diet (0 to 10 d), crumble starter diet (11 to 21 d), crumble grower diet (22 to 35 d), and pelleted finisher diet (35 to processing age). All diets were corn-soy based and formulated to meet the nutrient specifications of a typical commercial broiler diet (NRC, 1994; Rostagno et al., 2011). The feed was manufactured at Avidesa's feed mill (Buga, Valle, Colombia). The feed rations were packed in white bags with a strip of specific colour according to treatment to ensure that the farm staff was blinded to the treatments. The strips on each feed bag followed a colour code established for the trial, and each bag of feed was delivered as needed to each house. When feed was shipped to the farm by a truck and unloaded inside a bin,

each truck's compartment was identified with a tag following the same colour code. The amount of feed offered was recorded per house daily.

2.2.4 Data Recorded

2.2.4.1 Performance measures.

The variables measured for each house included BW, feed intake, and mortality. Feed consumption and mortality were recorded daily and summarized on a weekly basis. Cumulative mortality was expressed on a house basis and was calculated by dividing the number of dead birds by the number of chicks placed, and for weekly percentage by dividing the number of dead birds during the wk by the number of birds remaining alive at the end of the previous wk. All birds that were considered to have been unusual mortalities were recorded and necropsied in order to determine the probable cause of death.

At each of 0, 7, 14, 21, 28 and 35 d, approximately 1% of all birds in each house were weighed and BW recorded by house to determine weight gain and FCR. From d 21 the chicks were not fed at least 6 to 8 h prior to measuring BW. The birds were weighed individually with digital scales (Salter-Becknell ElectroSamson® Fairmont, MN; capacity 10 kg x 0.01 kg) according to Avidesa's standardized procedures (catching birds in different areas following a zig-zag line across the house). FCR was calculated for each house weekly as total feed consumed divided by weight gain.

Birds were shipped to the processing plant by trucks with a load capacity of 2,000 to 2,200 birds each. Total live weight per house was determined at the processing plant after truck arrival by dividing total live weight by the number of birds delivered to the slaughtering plant. Final average BW per house was determined at processing age by dividing total live weight divided by

the number of birds remaining alive at processing. Final FCR of each house was calculated based on total feed consumption divided by the final average BW.

2.2.5 Statistical Analyses.

Broiler performance data were analyzed as 4 - way ANCOVA with sex, strain, dietary MC and dietary MG as main effects using the procedure for linear mixed models (PROC MIXED) of SAS 9.3© for Windows (SAS Institute Inc., Cary, NC). For all statistical analyses, the house was the experimental unit; therefore ANCOVA was conducted using house means of performance parameters of each main effect and interactions among them. The number of houses per treatment corresponded to number of replicates. Variability due to broiler breeder source, geographical region, farms, and houses (floor type, floor level and environmental control) was considered as random terms. Since some variables can influence the dependent variables, the least square means were adjusted to a common covariate to increase precision and reduce error (residual). For the weekly analysis, HSI was included in the model as a covariate. For analysis at processing, HSI and processing age (PA) were included as covariates.

Differences between main effects, means, and their interactions were classified by pairwise comparisons, and unless otherwise noted, differences were considered significant at $P < 0.05$. The analysis for the weekly data was in accordance with the following model:

$$Y_{ijkl} = \mu + S_i + ST_j + MC_k + MG_l + (S\text{-}MC)_{ik} + (ST\text{-}MC)_{jk} + (MC\text{-}MG)_{kl} + (S\text{-}ST)_{ij} + (S\text{-}ST\text{-}MC)_{ijk} + (ST\text{-}MC\text{-}MG)_{ikl} + \beta\,(HSI_{ijkl} - HSIa) + E_{ijk1},$$

where μ was the population mean; S_i was the effect of sex ($i = 1$ to 2); ST_j was the effect of strain ($j = 1$ to 2); MC_k was the effect of dietary MC level ($k = 1$ to 2); MG_l was the effect of dietary MG extract level ($l = 1$ to 2); $(S\text{-}MC)_{ik}$, $(ST\text{-}MC)_{ik}$, $(MC\text{-}MG)_{kl}$, $(S\text{-}ST)_{ij}$, $(S\text{-}ST\text{-}MC)_{ijk}$, and $(ST\text{-}MC\text{-}MG)_{ikl}$ were the interactions of the main effects; $\beta\,(HSI_{ijkl} - HSIa)$ was a covariate coefficient

multiplied by the difference between individual HSI (HSI_{ijkl}) and average HSI (HSIa); and E_{ijk1} was the residual error.

The analysis for the processing data was in accordance with the following model:

$Y_{ijkl} = \mu + S_i + ST_j + MC_k + MG_l + (S\text{-}MC)_{ik} + (ST\text{-}MC)_{jk} + (MC\text{-}MG)_{kl} + (S\text{-}ST)_{ij} + (S\text{-}ST\text{-}MC)_{ijk} + (ST\text{-}MC\text{-}MG)_{ikl} + \beta (HSI_{ijkl} - HSIa) + \beta (PA_{ijkl} - PAa) + E_{ijk1}$,

where μ was the population mean; S_i was the effect of sex (i = 1 to 2); ST_j was the effect of strain (j = 1 to 2); MC_k was the effect of dietary MC level (k = 1 to 2); MG_l was the effect of dietary MG extract level (l = 1 to 2); $(S\text{-}MC)_{ik}$, $(ST\text{-}MC)_{ik}$, $(MC\text{-}MG)_{kl}$, $(S\text{-}ST)_{ij}$, $(S\text{-}ST\text{-}MC)_{ijk}$, and $(ST\text{-}MC\text{-}MG)_{ikl}$ were the interactions of the main effects; $\beta (HSI_{ijkl} - HSIa)$ was a covariate coefficient multiplied by the difference between individual HSI (HSI_{ijkl}) and average HSI (HSIa); $\beta (PA_{ijkl} - PAa)$ was a covariate coefficient multiplied by the difference between individual PA (PA_{ijkl}) and average PA (HSIa);and E_{ijk1} was the residual error.

2.3 RESULTS AND DISCUSSION

2.3.1 Production performance

As expected, males were significantly heavier than females during the trial, and the differences became larger with age (Table 2.1), due to the greater weight gain of males during the production cycle (Table 2.2). Females ate more feed than males from 7 to 14 and from 15 to 21 days of age, but from 22 to 35 d males had a higher feed intake than females (Table 2.3). Although they ate more, males had a lower FCR than females in each period and to processing due to the greater weight gain (Table 2.4). These differences in performance between sexes are in agreement with previous studies (López et al., 2011; Shim et al., 2012), and are related to sexual dimorphism that exists in broiler chickens (Young et al., 2001; Fanatico et al., 2005).

Strain B was heavier than strain A at d 7 (P = 0.0321), and at d 14 (P = 0.0192), but these differences disappeared after that (Table 2.1). Weight gain from 0 to 7 d was greater in strain B than strain A, but from d 14 to d 21 strain A had a higher weight gain than strain B; no differences were found thereafter (Table 2.2). From 0 to 7 d, strain B had higher feed intake than strain A; differences were not significant after that point (Table 2.3). From d 15 to d 21, FCR was significantly lower in strain A than strain B (Table 2.4). At processing, there were no significant differences in BW nor cumulative feed intake between strains, however strain A needed 31 g of feed less per kg of gain than strain B (Table 2.5) which would have a relevant impact on large broiler integrations since 70% of the production cost comes from feed (Moosavi et al., 2011). Other authors did not detect a significant difference between commercial broiler strains in final BW at similar processing ages to this trial (Goliomytis et al., 2003; López et al., 2011). In contrast, other reports have shown differences in BW (Brewer et al., 2012; Shim et al., 2012), probably because those comparisons were between broiler strains with stronger differences in growth rate. Furthermore, other investigators have also detected differences in FCR between commercial strains at similar processing ages to this field trial (Abdullah et al., 2010; Brewer et al., 2012; Shim et al., 2012).

Dietary MC supplementation provided 2,760 IU of 25-OH-D_3 per kg of feed in addition to the regular level of inclusion of vitamin D_3 (4,000 IU/kg) used by Avidesa. Accordingly, the experimental group received a higher level of vitamin D_3 activity (6,760 IU/kg) than the control group (4,000 IU/kg). Despite this there were no differences in production performance between the two dietary MC treatments at any age in this trial (Table 2.1; Table 2.2; Table 2.3; Table 2.4) nor at processing (Table 2.5). The high level of vitamin D_3 activity present in the two dietary MC treatments in the current research can be the reason for the lack of differences in performance, as

both diets contained vitamin D_3 activity well in excess of the requirement for broilers (NRC, 1994). In contrast, Świątkiewicz et al. (2006) found an increment in BW and a reduction in FCR (by 11.5% and 8.7% respectively) when 50% of the vitamin D_3 inclusion (3,500 IU/kg) was replaced with 25-OH-D_3 in the feed from 1 to 21 d of age. Moreover, chicks fed the combination of 2,500 IU/kg vitamin D_3 and 2,000 IU/kg 25-OH-D_3 had higher BW at d 21 and d 37 relative to birds fed 5,000 IU/kg of vitamin D_3 (Papešová et al., 2008). Our results did not suggest a linear effect of increasing levels of vitamin D_3 activity, contrary to Brito et al. (2010) who showed increases in broiler weight gain and feed efficiency when vitamin D_3 was increased from 800 IU to 5,550 IU/kg of feed. However, in the present study the level of total of vitamin D_3 activity of the MC treatment was higher than was in those studies. A greater biopotency of 25-OH-D_3 from 125 to 500 IU/kg of feed was found in comparison to vitamin D_3, but a linear effect was not observed with increasing levels of 25-OH-D_3 from 125 to 4,000 IU/kg of feed (Fritts and Waldroup, 2003). Despite the high level of vitamin D_3 activity included in MC dietary treatment, no signs of toxicity were seen, nor increased mortality, which is in agreement with the high level of safety of 25-OH-D_3 (Yarger et al., 1995b; Fritts and Waldroup, 2003).

The absence of differences between the dietary MC treatments and the Control are in agreement with previous reports. Pérez-Vendrell et al. (2001) did not find differences in broiler performance when 2 or 5 mg of CX per kg of feed were fed in comparison to the control group fed no CX, and Tunio et al., 2013 did not see any effect on broiler performance even when CX was supplemented a 25, 50 or 100 mg/kg.

Dietary MG treatments were applied only in 53% of male houses. Supplementation of MG did not influence BW or weight gain at d 28, d 35 (Table 2.1; Table 2.2) or at processing (Table 2.5). Notwithstanding, males fed MG had a significantly higher daily feed intake during the last

week of observation (from d 28 to d 35) than males with no MG supplementation (Table 2.3). Despite this, no difference in FCR was detected (Table 2.4). In contrast, supplementation of natural pigments including MG did not affect feed intake of broilers (Perez-Vendrell et al., 2001; Castañeda et al., 2005; Koreleski and Świątkiewicz, 2007; Rajput et al., 2012). In the current study, birds fed MG had significantly higher cumulative feed intake to processing age than birds not fed MG; however, MG had no effect on BW (Table 2.5). Consequently, males fed MG required 35 g more feed/kg of gain at processing age than males without MG supplementation (Table 2.5). That increase in FCR may have a large impact in economics of the poultry industry (Moosavi et al., 2011). Several factors can affect feed intake. Broilers housed at high environmental temperatures (26ºC) had reduced feed intake relative to birds at 21ºC (Olanrewaju et al., 2010). Diseases can also reduce feed intake (Ferket and Gernat, 2006). In the present study, most of the factors that may have affected feed intake were similar along all houses or were taken into account in the model as random variables or covariates; therefore, the reasons of the difference in feed intake between males supplemented and not with MG, are difficult to determine. It would be interesting to find out if that effect was related to physical changes in colour of feed presentation that might attract birds to eat more, or because physiologically MG extract increases appetite.

Dietary MG did not modify broiler productive parameters when added at same or higher level of inclusion of the present trial (Perez-Vendrell et al., 2001; Martínez-Peña et al., 2004; Castañeda et al., 2005; Muñoz-Díaz et al., 2012). Notwithstanding, broilers fed MG at 200 mg/kg of feed from d 4 to d 42 had a higher BW at processing (42 d) than birds with no MG supplementation; it was proposed that this effect may be due to a higher antioxidant efficiency of MG which contains the carotenoid lutein (Rajput et al., 2012). After lipopolysaccharide injection, dietary lutein at 50 mg/kg from d 1 reduced Interleukin-1ß mRNA levels and decreased TBARS

levels by 50% in liver samples of 50-d-old turkey poults in comparison to the group without lutein supplementation; in addition, BW was not reduced after injection in birds that received lutein, which was related to the higher antioxidant status (Shanmugasundaram and Selvaraj, 2011).

From d 29 to d 35, weight gain was similar between males of the two broiler strains when MG was not included in the diet; however, when MG was supplemented strain A males had a higher weight gain than strain B males (Figure 2.3). In the same period, FCR was similar between males of the two strains when MG was not supplemented in the diet; however, when MG was included, the strain B had a higher FCR than strain A (Figure 2.4), which was related to the lower weight gain of strain B when fed MG. There are no previous reports of this interaction, however this situation shows that broiler strains respond different to dietary MG in regards to feed efficiency and weight gain, which could be the reason that previous studies have not seen similar results. Dietary MG negatively influenced weight gain and FCR of strain B, but not strain A. As there are different growth rates between strains (Goliomytis et al., 2003; Abdullah et al., 2010), there may also be differences in metabolic responses to various feed additives such as MG.

From 29 to 35 d of age, dietary MC reduced weight gain in males with no dietary MG supplementation, but had no effect in males fed MG. Weight gain in males that received MC was increased when fed MG, but had no effect in males without MC (Figure 2.3). From 29 to 35 d, dietary MC from placement to 21 d of age increased FCR in males not fed MG, but had no effect in males fed MG (Figure 2.4). This effect is related to the reduction in weight gain that MC caused in males with no MG supplementation. In males, the early high level of vitamin D_3 activity (6,760 IU/kg) reduced weight gain of males during the fifth week of life; however, that effect disappeared when males were fed MG. The effect of dietary MG on weight gain and feed efficiency of strain A birds and weight gain of males fed MC could be related to a higher antioxidant status

(Shanmugasundaram and Selvaraj, 2011); moreover, this finding can indicate an additive effect between the carotenoids presents in MG and MC. As MC is a novel additive mainly studied in broiler breeders, there are no previous reports about these interactions.

Dietary MC reduced cumulative feed intake in strain A males, but had no effect on strain A females, nor strain B birds. Females of strain A and B had a similar feed intake when fed MC, but strain A females had lower feed intake than strain B females when not fed MC (Figure 2.5). Feed intake of strain A broilers was more responsive to dietary MC than strain B, but that effect was different according to bird sex. Therefore, carotenoid supplementation (CX and MG) affected broiler performance, according to genetics and gender, but there were no previous published data available about this kind of interaction.

2.3.2 Effects on Livability

From the second wk, mortality was consistently lower in females than males (Table 2.6). Higher growth rates have been correlated to a higher mortality in broilers (Gonzales et al., 1998; Havenstein et al., 2003; Shim et al., 2012), likely because males have higher growth rates than females (Fanatico et al., 2005; Zuidhof, 2005; Murawska et al., 2011), and are more prone to metabolic diseases than females (Julian, 2005).

During the whole trial there were no differences in livability between strains (Table 2.6). This may be related to the absence of differences in weight gain or final BW between broiler strains and consistent application of established procedures by Avidesa of disease control.

From d 29 to d 35, dietary MC reduced mortality relative to birds not fed MC (Table 2.6). At the same period, MG increased mortality in males fed MC, but had no effect in males without MC supplementation; moreover, dietary MC reduced mortality in males without dietary MG, but had no effect in males fed MG (Table 2.6). Previous studies have shown that the two active

compounds of MC may influence livability. At d 21 post-hatching, increased livability and higher antioxidant capacity was seen in chicks from eggs of breeders supplemented with 6 mg of CX per kg of feed relative to eggs from breeders without CX (Zhang et al., 2011). Moreover, dietary 25-OH-D_3 at 2,760 IU/kg from 0 to 21 d, and 1,380 IU/kg from 22 to 39 d increased phagocytosis activity by 28.5% in comparison to birds fed vitamin D_3 at 3,000 IU/kg of feed (Chou et al., 2009). These authors also reported that chicks fed 25-OH-D_3 and exposed to *Salmonella typhimurium* E29 at 7 and 14 d had higher antibody titers at d 21 than those fed only with vitamin D_3 and given the same bacterial challenge. These findings indicate that 25-OH-D_3 can increase immune response in broilers. More recently, Cho et al. (2013) found an increased phagocytic capacity in chicks from hens fed MC relative to hens without supplementation of MC. A higher antioxidant status and a greater immune response could positively influence livability, and might be the reasons for the effect of MC on livability in this study. However, dietary MG reduced livability of males fed MC. In broilers, a greater weight gain has been linked to a higher mortality (Gonzales et al., 1998; Havenstein et al., 2003; Shim et al., 2012); therefore, the greater growth rate seen in males supplemented with MC and MG could have negatively influenced livability.

2.3.3 Conclusion

Overall, dietary inclusion of the combination of 25-OH-D_3 and CX did not increase weight gain nor feed efficiency; in contrast, it reduced weight gain of males from d 29 to d 35. High vitamin D_3 activity during the first three weeks of age (6,760 IU/kg) may negatively affects weight gain in males. Dietary MG supplementation in the grower and finisher diets increased feed intake from d 29 to d 35 and reduced feed efficiency to processing age; however, MG increased BW in strain A males and in males supplemented with MC. This effect in weight gain might be related to a higher antioxidant status. Dietary MC increased livability from d 29 to d 35. Both active

compounds in MC may influence livability through different mechanisms (Chou et al., 2009; Zhang et al., 2011). Due to the logistics of Avidesa, it was not possible to include each of the two compounds separately in order to see the effect independently and determine the synergy between them. This situation was taken into account in Chapter 5 where the MC components were compared separately as well as together. However, mortality was increased when males fed MC from d 0 to approximately d 21 and then MG from approximately d 22 to processing, which might be related to the associated higher growth rate. According to our results, MC reduced weight gain in males from d 29 to d 35, but this effect disappeared when males had a subsequent MG supplementation; however, the combination of the two additives reduced livability during that period.

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2.5 TABLES

Table 2.1 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on BW adjusted to a common heat stress index (HSI)[3] with analysis of covariance.

Effect			0 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	29 to 35 d
		n[4]	g/b/d				
S	Female	173	172.1[b]	412.8[b]	814.6[b]	1,307[b]	1,817[b]
	Male	91	173.6[a]	422.2[a]	845.2[a]	1,404[a]	2,014[a]
	Pooled SEM		4.3	3.5	10.5	12.2	24.8
ST	A	104	169.5[b]	411.0[b]	827.1	1,355	1,921
	B	160	176.2[a]	424.0[a]	832.8	1,356	1,911
	Pooled SEM		4.3	3.5	10.5	12.4	25.2
MC	0	134	171.8	417.7	831.9	1,358	1,926
	250	130	173.9	417.3	827.9	1,353	1,907
	Pooled SEM		4.3	3.5	10.5	13.5	26.9
MG	0	91	-	-	-	1,420	2,037
	1.0	102	-	-	-	1,422	2,044
	Pooled SEM		-	-	-	24.2	27.7
Covariate	HSI [g/b/(°C x d)]		-0.004	0.003	0.001	0.001	0.001
Sources of variation			P-values				
	HSI		0.6694	0.8591	0.7249	0.0975	0.6092
	S		0.0306	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.0321	0.0192	0.3863	0.9661	0.3393
	MC		0.2681	0.8921	0.5283	0.6140	0.2427
	MG		-	-	-	0.7700	0.4050
	S x ST		0.4588	0.4967	0.3741	0.3598	0.5749
	S x MC		0.6546	0.3785	0.3641	0.6381	0.1745
	ST x MC		0.1025	0.9172	0.4121	0.9763	0.8944
	ST x MG		-	-	-	0.1416	0.4576
	MC x MG		-	-	-	0.8100	0.1060
	S x ST x MC		0.9498	0.6041	0.1822	0.3169	0.5489
	ST x MC x MG		-	-	-	0.6190	0.1740

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Poultry house as experimental unit.

Table 2.2 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on weight gain adjusted to a common heat stress index (HSI)[3] with analysis of covariance.

Effect			0 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	29 to 35 d
		n[3]	g/b/d				
S	Female	173	18.9	34.4[b]	57.0[b]	71.0[b]	73.6[b]
	Male	91	19.1	35.5[a]	60.0[a]	80.8[a]	87.5[a]
	Pooled SEM		0.6	0.7	1.0	0.9	2.1
ST	A	104	18.5[b]	34.6	59.0[a]	76.4	81.3
	B	160	19.5[a]	35.3[a]	58.0[b]	75.3	79.8
	Pooled SEM		0.6	0.7	1.0	0.9	2.1
MC	0	134	18.8	35.1	58.7	76.1	81.6
	250	130	19.1	34.8	58.3	75.6	79.5
	Pooled SEM		0.6	0.7	1.0	1.0	2.2
MG	0	91	-	-	-	81.2	87.1
	1.0	102	-	-	-	81.1	88.0
	Pooled SEM		-	-	-	2.8	1.6
Covariate	HSI [g/b/(°C x d)]		-0.001	0.001	0.002	0.001	-0.001
Sources of variation			P-values				
	HSI		0.6694	0.5984	0.5191	0.1543	0.8960
	S		0.0306	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.0321	0.0638	0.0336	0.2011	0.1413
	MC		0.2681	0.4900	0.6104	0.6735	0.6735
	MG		-	-	-	0.8860	0.3290
	S x ST		0.4588	0.7352	0.1709	0.4024	0.2121
	S x MC		0.6546	0.5241	0.6191	0.2672	0.0591
	ST x MC		0.1025	0.3935	0.3445	0.4849	0.6572
	ST x MG		-	-	-	0.1860	0.0412
	MC x MG		-	-	-	0.8707	0.0370
	S x ST x MC		0.9498	0.6000	0.2578	0.4569	0.7796
	ST x MC x MG		-	-	-	0.6627	0.1906

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Poultry house as experimental unit.

Table 2.3 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on feed intake adjusted to a common heat stress index (HSI)[3] with analysis of covariance.

Effect			0 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	29 to 35 d
		n[3]	g/b/d				
S	Female	173	21.4	49.2[a]	85.1[a]	115.4[b]	150.3
	Male	91	21.3	48.6[b]	84.2[b]	122.6[a]	151.0
	Pooled SEM		0.2	0.3	0.5	1.8	1.0
ST	A	104	21.2[b]	48.8	84.3	119.3	156.5[a]
	B	160	21.5[a]	48.9	84.9	118.7	144.7[b]
	Pooled SEM		0.2	0.3	0.5	1.8	1.0
MC	0	134	21.3	48.8	84.5	119.4	150.8
	250	130	21.4	48.9	84.8	118.6	150.5
	Pooled SEM		0.2	0.3	0.5	1.8	1.0
MG	0	91	-	-	-	122.9	157.5[b]
	1.0	102	-	-	-	123.2	158.9[a]
	Pooled SEM		-	-	-	2.8	3.1
Covariate	HSI [g/b/(°C x d)]		-0.001	0.001	0.001	0.004	0.001
Sources of variation			P-values				
	HSI		0.2231	0.4049	0.8600	0.1443	0.1421
	S		0.5338	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.0050	0.3905	0.0991	0.2217	0.6503
	MC		0.5817	0.9067	0.4220	0.0880	0.1967
	MG		-	-	-	0.4951	0.0097
	S x ST		0.9154	0.9953	0.2665	0.2063	0.1777
	S x MC		0.8597	0.2683	0.0527	0.0115	0.2543
	ST x MC		0.6923	0.5410	0.0541	0.3156	0.6113
	ST x MG		-	-	-	0.6287	0.1743
	MC x MG		-	-	-	0.8193	0.4007
	S x ST x MC		0.0308	0.0637	0.2025	0.3471	0.6316
	ST x MC x MG		-	-	-	0.6220	0.1271

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Poultry house as experimental unit.

Table 2.4 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on FCR adjusted to a common heat stress index (HSI)[3] with analysis of covariance.

Effect			0 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	29 to 35 d	Cumulative
		n[4]	g:g					
S	Male	173	1.122[b]	1.364[b]	1.406[b]	1.515[b]	1.799[b]	1.501[b]
	Female	91	1.136[a]	1.429[a]	1.498[a]	1.623[a]	1.985[a]	1.594[a]
	Pooled SEM		0.021	0.033	0.021	0.027	0.052	0.031
ST	A	104	1.151	1.404	1.437[b]	1.559	1.865[b]	1.542
	B	160	1.107	1.389	1.467[a]	1.578	1.919[a]	1.553
	Pooled SEM		0.025	0.034	0.021	0.027	0.052	0.032
MC	0	134	1.136	1.390	1.447	1.569	1.870	1.542
	250	130	1.122	1.403	1.458	1.569	1.915	1.553
	Pooled SEM		0.022	0.035	0.023	0.029	0.055	0.031
MG	0	91	-	-	-	1.519	1.809	1.509
	1.0	102	-	-	-	1.525	1.809	1.509
	Pooled SEM		-	-	-	0.0310	0.058	0.025
Covariate	HSI [g:g/(°C x d)]		-0.004	-0.001	-0.004	-0.001	0.002	0.001
Sources of variation			P-values					
	HSI		0.7022	0.5090	0.6843	0.3306	0.4510	0.5202
	S		0.0465	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.0927	0.1872	0.0106	0.3234	0.0491	0.1818
	MC		0.3773	0.4512	0.4891	0.9877	0.2509	0.3869
	MG		-	-	-	0.7128	0.9785	0.9553
	S x ST		0.4938	0.8063	0.7629	0.7507	0.1122	0.1092
	S x MC		0.5628	0.9914	0.4988	0.0373	0.2425	0.8976
	ST x MC		0.2326	0.4567	0.9840	0.5931	0.6931	0.8639
	ST x MG		-	-	-	0.2267	0.0254	0.1777
	MC x MG		-	-	-	0.6641	0.0240	0.1234
	S x ST x MC		0.0825	0.6770	0.6464	0.8000	0.9488	0.9436
	ST x MC x MG		-	-	-	0.4502	0.4544	0.3445

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Poultry house as experimental unit.

Table 2.5 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on BW, cumulative feed intake (FI), and FCR at processing. Dependent variables adjusted to a common heat stress index (HSI)[3] and a common processing age (PA)[4] with analysis of covariance.

Effect			Age	BW	Cumulative FI	FCR
		n[5]	d	g/b		g:g
S	Female	173	40.8	2,257[b]	4,075[b]	1.793[a]
	Male	91	45.0	2,615[a]	4,378[a]	1.667[b]
	Pooled SEM			34.9	29.6	0.032
ST	A	104	42.7	2,446	4,210	1.715[b]
	B	160	42.9	2,427	4,243	1.746[a]
	Pooled SEM			35.4	27.1	0.032
MC	0	134	42.7	2,438	4,228	1.728
	250	130	42.9	2,434	4,225	1.733
	Pooled SEM			36.3	26.6	0.033
MG	0	91	41.9	2,826	4,859[b]	1.734[b]
	1.0	102	45.0	2,831	4,961[a]	1.769[a]
	Pooled SEM			31.7	56.1	0.029
Covariate	HSI [g/b/(°C x d)]			0.001	0.003	0.001
	PA (g/d)			0.068	0.174	0.021
Sources of variation				P-values		
	HSI			0.3690	0.0583	0.5266
	PA			< 0.0001	< 0.0001	< 0.0001
	S			< 0.0001	< 0.0001	< 0.0001
	S			0.2743	0.1956	0.0134
	ST			0.8349	0.9065	0.7190
	MC			0.6616	< 0.0001	< 0.0001
	MG			0.1519	0.3273	0.5637
	S x ST			0.6745	0.0276	0.1328
	S x MC			0.4301	0.2812	0.9089
	ST x MC			0.9430	0.7513	0.9202
	ST x MG			0.4265	0.7134	0.2082
	MC x MG			0.0638	0.0096	0.7861
	S x ST x MC			0.1245	0.0647	0.7010

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ PA – 21 (d).

[4]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[5]Poultry house as experimental unit.

Table 2.6 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on mortality adjusted to a common heat stress index (HSI)[3] and a common processing age (PA)[4] with analysis of covariance.

Effect			0 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	29 to 35 d	Total
		n[5]	%					
S	Male	173	0.99	0.63[b]	0.46[b]	0.38[b]	0.44[b]	3.23[b]
	Female	91	1.03	0.68[a]	0.59[a]	0.55[a]	0.67[a]	5.02[a]
	Pooled SEM		0.07	0.05	0.04	0.03	0.13	0.58
ST	A	104	0.96	0.57	0.49	0.47	0.62	4.20
	B	160	1.06	0.73	0.57	0.46	0.50	4.04
	Pooled SEM		0.09	0.06	0.04	0.04	0.13	0.58
MC	0	134	1.00	0.63	0.52	0.46	0.66[a]	4.09
	250	130	1.02	0.67	0.54	0.47	0.46[b]	4.16
	Pooled SEM		0.08	0.05	0.04	0.04	0.13	0.59
MG	0	91	-	-	-	0.57	0.66	5.33
	1.0	102	-	-	-	0.56	0.66	5.70
	Pooled SEM		-	-	-	0.04	0.13	0.58
Covariate	HSI [%/(°C x d)]		-0.002	0.002	0.001	-0.004	-0.004	0.003
	PA (% / d)		-	-	-	-	-	-0.053
Sources of variation			P-values					
	HSI		0.9554	0.3186	0.7973	0.0600	0.4339	0.8479
	PA		-	-	-	-	-	0.3553
	S		0.2268	0.0237	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.2744	0.0508	0.1256	0.8354	0.1181	0.5658
	MC		0.6608	0.1906	0.6423	0.8098	0.0175	0.8083
	MG		-	-	-	0.7236	0.9278	0.1849
	S x ST		0.2116	0.3270	0.4467	0.6328	0.1609	0.0247
	S x MC		0.8341	0.9368	0.2284	0.9868	0.2126	0.0844
	ST x MC		0.4841	0.2407	0.6426	0.7017	0.8917	0.8868
	ST x MG		-	-	-	0.6562	0.1333	0.8780
	MC x MG		-	-	-	0.1595	0.0249	0.9595
	S x ST x MC		0.8127	0.3718	0.0476	0.8058	0.2552	0.2431
	ST x MC x MG		-	-	-	0.9042	0.8412	0.1188

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogota D.C., Colombia.

[3]Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ PA – 21 (d).

[4]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[5]Poultry house as experimental unit.

2.6 FIGURES

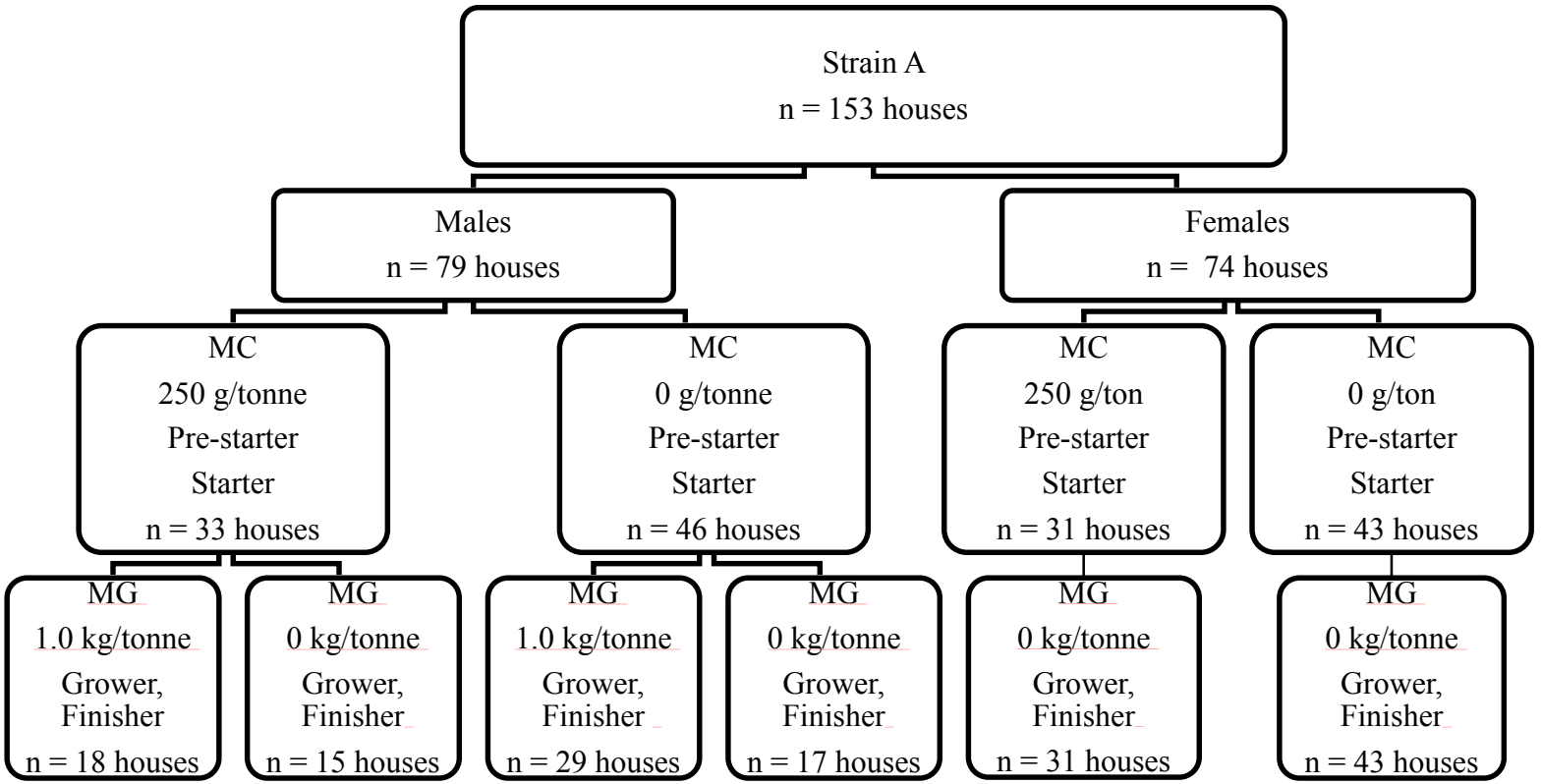

Figure 2.1. Experimental design as implemented in strain A.
Broiler males of strain A were fed with one of two levels of dietary MaxiChick® (MC; 0 or 250 g/tonne) in the pre-starter and starter feeding phase (0 to approximately 21 d), and one of two levels of dietary Marigold extract (MG; 0 or 1.0 kg/tonne) in the grower and finisher feeding phase (approximately 22 d to processing). Broiler females of strain A were fed with one of two levels of dietary MC (0 or 250 g/tonne) in the pre-starter and starter feeding phase (0 to approximately 21 d), and one level of dietary MG (0 kg/tonne) in the grower and finisher feeding phase (approximately 22 d to processing). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

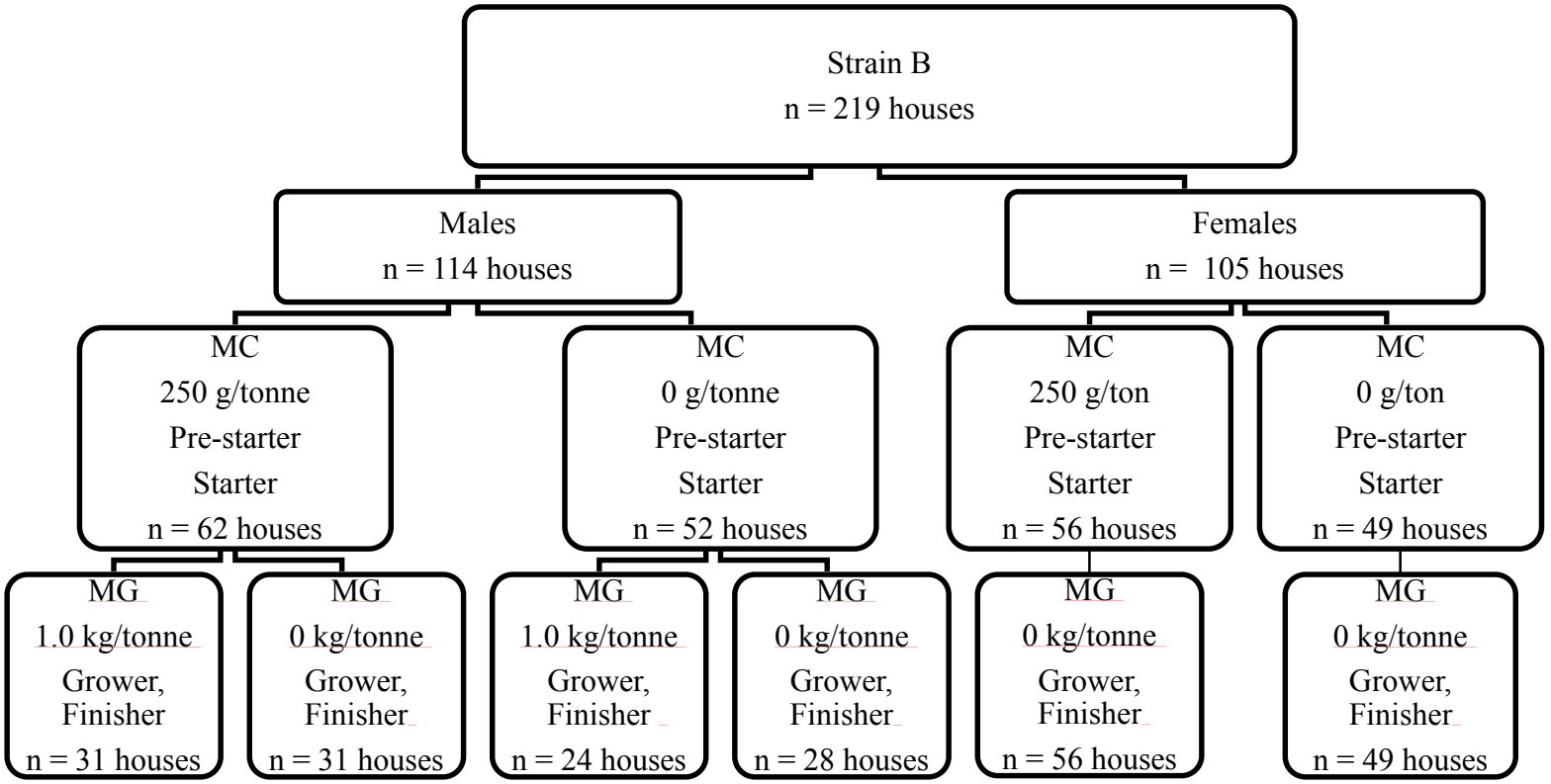

Figure 2.2. Experimental design as implemented in strain B.
Broiler males of strain B were fed with one of two levels of dietary MaxiChick® (MC; 0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d), and one of two levels of dietary Marigold extract (MG; 0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing). Broiler females of strain B were fed with one of two levels of dietary MC (0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d), and one level of dietary MG (0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

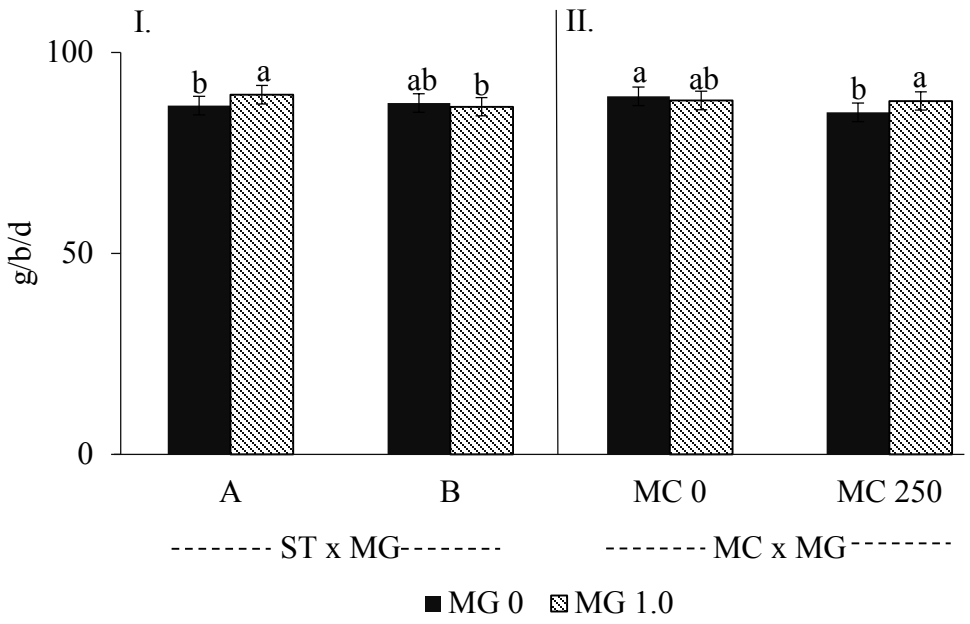

Figure 2.3. Weight gain from 29 to 35 d adjusted to a common heat stress index with analysis of covariance.
I. Strain (ST) x dietary marigold extract (MG; P = 0.0412). Broiler males of strain A and B were fed with one of two levels of dietary MG (0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing).
II. Dietary MaxiChick® (MC) x dietary MG (P = 0.0370). Broiler males were fed with one of two levels of dietary MC (0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d), and two levels of dietary MG (0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing), that provided a xanthophyll content ranging from 12 to 15 mg/kg of feed. Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

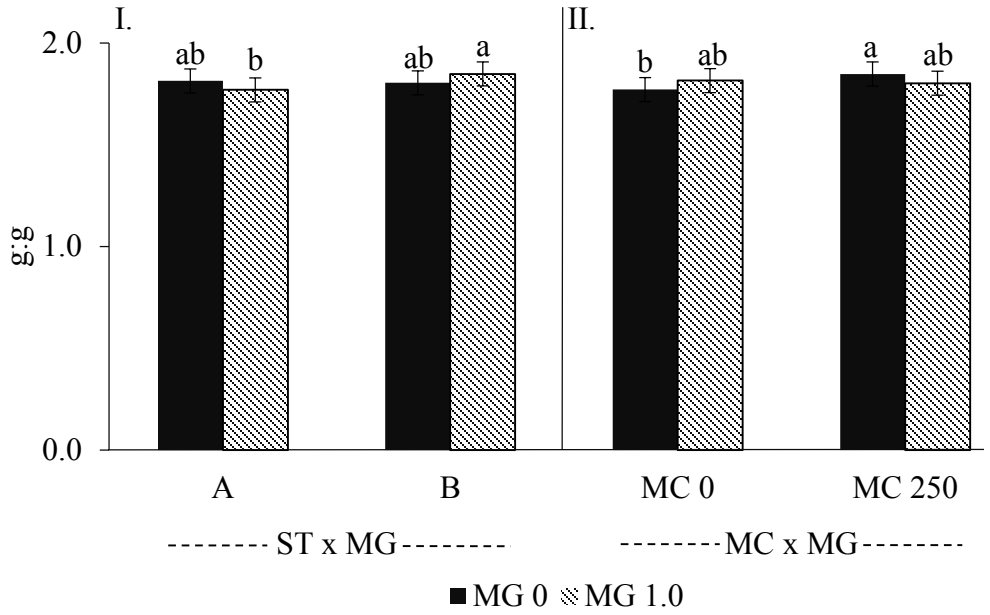

Figure 2.4. FCR from 29 to 35 d adjusted to a common heat stress index with analysis of covariance.
I. Strain (ST) x dietary marigold extract interaction (MG; P = 0.0254). Broiler males of strain A and B were fed with one of two levels of dietary MG (0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing).
II. Dietary MaxiChick® (MC) x dietary MG interaction (P = 0.0240). Broiler males were fed with one of two levels of dietary MC (0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d), and two levels of dietary MG (0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing). Heat stress index = [daily maximum house temperature −daily recommended temperature (°C)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

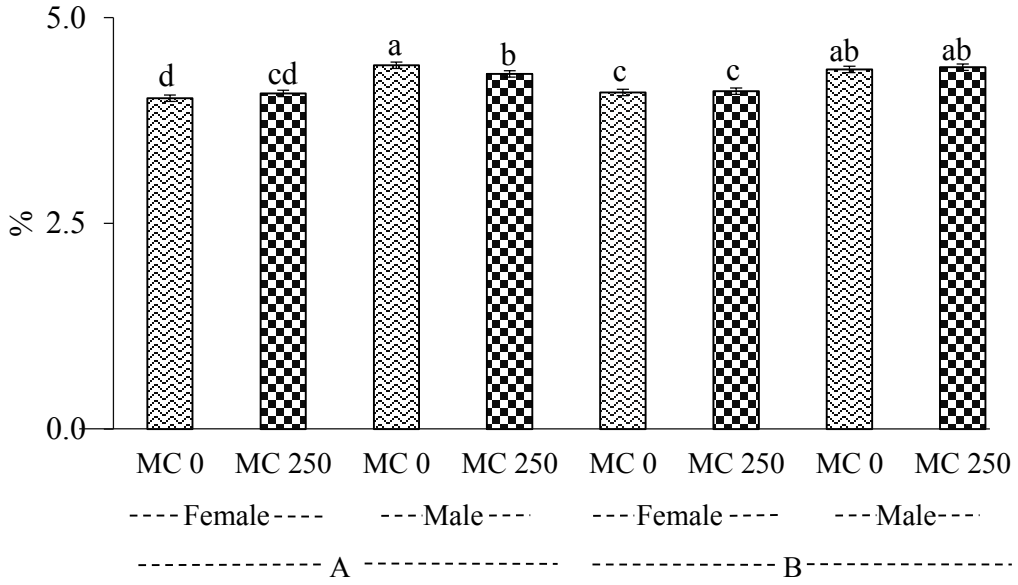

Figure 2.5. MaxiChick® (MC) x sex x strain interaction for cumulative feed intake adjusted to a common heat stress index and a common processing age with analysis of covariance (P = 0.0096). Strain A and B broilers were fed with one of two levels of dietary MC (0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d). Heat stress index = [daily maximum house temperature − daily recommended temperature (ºC)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

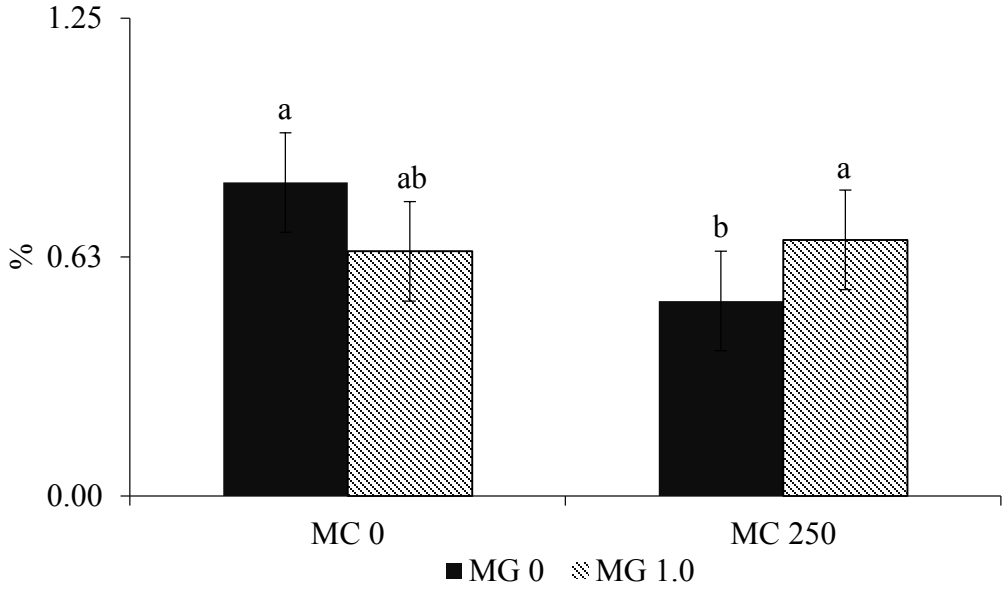

Figure 2.6. Dietary MaxiChick® (MC) x dietary marigold extract (MG) interaction for mortality from 29 to 35 d adjusted to a common heat stress index with analysis of covariance (P = 0.0249). Broiler males were fed with one of two levels of dietary MC (0 or 250 g/tonne) during the pre-starter and starter feeding phase (0 to approximately 21 d), and one of two levels of dietary MG (0 or 1.0 kg/tonne) during the grower and finisher feeding phase (approximately 22 d to processing). Heat stress index = [daily maximum house temperature −daily recommended temperature (ºC)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

3. EFFECT OF DIETARY 25-OH-D_3 AND CANTHAXANTHIN ON YIELD, SKIN COLOUR AND BONE CHARACTERISTICS OF BROILERS IN COMMERCIAL PRODUCTION CONDITIONS IN COLOMBIA

ABSTRACT

Yield, skin colour and bone characteristics were assessed to determine the effects of a combination of dietary 25-hydroxy vitamin D_3 (25-OH-D_3) and canthaxanthin (CX). A production cycle of 4,922,130 broilers reared sex-separately of two commercial broiler strains (A and B) was followed in each of 372 commercial poultry houses in Colombia. The Control diet contained 4,000 IU of vitamin D_3 per kg of complete feed. The treatment diet (MC) contained 6 mg of CX, 2,760 IU of 25-OH-D_3, plus 4,000 IU of vitamin D_3 per kg of complete feed from 0 to approximately 21 d. From approximately d 22 to processing age, marigold extract (MG) at 1.0 g per kg of feed with a total xanthophyll content of 12 to 15 mg/kg of feed was supplemented to 53% of the males. The average age at processing was 40.8 d for females and 45.0 d for males. From half of the houses for each treatment, 20 birds were chosen randomly before slaughter to assess yield and skin colour. From these birds, 5 carcasses were randomly selected to assess bone strength. Breeder source, geographical region, farm, and housing type were included as random terms in the data analysis. Heat stress index and processing age were included as covariates Weights and yield of carcass, whole breast, breast fillet, and thighs were highest in strain A, which had lower intestine yield. MC reduced most of the carcass traits and increased carcass water absorption, especially in strain A. MG increased breast weight, and weight and yield of carcass, drumstick, and thigh, and reduced intestine yield. MG reduced skin lightness and redness, but increased yellowness. MC increased bone breaking stress; bone breaking strength was nearly increased ($P = 0.0768$). MC increased bone breaking strength in both sexes of strain B, but reduced it in strain A males; MC had no effect

in strain A females. In conclusion, a high vitamin D_3 activity (6,760 IU/kg of feed) reduced meat yield. The increased yield caused by dietary MG might be related to a higher antioxidant status or an enhanced availability of metabolizable energy due to a lower intestine yield. The increased bone quality with MC supplementation, mainly in strain B; likely due to the inclusion of 25-OH D_3.

Key words: Yield, 25-hydroxy vitamin D_3, canthaxanthin, performance, bones.

3.1 INTRODUCTION

After intestinal absorption, vitamin D_3 is transported in the blood to the liver where it is converted into 25-OH-D_3 (calcidiol), the major circulating form of vitamin D_3 (Bar et al., 1980). This metabolite is then transported to the kidney and converted into l,25-$(OH)_2$-D_3, (calcitriol) which the active form of vitamin D_3 (Soares et al., 1995). Intestinal absorption of 25-OH-D_3 is more efficient (Nemere et al., 1994; Phadnis and Nemere, 2003), has a higher retention rate (Bar et al., 1980), lower daily excretion rate (Chou et al., 2009), and higher biological activity (Soares et al., 1978) compared to vitamin D_3. Breast is the most valuable portion of the chicken carcass (Agriculture and Agri-Food Canada, 2014); therefore, small differences in breast yield can have significant economic impact. Supplemental 25-OH-D_3 as a partial or sole source of dietary vitamin D_3 activity increased breast yield of broiler chickens, especially when included throughout the grow-out period (Yarger et al., 1995a; Mireles et al., 1996; Fritts and Waldroup, 2003; Vignale et al., 2013). Due to rapid growth, skeletal disorders have appeared, which reduces profits and welfare in broiler operations (Knowles et al., 2008; Sun et al., 2013). Dietary 25-OH-D_3 inclusion reduced severity of tibial dyschondroplasia (TD) and increased bone ash (Rennie and Whithead, 1996; Fritts and Waldroup, 2003; Parkinson and Cransberg, 2004) and bone breaking strength (Saunders-Blades, 2008) in comparison to broilers fed with vitamin D_3 at same level of inclusion.

Carotenoids including canthaxanthin (CX) can reduce lipid peroxidation and increase antioxidant capacity in birds (Surai et al., 2003; Zhang et al., 2011; Shanmugasundaram and Selvaraj, 2011; Rosa et al., 2012). In some poultry markets such as Mexico and Colombia, CX has been used in combination with other sources of natural carotenoids such as marigold extracts (MG, *Tagetes erecta*) for skin and egg pigmentation (Pérez-Vendrell et al., 2001; Cho et al., 2013). There is no evidence to date that carotenoid supplementation influences meat yield; however, it has been proposed that dietary lutein (an oxycarotenoid) inclusion can increase BW and reduce inflammation as a result of a higher antioxidant status (Shanmugasundaram and Selvaraj, 2011; Rajput et al., 2012). Supplemental CX increased mitigated osteoporosis in rats (Lin-Peng et al., 2009), and stimulated in vitro differentiation of osteoblasts (Park et al., 1997).

A new additive is available that combines 6 mg CX and 69 µg 25-OH-D_3 per kg of complete feed (MaxiChick® (MC); DSM Nutritional Products, (DSM Nutritional Products, Basel, Switzerland), that has been tested mainly in broiler breeders (Rosa et al., 2010a; 2010b). Embryo mortality was reduced (Rosa et al., 2010b; Cho et al., 2013a), sperm quality was increased (Rosa et al., 2010a), and phagocytic capacity was increased (Cho et al., 2013b) in broilers breeders fed MC. There are no studies of MC effects on broilers. The objectives of the current study were to investigate the effects of the dietary MC during the first three weeks of age on meat yield, internal organ weights, skin colour and bone characteristics of broilers under commercial conditions in Colombia. It was hypothesized that dietary MC would increase meat yield, internal organ weights, bone strength and influence skin colour of broilers as compared to a control diet with no MC.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design.

The experiment was conducted in a commercial broiler vertical integration in Colombia (Avidesa, MacPollo de Occidente) as a 2 x 2 x 2 x 2 incomplete factorial arrangement. One whole broiler cycle of 4,922,130 broilers of both sexes that were placed in 58 farms (n = 372 houses) was evaluated. The treatments were: two commercial broiler strains (A, B), two sexes, two dietary MC levels (0 and 250 g/tonne of feed), and two marigold extract levels (MG; 0 or 1.0 kg/tonne of feed). The Control diet contained a basal level of 4,000 IU vitamin D_3 per kg of complete feed. Dietary MC provided 69 μg of 25-OH-D_3 (equivalent to 2,760 IU of vitamin D_3 activity), 6 mg of CX, and the basal level of vitamin D_3 per kg of complete feed in the pre-starter and starter diets from 0 to approximately 21 d. Dietary MG (xanthophyll content of 12 to 15 mg/kg of feed) was given to 102 houses (53%) of males in the grower and finisher diets according to Colombian regional market requirements for yellow-skinned chickens. For treatment distribution, animals and housing, and broiler management followed in this trial refer to Chapter 2.

3.2.2 Data Recorded

3.2.2.1 Carcass measures.

Before slaughter, broilers were subjected to a total feed withdrawal of 8 to 12 h, including 1 to 4 h of transportation and holding time at the processing plant. In the farm, water was not removed from the birds until they were loaded in plastic crates (59 x 84 x 26 cm; 25 kg capacity corresponding to approximately 8 males or 11 females) for transportation. If the shipment was during the day, crates contained up to 22 kg, and during night up to 25 kg of live birds. The birds were transported for between 30 minutes to 3 hours on trucks having 2,000 to 2,200 broiler capacity from each house to Avidesa's processing facility.

From 50% of the houses, 20 to 23 birds (3,609 birds in total) were randomly selected at the processing plant before slaughter to represent the treatment corresponding to each house. To ensure a uniform representation from within each house, these birds were removed at random intervals when the cages were being unloaded from the truck just prior to slaughter. Immediately before processing, a numbered wing-tag was placed in the left wing of these birds to preserve identity, and they were individually weighed.

All birds were subsequently processed under commercial conditions using electrical stunning (20 to 26mv, 15 s). Birds were bled for 180 s, and then carcasses were passed through two scalding tanks filled with water at a temperature of 56.6°C for 130 s, and 56.8°C for 140 s. For yellow-skinned broilers the scald temperature was set at 52 to 53°C to allow retention of the epidermis. After scalding, birds were defeathered by rotating rubber fingers and then the heads were removed. The line speed was 98 birds per minute. After being defeathered, the wing-tagged birds were removed from the processing line and manually eviscerated.

Heart, liver, intestine (duodenum to rectum), abdominal fat, and spleen weights from each wing-tagged bird were recorded, and yield was calculated as percentage of carcass weight [(organ weight/carcass weight) × 100 %]. Fat pad was removed from the abdominal cavity excluding the gizzard fat and was weighed. The right tarsometatarsus (shank) of 5 carcasses randomly selected from the wing-tagged carcasses was individually marked with the corresponding number of the wing tag, and frozen for bone strength analysis in an external laboratory. Each of these procedures were always conducted by the same personnel.

After manual evisceration, the wing-tagged carcasses were returned to the processing line and were pre-chilled at 18°C for 25 min in chlorine water, and then chilled at 2°C for 60 to 72 min in chlorine water (50 ppm) min to allow the carcass to reach a core temperature of 4 °C. Carcass

water absorption (WA) was calculated as the increase in carcass weight by moisture retention after pre-chilling and chilling. After chilling, wing-tagged carcasses were removed from the processing line for manual cut-up, and for skin colour measurements. The remaining birds continued through the regular process and normal inspection. The wing-tagged carcasses were cut up to obtain whole breast (Pectoralis major and Pectoralis minor, bone, skin), breast fillet (Pectoralis major), legs (thighs and drums separately), wings, and barrel (rib cage and vertebrae). The breast fillet was manually deboned. Carcass yield was calculated as the percent of live BW [(carcass weight / live BW) × 100 %].Yield for each carcass component was calculated as the percent of eviscerated carcass [(carcass portion/eviscerated carcass weight) × 100 %] per bird.

3.2.2.2 Skin colour measures.

Colour measurement was conducted using a Minolta Chroma meter CR-300, which expresses colour in terms of CIE colour system values (Commission Internationale de l'Eclairage) for lightness (L*), redness (a*), yellowness (b*). The colorimeter was calibrated throughout the trial using a standard white ceramic tile. Skin colour measurements of each wing-tagged carcass (one reading per carcass) were taken after chilling on the right medial breast surface avoiding any feather tract or major blood vessels according to the method described by Castañeda et al. (2005), and Huezo et al. (2007). Only areas free from obvious defects (bruises, discolorations, hemorrhages, or any other condition that might have affected uniform colour reading) were selected for colour measurements.

3.2.2.3 Bone characteristics.

The right tarsometatarsus (shank) of five wing-tagged birds per sampled house was removed (827 in total) with flesh and skin intact and then were individually marked, packed in

plastic bags, frozen at -10ºC and sent to an external laboratory for bone measurements (Lepton, S.A. Specialized Chemical Analysis and Support, Bogotá D.C., Colombia).

Before breaking, the shanks were thawed in a fridge at 4 to 6ºC for 24 hours and were marked at the midpoint and broken using a Texture Analyzer machine (model TA-XT plus, Stable Micro Systems Ltd, Vienna Court, Lammas Road, Godalming, Surrey, UK) to measure bone breaking strength in KgF (maximum force required to break the bone) under the compression method of "return to start" (Fleming et al., 1998; Park et al., 2003). The shank was held by 2 brackets (spaced 36.4 mm apart) and the physical power was applied to the midpoint of the bone by a static load cell (5 kN) and a trigger force of 20.0 g with a test speed of 2 mm/s.

3.2.3 Statistical Analyses.

Carcass traits, skin colour and bone characteristics data were analyzed as 4 way ANCOVA with sex, strain, dietary MC and dietary MG as primary sources of variation using the procedure for linear mixed models (PROC MIXED) of SAS 9.3© for Windows (SAS Institute Inc., Cary, NC). The experimental unit was the individual bird for all statistical analyses; the ANCOVA of the data was conducted by using means of processing traits, skin colour, and bone measurements of each main effect and interactions among them. Variability due to broiler breeder source, geographical region, farms, and houses (floor type, floor level and environmental control), was considered using random terms in the model. This broiler operation determines processing age (PA) according to a different BW objective for males and females, taking into account sales demand and logistics of the company. The overall average age at processing was 40.8 days for females with a range of 37 to 47 days, and 45.0 days for males with a range of 40 to 48 days. Variability due to environmental temperatures, a heat stress index (HSI) average was calculated for each house (refer to Chapter 2). For this variation, PA and HSI were considered as a covariates

in the model. Covariate is a variable that can influence the dependent variables. To increase precision and reduce error (residual), the least square means were adjusted to a common covariate. Pearson correlation coefficients were calculated for skin redness and yellowness using the CORR procedure of SAS 9.3© for windows (SAS Institute Inc., Cary, NC)

The least squares means were calculated for all variables in the study, and the related LSD were calculated to determine significant differences and interactions between main effects. Differences were considered significant at $P < 0.05$. The analysis was in accordance with the following model:

$$Y_{ijkl} = \mu + S_i + ST_j + MC_k + MG_l + (S\text{-}MC)_{ik} + (ST\text{-}MC)_{jk} + (MC\text{-}MG)_{kl} + (S\text{-}ST)_{ij} + (S\text{-}ST\text{-}MC)_{ijk} + (ST\text{-}MC\text{-}MG)_{ikl} + \beta\,(HSI_{ijkl} - HSIa) + \beta\,(PA_{ijkl} - PAa) + E_{ijk1},$$

where μ was the population mean; S_i was the effect of sex ($i = 1$ to 2); ST_j was the effect of strain ($j = 1$ to 2); MC_k was the effect of dietary MC level ($k = 1$ to 2); MG_l was the effect of dietary MG extract level ($l = 1$ to 2); $(S\text{-}MC)_{ik}$, $(ST\text{-}MC)_{ik}$, $(MC\text{-}MG)_{kl}$, $(S\text{-}ST)_{ij}$, $(S\text{-}ST\text{-}MC)_{ijk}$, and $(ST\text{-}MC\text{-}MG)_{ikl}$ were the interactions of the main effects; $\beta\,(HSI_{ijkl} - HSIa)$ was a covariate coefficient multiplied by the difference between individual HSI (HSI_{ijkl}) and average HSI (HSIa); $\beta\,(PA_{ijkl} - PAa)$ was a covariate coefficient multiplied by the difference between individual PA (PA_{ijkl}) and average PA (HSIa);and E_{ijk1} was the residual error.

3.3 RESULTS

3.3.1 Broiler Carcass Traits at Processing

Dietary MC significantly reduced carcass weight in strain A males only (Table 3.1; Table 3.2). In both strains, females had significant higher carcass yield than males; strain A broilers had higher carcass yield than strain B (Table 3.3; Table 3.4). Within each sex by strain combination, dietary MC had no effect on breast weight at processing. Strain A females fed MC had similar

breast weight as the strain B females not fed MC (Table 3.2). Breast filet weights were 13.4% higher in males than females, and 11.4% higher in strain A than in strain B (Table 3.1). However, females had 2.31% higher breast yield than males (Table 3.3). Females had higher carcass yield and breast filet yield than males. Females and males of strain A had higher carcass yield and breast filet yield than females and males of strain B respectively (Table 3.4). Males had higher thigh weights than females (Table 3.1). Dietary MC had a tendency to reduce thigh weights in strain A regardless of bird sex, and in strain B males ($P = 0.0578$; Table 3.2). Supplemental MC reduced thigh yield in strain A females, and increased it in strain B females; MC affected males of both strains in a similar manner (Table 3.2). Dietary MC reduced drum weights in strain A males, but had no effect on any other strain by sex group; males of both strains had significantly higher drum weights than females (Table 3.2). Dietary MC reduced drum yield in strain A regardless of sex, but increased it in strain B males; MC had no effect on drum yield in strain B females (Table 3.2). Dietary MC reduced wing weights in strain B females, but had no effect on any other strain by sex group; moreover, in both strains, males had significantly higher wing weights than females; when comparing sexes within strains there were no differences (Table 3.2). Strain B females had significantly higher wing yield than strain A females; there were no differences in wing yield between sexes within each strain (Table 3.4). Supplemental MC had a tendency to reduce wing yield in strain B females ($P = 0.0750$; Table 3.2). Dietary MC increased WA of carcasses in strain A males, but had no effect on any other strain by sex group (Table 3.2).

Dietary MG was provided to only 53% of the male houses. Supplementation of MG increased carcass weight in strain B, but had no effect in strain A. When not fed MG, strain A males had higher carcass weights than strain B males; in contrast carcass weights were similar when both strains were fed MG (Table 3.5). Supplemental MG increased carcass yield in both

strains. Independent of dietary MG, strain A had higher carcass yield than strain B (Table 3.5). Dietary MG increased breast weights by 2.6% in comparison to birds fed diets without MG (Table 3.1). Supplemental MG reduced breast filet weights of birds fed MC, but had no effect in males not fed MC. Notwithstanding, MC reduced breast filet weights of birds when fed MG, but had no effect in birds not fed MG (Table 3.6). Dietary MG reduced breast filet yield of birds fed MC, but had no effect in birds not fed MC; MC did not affect breast filet independent of dietary MG (Table 3.6). Breast filet yield was significantly reduced in strain B by dietary MG; but MG did not affect breast filet yield in Strain A (Table 3.5). Dietary MG increased thigh weights in strain A when not fed MC, and also in strain B when fed MC; however, MG had no effect in strain A when fed MC nor in strain B when not fed MC. Moreover, MC reduced thigh weights in strain B males when not fed MG; but had no effect on any other strain by MG group (Table 3.7). The same significant interaction was found in thigh yield (Table 3.7). Dietary MG increased drum weights in strain B, but not in strain A (Table 3.5). Supplemental MG increased drum yield by 1.3 % in comparison to birds fed diets without MG (Table 3.3). Dietary MG had a tendency to reduce wing weights in males not fed MC, but did not when they were fed MC ($P = 0.0780$; Table 3.6). Dietary MC had a tendency to reduce wing weights of birds fed MG, but had no effect in birds fed diets without MG ($P = 0.0780$; Table 3.6). Supplemental MG, increased wing weights in strain B; but had no effect in strain A (Table 3.5). Dietary MG reduced wing yield in males fed MC, but had no effect in birds not fed MC. Supplemental MC did not affect wing yield regardless of dietary MG (Table 3.6). Males fed MG had lower carcass WA than males not fed MG (Table 3.3).

3.3.2 Percentage of Relative Weight of Organs

Dietary MC increased heart yield in strain A males, but not in strain A females; MC had no effect on heart yield in strain B broilers (Table 3.2; Table 3.8). When not fed MC, females and

males of strain A had similar heart yield; but when strain A birds were fed MC, males had higher heart yield than females. In contrast, when broilers of strain B were not fed MC, males had higher heart yield than females, but when strain B broilers fed MC the difference between sexes disappeared (Table 3.2). Supplemental MG reduced heart yield in strain B; but had no effect in strain A. When not fed MG, strain B had higher heart yield than strain A; but heart yield was similar when both strains were fed MG (Table 3.5).

In strain A, dietary MC reduced liver yield in females relative to males, but had no interaction with sex for strain B (Table 3.2). Dietary MG reduced liver yield of birds not fed MC, but had no effect in birds fed MC; moreover, MC had no effect independent of dietary MG (Table 3.6). Liver yield was reduced by 3.9 % in strain B birds when fed MG, but was not affected by MG in strain A. Independent of dietary MG, strain B had higher relative liver weight than strain A (Table 3.5).

Dietary MC increased spleen yield in both sexes of strain A, but had no effect in strain B birds (Table 3.2). Dietary MG reduced spleen yield in strain A birds fed MC, but had no effect on any other strain by MC group (Table 3.7). Regardless of dietary MG, MC increased spleen yield in strain A. Dietary MC reduced spleen yield in strain B birds not fed MG, but had no effect when strain B birds fed MG (Table 3.7).

Dietary MC increased relative intestine weights only in strain B females (Table 3.2). Supplemental MG reduced relative intestine weight of males independent of dietary MC (Table 3.6).

Females had higher fat pad yield than males at processing (Table 3.8). Females of both strains had a tendency to have higher fat pad yield than males ($P = 0.0593$; Table 3.4). Strain B females had a tendency to have higher fat pad yield than strain A females; there were no differences

between males (P = 0.0593; Table 3.4). Dietary MC increased relative fat pad weights in strain A, but had no effect in strain B (Figure 3.1). Moreover, when not fed MC, strain B birds had higher fat pad yield than strain A, but fat pad yield was similar when both strains were fed MC (Figure 3.1). Dietary MG had a tendency to increase fat pad yield at processing (P = 0.0616; Table 3.8).

3.3.3 Skin colour

Dietary MC to approximately 21 d of age reduced skin lightness in males; but had no effect in females (Figure 3.2). When not fed MC, skin lightness was higher in males than females, but was similar when both sexes were fed MC (Figure 3.2). Birds fed MG had significant lower skin lightness than birds fed diets without MG (Table 3.9).

Supplemental MC reduced skin redness in strain A males, but increased it in strain B males; MC had no effect in females of either strain (Table 3.2). Dietary MG significantly reduced skin redness in both strains independent of dietary MC; MC significantly increased skin redness in strain A birds fed MG and in strain B birds not fed MG (Table 3.7). Dietary MC had no effect in strain A birds not fed MG and strain B birds fed MG (Table 3.7).

Dietary MC increased skin yellowness in strain A birds regardless of sex, but in contrast reduced it in strain B males; MC had no effect in strain B females (Table 3.2). Skin yellowness was increased by MG in both strains independently of dietary MC (Table 3.7). Supplemental MC reduced skin yellowness in strain A birds fed MG, but had no effect on the other strain by MG groups (Table 3.7).

3.3.4 Bone characteristics

Dietary MC increased bone breaking strength in both sexes of strain B, in contrast it reduced the same trait in strain A males. Dietary MC had no effect in strain A females (Table 3.2). Bone breaking stress was increased by 8.8% when broilers were fed MC (Table 3.10).

Supplemental MG reduced bone breaking strength in strain A birds not fed MC, but had no effect in any other strain by MC group (Table 3.7). In males, dietary MC increased bone breaking strength in strain A fed MG, but had no effect in other strain by MG groups (Table 3.7). Males had higher bone breaking stress than females at processing age (Table 3.10). Dietary MG had a tendency to reduce bone breaking stress (P = 0.0856; Table 3.10).

3.4 DISCUSSION

3.4.1 Broiler Carcass Traits at Processing

Males had higher carcass part weights than females, which is similar to previous studies where similar differences have been reported in those traits (Abdullah et al., 2010; López et al., 2011; Brewer et al., 2012; Shim et al., 2012), due to sexual dimorphism in broiler chickens (Fanatico et al., 2005; Murawska et al., 2011). Females had greater carcass and breast yield compared to males, while drum yield was greater for males. These results agree with previous studies (Young et al., 2001; Abdullah et al., 2010; López et al., 2011). This is related to the different growth dynamic that exists between sexes in broilers; as an example, in females the proportion of breast muscle increases with BW more than in males (Zuidhof, 2005).

Strain A birds had a higher breast filet weights and carcass and breast yields than strain B birds. There was no overall difference in WA between strains, which indicates that the higher values in yield and weights were not related to a higher moisture retention after chilling in strain A, demonstrating a real higher meat production in strain A in comparison to strain B. Differences between strains in these traits have been reported in several previous studies (Mehaffey et al., 2006; Abdullah et al., 2010; Shim et al., 2012).

In broilers, WA by water-chilled carcasses is highly variable (Young and Smith, 2004), and is related to weight (Kato et al., 2013). Smaller carcasses absorb more water, because per unit

of BW have larger contact surface with water (Essary and Dawson, 1965; Carciofi and Laurindo 2007; Huezo et al., 2007). In the current study, all carcasses were evaluated after the same immersion chilling and pre-chilling procedure. In strain A males, dietary MC increased carcass WA by 20.9%.

In a diet containing a total of 4,000 IU of vitamin D_3 activity, replacement of 2,760 IU or 1,500 IU of vitamin D_3 with 25-OH-D_3 during the grow-out period increased WA in breast meat by 21.4% and 24.4% respectively, and WA in leg meat by 16.2% and 11.6%, respectively (Michalczuk et al., 2010), although the mechanism was not determined. In the current study, MC influenced meat WA depending on bird sex and strain, and that effect is likely related to the inclusion of 25-OH-D_3 in MC. However, in strain A males, dietary MC reduced carcass weight by 3.2%; therefore, the higher WA can be also related to the smaller carcass of these birds relative to strain A males not fed MC.

With the exception of the increased thigh yield in strain B females and the increased drum yield in strain B males, MC reduced most of the carcass traits, especially in strain A. According to the available literature, the effects of 25-OH-D_3 on processing yield have not been consistent. However, inclusion of 25-OH-D_3 as partial or sole source of dietary vitamin D_3, at a dose of 2,760 IU/kg in broilers diets has been previously shown increase meat yield (Yarger et al., 1995a; Saunders-Blades, 2008; Brito et al., 2010). In the current research, the MC group received 69 µg/kg of 25-OH-D_3 from d 1 to d 21 in addition to 4,000 IU of vitamin D_3, for a total of 6,760 IU/kg of vitamin D_3 activity. These results are in contrast to a recent report of a higher meat yield in broiler fed 69 µg/kg of 25-OH-D_3 in combination with 2,760 IU/kg of vitamin D_3 throughout the grow-out period in comparison to broilers fed 5,520 IU/kg vitamin D_3 only (Vignale et al., 2013). Other authors have also not found differences in meat yield due to dietary 25-OH-D_3 at

different levels of inclusion or in combination with vitamin D_3 (Angel et al., 2006; Michalczuk et al., 2010). However it is important to note that the evidence indicates that 25-OH-D_3 has a greater biopotency than vitamin D_3 when compared at low levels of inclusion (Fritts and Waldroup, 2003). This argument may be the reason for the lack of response in this trial in comparison to other studies that have reported higher meat yield when birds were fed 25-OH-D_3 at lower dietary levels than the current study (Yarger et al., 1995a: 2760 IU/kg; Saunders-Blades, 2008: 2760 IU/kg). For instance, Brito et al. (2010) reported a mean increment of 0.9% in carcass yield in favor of supplemental 25-OH-D_3 during three periods (d 1 to 21, d 22 to 38, and d 39 to 45) at different doses compared to vitamin D_3 at the same level of activity. Also in that study, the highest carcass yield was obtained with dietary inclusion of 1,500, 1,200, and 750 IU of 25-OH-D_3 per kg of feed at the three periods of inclusion respectively. The reduction in some carcass yields seen in the present study may indicate that dietary 25-OH-D_3 at 2.4 times of the level commonly used in the broiler industry (69 µg/kg; Roberson et al., 2005) negatively affects meat yield. That is in contrast to the reported level of safety of 25-OH-D_3 that some evidence of toxicity such as weight loss and mild renal calcification are seen only up to 10 times of a basal level of 69 µg/kg (2,760 IU/kg), (Yarger et al., 1995b).

The response to the combination of dietary MC from d 0 to d 21 and dietary MG in males from d 22 to processing age was different depending on bird strain. For instance, some carcass traits such as thigh weight and yield were increased in strain B males, but others such as weights and yields of breast filet and wing were reduced. There are no reports indicating that natural or synthetic carotenoid supplementation can increase or reduce meat yield. One main component of MC is CX; therefore, it would be interesting to determine if these interactions are between CX and the carotenoids present in MG, and if there is an additive or contrary effect between them on

carcass traits. Since MC is a new additive that has been mainly tested in broiler breeders, there are no reports about those interactions in broilers, further studies are needed to clarify these effects.

In the current study, the effect of dietary MG on meat yield depended on the bird strain. With the exception of breast filet yield that was reduced by 6.3% in strain B males, MG had a positive effect on carcass traits, especially in strain B. Just as there are different growth rates between broiler strains (Goliomytis et al., 2003; Abdullah et al., 2010), there may also be different metabolic responses to dietary carotenoids. Previous publications have concluded that neither synthetic nor natural pigment sources affect broiler meat yield (Pérez-Vendrell et al., 2001; Martínez-Peña et al., 2004; Muñoz-Díaz et al., 2012). Notwithstanding, the increase in carcass traits seen in this study may be related to the antioxidant effect of lutein, which is the most abundant carotenoid in MG (Hadden et al., 1999). Dietary supplementation of 200 mg of lutein per kg of feed from 4 to 42 d, increased BW of broilers in comparison to the control group without lutein (Rajput et al., 2012). In 50-d-old turkey poults injected with lipopolysaccharide, liver malondialdehyde concentration was decreased from 1,400 nmol/g to 700 nmol/g, and BW was not affected when fed 50 mg of lutein per kg of feed from 1 d of age, in comparison to birds not fed lutein (Shanmugasundaram and Selvaraj, 2011).

In the present study, dietary MG reduced male carcass WA relative to the males not fed MG (Table 3.6). This means that the higher values in weights and yields of carcass and parts that were seen in broilers fed MG did not depend on increased moisture retention after chilling, and in fact this situation represents a greater meat production when MG was included in broiler diets.

3.4.2 Percentage of Relative Weight of Organs

Dietary MC increased relative heart weight in strain A males. In a diet containing a total of 4,000 IU of vitamin activity, replacement of 2,760 IU of vitamin D with 25-OH-D_3 increased

heart weight relative to carcass weight, although the reason was not determined (Michalczuk et al., 2010). A greater heart size is related to higher growth rate (Bouyeh, 2012), and proportional heart growth in broilers is greater at earlier ages (Murawska et al., 2011). However, in this study, strain A had a lower growth rate that strain B at early ages (Chapter 2; Table 2.1). This finding is opposite to a previous report were no differences were found in heart relative weight between similar strains (Marcato et al., 2010). Brooding conditions and altitude can cause differences in heart size (Julian, 2000; Arce-Menocal et al., 2009; Özkan et al., 2010); however, in this trial both strains had similar management, housing, and environment.

Dietary MG increased carcass weight. Similar results have not been reported previously. The liver is considered the main metabolic organ of body, and different factors such as nutrition, toxins and diseases might interfere with its function (Marcato et al., 2010). The change in liver size may reflect changes in metabolic activity. Liver yield was reduced by dietary MC in strain A females; and dietary MG reduced liver yield in strain B and in birds not fed MC. This might suggest that MC and MG can influence the liver metabolic activity depending on bird sex and strain. However, more data such as liver enzymatic activity, RNA:DNA, RNA:protein, and protein:DNA in liver samples (Palo et al., 1995) would be useful to clarify this situation, but they were not evaluated in the present trial.

Relative spleen weight was increased in strain A when fed MC, but it was reduced when birds were fed with MC to approximately 21 d of age and MG thereafter. Spleen weight has been used for evaluation of immune status in chickens (Sandercock et al., 2009; Zhou et al., 2009). There were no difference in mortality between strains (Chapter 2, Table 2.6). Dietary MG increased mortality especially at the end of the grow-out period in birds fed MC (Chapter 2; Figure

2.6); however, spleen yield was reduced in strain A birds fed MC. It may suggest that immune status in these birds was not increased.

In mammals and birds, the gut is one of the most energy-demanding organs (Yason et al., 1987; Derting and Bogue, 1993; Moghaddam et al., 2012). Dietary MC increased intestine yield in strain B females. At d 7, broilers fed 25-OH-D_3 at 2,760 IU/kg and 1,380 IU/kg of feed in starter and grower diets had lighter small intestine weights than those of the control group fed vitamin D_3 at 3,000 IU/kg of feed ($P < 0.1$; Chou et al., 2009). These authors proposed that supplemental 25-OH-D_3 results in lighter small intestines and consequently may lower the dietary energy need of broilers.

Dietary MG reduced intestine yield of males independent of dietary MC. Lighter intestines can reduce dietary energy needs for gut maintenance, leaving more energy for other purposes (Moghaddam et al., 2012). Therefore, the lower intestine yield when males were fed MG could be the reason for increasing weights and yields of carcass, carcass parts, and fat pad ($P = 0.0616$) due to an enhanced availability of metabolizable energy.

Fat pad yield was higher in females than males since females accumulate fat faster and in higher quantities than males (Plavnik and Hurwitz, 1982; Zuidhof, 2005; Murawska et al., 2011). Dietary MC increased fat pad yield in strain A by 8.8 % (Figure 3.1). No differences in fat pad yield were found replacing 1,500 and 2,760 IU of a total of 4,000 IU vitamin D_3 /kg of feed with 25-OH-D_3 during the grow-out period (Michalczuk et al., 2010). Abdominal fat is considered as waste in the poultry industry (Zhou et al., 2009). Further studies are needed to clarify this undesirable effect on strain A, and to verify if this effect was due to CX or 25-OH-D_3, or the combination of the two additives.

3.4.3 Skin colour

Dietary MC influenced skin carcass colour depending on bird sex and strain. Females are more responsive in skin yellowness when fed carotenoids than males (Sirri et al., 2010), which is related to a greater ability to accumulate sub-cutaneous fat (Murawska et al., 2011). Moreover, there are differences in ability to deposit carotenoid pigments among broiler strains, which are related to a different genetic capacity for carotenoid deposition, feed intake and fat deposition (Fletcher, 2002; Santiago et al., 2005; Sirri et al., 2010). The effects of MC on skin pigmentation were probably related to the presence of CX since there is no evidence that 25-OH-D_3 affects skin pigmentation. The effectiveness of CX for pigmenting skin of broilers has been demonstrated in the past (Couch et al., 1971; Janky and Harms, 1983; Pérez-Vendrell et al., 2001; Muñoz-Díaz et al., 2012). However, a residual effect of CX after three weeks without dietary supplementation is unlikely, since the degree of pigmentation is reduced over time after carotenoid supplementation is suspended. When broilers are pigmented for commercial purposes, carotenoids have to be included in the diet for 2.5 to 3 weeks before slaughtering because in that period feed intake is higher than early ages, which results in a greater pigment deposition (Pérez-Vendrell et al., 2001; Castañeda et al., 2005; Muñoz-Díaz et al., 2012).

Lutein and zeaxanthin are yellow xanthophylls, and are the main carotenoids presents in MG (Hadden et al., 1999; Pérez-Vendrell et al., 2001). As expected, dietary MG increased yellowness, but reduced skin lightness and redness, in agreement with previous reports (Pérez-Vendrell et al., 2001; Castañeda et al., 2005; Rajput et al., 2012). In the current study, redness and yellowness were negatively correlated ($r = -0.6946$; < 0.0001). The increased redness and reduced yellowness when birds were fed MC and then MG was probably because CX is a red pigment.

3.4.4 Bone characteristics

Differences between sexes in bone characteristics have been reported in the past in broilers (Shaw et al., 2010; Yalçin et al., 2001), due to differences in size, growth rate, sexl hormone levels (Rath et al., 2000), and allometric development (Murawska et al., 2011). Differences in growth rate were also found between sexes in this study (Chapter 2; Table 2.2).

Bone breaking stress is the force required to break the bone accounting the cross-sectional area (g/mm^2 or kg/cm^2; Patterson et al., 1986). To calculate this parameter, the transversal area ($A=\pi r^2$) of each shank was determined taking into account the two outside diameters at the midpoint (perpendicular and parallel to the direction of the applied force). This evaluation accounts for the variation in diameters of bones among birds, and is more specific for evaluations where birds are processed at different ages, and bones may have different diameters (Patterson et al., 1986; Rath et al., 2000). Supplemental MC from d 0 to d 21 increased bone breaking stress regardless of strain and sex, and also increased bone breaking strength mainly in strain B. There is no evidence in the literature that carotenoids affect bone strength. In contrast, vitamin D_3 and its metabolites are clearly involved in mineralization and bone formation (Rama-Rao et al., 2006; 2009; Khan et al., 2010); therefore, the differences detected in these traits were likely due to the effect of 25-OH-D_3 in MC. At 42 d femur breaking strength was increased by 18.5 % and 17.5 % when broilers were fed 2,760 IU/kg of 25-OH-D_3 during the entire grow-out period or from 0 to 28 d, respectively, in comparison to dietary vitamin D_3 at the same level of inclusion (Saunders-Blades, 2008). The greater biopotency of 25-OH-D_3 than vitamin D_3 was more noticeable at low levels of inclusion (Fritts and Waldroup, 2003). In addition, dietary 25-OH-D_3 was more effective in increasing bone ash and reducing severity of TD when Ca and P were restricted in the diet (Bar et al., 2003; Ledwaba and Roberson, 2003). Nonetheless, in this study bone traits were positively

influenced with a high level of inclusion of 6,270 IU/kg of 25-OH-D_3 in comparison to 4,000 IU/kg of dietary vitamin D_3, and Ca and P levels were not restricted.

When strain B birds were fed MG but not MC, bone breaking strength was reduced; however the same trait was increased when birds were fed MC and then MG. This interaction is likely related to the presence of 25-OH-D_3 in MC. However, it can also indicate an additive effect between carotenoids presents in MC (CX) and in MG (lutein and zeaxanthin) through an increased antioxidant status or a higher osteoblast differentiation that facilitates bone formation. In vitro, retinol and carotenoids (including CX) stimulated differentiation of mouse osteoblastic cells (Park et al., 1997). Lin-Peng et al., (2009) reported that CX increased bone quality and reduced osteoporosis of ovariectomized rats. Supplemental MC has been mainly tested in broiler breeders; therefore, there are no reports about those interactions in broilers, and further studies are needed to clarify these effects.

3.4.5 Conclusion

Overall, strain A had greater meat yield than strain B and also higher feed efficiency at processing age (Chapter 2; Table 2.5). Early MC inclusion in broiler diets increased bone quality, likely due to the presence of 25-OH-D_3 in MC. The inclusion of MC has the potential to influence carcass parts yield, and organ weights at processing depending on bird sex, and broiler strain. MC reduced most of the carcass traits especially in strain A. Further studies are needed to clarify these effects comparing dietary MC at lower and similar levels of vitamin D_3 activity, having a partial replacement of vitamin D_3 activity with MC, or including MC in broiler diets throughout the entire production period. In this trial, CX and 25-OH-D_3 were not fed separately, so it was not possible to identify a synergy between canthaxanthin and 25-OH-D_3 in broiler performance or carcass traits; however those components were assessed separately in Chapter 5. A total vitamin D_3 activity of

6,760 IU/kg may reduce meat yield. Although there was not an independent effect of MC on size of visceral organs and skin colour, the effect of early dietary MC supplementation on visceral organs weights and skin pigmentation depended on bird sex, breed, and late dietary MG inclusion.

Dietary MG supplementation in the grower and finisher diets increased meat yield, probably through a higher antioxidant status or an enhanced availability of metabolizable energy; however, dietary MG increased FCR (Chapter 2; Table 2.5). The effect of dietary MG on meat yield and performance may be dependent on MC dietary inclusion at early ages and broiler strain.

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3.6 TABLES

Table 3.1 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on BW and absolute weight of carcass traits at processing age (PA)[3]. Dependent variables adjusted to a common heat stress index (HSI)[4] and a PA with analysis of covariance.

Effect			BW	Carcass	Breast[5]	Breast filet[6]	Thighs	Drums	Wings
		n[7]	g						
S	Female	1609	2,287[b]	1,606[b]	637.8[b]	360.6[b]	305.2[b]	225.4[b]	185.3[b]
	Male	893	2,709[a]	1,884[a]	735.5[a]	409.1[a]	351.7[a]	276.8[a]	216.8[a]
	Pooled SEM		41.1	32.0	15.8	10.6	15.4	10.9	7.0
ST	A	1143	2,515	1,771[a]	708.5[a]	405.6[a]	336.9[a]	249.8	200.8
	B	1359	2,481	1,718[b]	664.8[b]	364.1[b]	320.1[b]	252.3	201.3
	Pooled SEM		41.8	33.6	16.0	11.2	15.4	10.6	7.4
MC	0	674	2,515	1,761	690.0	390.2	332.8	254.7	203.3
	250	685	2,480	1,728	683.4	379.5	324.2	247.4	198.7
	Pooled SEM		41.6	33.6	16.1	11.3	15.7	10.7	7.1
MG	0	451	2,900[b]	2,045[b]	799.2[b]	471.0	370.1[b]	300.0	233.8
	100	569	2,946[a]	2,092[a]	819.6[a]	465.0	391.1[a]	308.9	236.3
	Pooled SEM		76.2	53.9	26.8	19.3	14.3	236.3	10.4
Covariates	HSI [g/(°C x d)]		61.24	49.54	18.91	13.32	7.32	6.39	4.29
	PA (g/d)		-0.0001	-0.001	-0.041	0.031	-0.081	-0.006	0.023
Sources of variation			P-values						
	HSI		0.9992	0.9913	0.4422	0.4537	0.0113	0.7803	0.1699
	PA		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	S		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	ST		0.2135	0.0186	0.0005	< 0.0001	0.0077	0.6790	0.9242
	MC		0.1126	0.0622	0.4712	0.1681	0.1729	0.0743	0.1672
	MG		0.0202	0.0003	0.0018	0.2156	< 0.0001	0.2744	0.1659
	S x ST		NS	*	*	NS	*	NS	*
	S x MC		NS	NS	NS	NS	NS	**	NS
	ST x MC		NS	NS	NS	NS	NS	***	NS
	ST x MG		NS	*	NS	**	*	*	*
	MC x MG		NS	NS	NS	*	*	NS	**
	S x ST x MC		*	*	*	NS	**	***	*
	ST x MC x MG		NS	NS	NS	NS	*	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[3]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[4]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[5]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[6]Breast filet: Pectoralis major.

[7]Bird as experimental unit.

Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant: P > 0.05.

Table 3.2 Significant and nearly significant interaction effects between MaxiChick® (MC)[1], sex (S), and strain (ST) on carcass traits and bone characteristics at processing age (PA)[2]. Dependent variables adjusted to a common heat stress index (HSI)[3] and a common PA with analysis of covariance.

ST	A				B				Pooled SEM	P-values
S	Female		Male		Female		Male			
MC	0	250	0	250	0	250	0	250		
BW (g)	2300^{c}	2298^{c}	2768^{a}	2692^{b}	2295^{c}	2254^{c}	2698^{ab}	2677^{b}	45.1	0.0161
Carcass (g)	1626^{c}	1618^{c}	1953^{a}	1888^{b}	1607^{cd}	1571^{d}	1857^{b}	1837^{b}	36.1	0.0154
Breast[4] (g)	655^{c}	651^{c}	776^{a}	757^{a}	626^{d}	626^{d}	704^{b}	710^{b}	17.4	0.0392
Thighs (g)	318	305	367	357	299	299	347	335	16.0	0.0578
Thighs[5] (%)	19.3^{a}	18.4^{cd}	18.8^{bc}	18.6^{bcd}	18.1^{de}	18.7^{abc}	18.0^{de}	18.1^{de}	0.63	0.0008
Drums (g)	229^{d}	219^{d}	290^{a}	262^{c}	227^{d}	227^{d}	274^{bc}	282^{ab}	11.2	< 0.0001
Drums[5] (%)	14.2^{c}	13.6^{e}	14.9^{ab}	13.9^{de}	14.2^{c}	14.6^{bc}	14.7^{b}	15.4^{a}	0.69	0.0008
Wings (g)	183^{bc}	183^{bc}	222^{a}	215^{a}	192^{b}	182^{c}	216^{a}	213^{a}	7.7	0.0036
Wings[5] (%)	11.6	11.6	11.7	11.6	12.2	12.0	11.8	11.8	0.55	0.0750
Water Absorption[6] (%)	9.9^{a}	9.9^{a}	6.7^{c}	8.1^{b}	10.2^{a}	10.1^{a}	7.3^{bc}	7.1^{bc}	0.44	0.0075
Heart[5] (%)	0.844^{bcd}	0.830^{d}	0.836^{cd}	0.883^{ab}	0.832^{cd}	0.868^{bc}	0.916^{a}	0.889^{ab}	0.040	< 0.0001
Liver[5] (%)	3.04^{de}	2.95^{e}	2.98^{de}	3.07^{cd}	3.16^{bc}	3.20^{ab}	3.30^{a}	3.22^{ab}	0.065	0.0002
Spleen[5] (%)	0.157^{c}	0.172^{ab}	0.152^{c}	0.179^{ab}	0.169^{bc}	0.172^{ab}	0.184^{a}	0.171^{ab}	0.012	0.0074
Intestine[5] (%)	6.54^{cd}	6.20^{d}	6.67^{bc}	6.67^{bc}	7.09^{b}	7.49^{a}	7.80^{a}	7.60^{a}	0.416	< 0.0001
Skin redness[7] (a*)	2.66^{bc}	2.45^{c}	3.17^{a}	2.69^{bc}	2.35^{c}	2.24^{c}	2.32^{c}	3.12^{ab}	0.21	< 0.0001
Skin yellowness[7] (b*)	5.21^{bc}	6.23^{a}	4.47^{c}	6.38^{a}	5.30^{bc}	5.05^{bc}	4.79^{c}	3.09^{d}	1.01	< 0.0001
Bone breaking strength[8] (KgF)	20.1^{cde}	21.3^{cde}	25.6^{a}	22.3^{bcd}	19.0^{e}	22.9^{abc}	20.0^{de}	24.8^{ab}	1.4	0.0267

[a-e]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[3]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Breast: Pectoralis major, p. minor, bone and skin included.

[5]Yield as the percent of eviscerated carcass weight per bird.

[6]Percent of increase in the carcass weight by moisture retention after pre-chilling and chilling.

[7]Measurements were taken after chilling on the right medial breast surface.

[8]Measurements taken from right tarsometatarsus (shank) with flesh and skin intact.

Table 3.3 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on carcass yield[3], carcass parts yield[4] and carcass water absorption (WA)[5] at processing age (PA)[6]. Dependent variables adjusted to a common heat stress index (HSI)[7] and a common PA with analysis of covariance.

Effect			Carcass	Breast[8]	Breast Filet[9]	Thighs	Drums	Wings	WA
		n[10]	% of Live BW	% of Eviscerated Carcass Weight					— (%) —
S	Female	1609	69.6[a]	39.8[a]	23.0[a]	18.6	14.2[b]	11.8	10.0[a]
	Male	893	69.0[b]	38.9[b]	22.1[b]	18.4	14.7[a]	11.7	7.3[b]
	Pooled SEM		0.4	0.3	0.7	0.6	0.7	0.5	0.3
ST	A	1143	69.9[a]	40.0[a]	23.4[a]	18.8[a]	14.2	11.6	8.6
	B	1359	68.7[b]	38.7[b]	21.8[b]	18.2[b]	14.7	12.0	8.7
	Pooled SEM		0.4	0.4	0.7	0.6	0.7	0.5	0.3
MC	0	674	69.4	39.2	22.7	18.5	14.5	11.8	8.5
	250	685	69.2	39.5	22.5	18.5	14.4	11.7	8.8
	Pooled SEM		0.4	0.3	0.7	0.6	0.7	0.5	0.3
MG	0	451	70.6[b]	38.8	22.9[a]	18.1[b]	14.8[b]	11.6	6.9[a]
	100	569	71.2[a]	39.0	22.0[b]	18.8[a]	15.0[a]	11.5	6.5[b]
	Pooled SEM		0.8	0.5	0.8	0.3	0.9	0.2	0.5
Covariates	HSI [%/(°C x d)]		0.003	- 0.002	0.001	-0.089	0.001	0.002	-0.0001
	PA (%/d)		0.0002	0.019	0.013	-0.006	-0.048	-0.1	-0.001
Sources of variation			P-values						
	HSI		0.8382	0.2029	0.5312	< 0.0001	0.5245	0.0284	0.9328
	PA		< 0.0001	0.6448	0.0055	0.0120	0.0447	< 0.0001	0.0085
	S		< 0.0001	< 0.0001	< 0.0001	0.1075	< 0.0001	0.2692	< 0.0001
	ST		< 0.0001	0.0028	0.0041	0.0246	0.1547	0.1129	0.9368
	MC		0.4354	0.2890	0.5156	0.8574	0.5739	0.6930	0.4955
	MG		< 0.0001	0.4077	0.0001	< 0.0001	0.0330	0.2069	0.0257
	S x ST		*	NS	*	NS	NS	*	NS
	S x MC		NS	NS	NS	NS	NS	NS	*
	ST x MC		NS	NS	*	*	***	NS	NS
	ST x MG		*	NS	*	*	NS	NS	NS
	MC x MG		NS	NS	*	***	NS	*	NS
	S x ST x MC		NS	NS	NS	*	*	**	*
	ST x MC x MG		NS	NS	NS	***	NS	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]Yield as the percent of live BW per bird.

[2]Yield as the percent of eviscerated carcass weight per bird.

[3]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[4]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[5]Water absorption: Percent of increase in the carcass weight by moisture retention after pre-chilling and chilling

[6]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[7]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[8]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[9]Breast filet: Pectoralis major.

[10]Bird as experimental unit.

Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant: P > 0.05.

Table 3.4 Significant and nearly significant interaction effects between sex (S) and strain (ST) on carcass yield[1], carcass parts yield[2] at processing age (PA)[3] Dependent variables adjusted to a common heat stress index (HSI)[3] and a common PA with analysis of covariance.

ST	A		B		Pooled SEM	P-values
S	Female	Male	Female	Male		
Carcass (%)	70.1[a]	69.6[b]	69.2[c]	68.3[d]	0.4	0.0096
Breast filet (%)	24.0[a]	22.8[b]	22.1[b]	21.4[c]	0.7	0.0413
Wing (%)	11.5[b]	11.6[ab]	12.1[a]	11.8[ab]	0.5	0.0002
Fat Pad (%)	2.45	2.04	2.56	2.04	0.094	0.0593

[a-d]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]Yield as the percent of live BW per bird.

[2]Yield as the percent of eviscerated carcass weight per bird.

[3]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[4]Heat stress index = [daily maximum house temperature − daily recommended temperature (ºC)]/ processing age – 21 (d).

Table 3.5 Significant interaction effects between dietary marigold extract (MG)[1] and strain (ST) on carcass traits at processing age (PA)[2]. Dependent variables adjusted to a common heat stress index (HSI)[2] and a common PA with analysis of covariance.

	ST	A		B		Pooled SEM	P-values
	MG	0	1.0	0	1.0		
Carcass (g)		2,086[a]	2,107[a]	2,004[b]	2,077[a]	56.2	0.0372
Carcass[4] (%)		71.5[b]	71.8[a]	69.7[d]	70.7[c]	0.8	0.0027
Breast filet[5,6] (%)		23.1[a]	23.1[a]	22.4[a]	21.0[b]	1.2	0.0002
Drums (g)		301[b]	303[ab]	299[b]	315[a]	26.1	0.0131
Wings (g)		239[a]	236[a]	228[b]	236[a]	10.6	0.0035
Heart[6] (%)		0.775[b]	0.764[b]	0.845[a]	0.794[b]	0.051	0.0296
Liver[6] (%)		2.75[c]	2.80[c]	3.07[a]	2.95[b]	0.127	0.0011

[a-d]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[2]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[3]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[4]Yield as the percent of live BW per bird.

[5]Breast filet: Pectoralis major.

[6]Yield as the percent of eviscerated carcass weight per bird.

Table 3.6 Significant interaction effects between dietary MaxiChick® (MC)[1] and dietary marigold extract (MG)[2] on carcass traits at processing age (PA)[3]. Dependent variables adjusted to a common heat stress index (HSI)[4] and a common PA with analysis of covariance.

	MC	0		250		Pooled SEM	P-values
	MG	0	1.0	0	1.0		
Breast filet[5] (g)		470[a]	475[a]	472[a]	455[b]	20.3	0.0128
Breast filet[5,6] (%)		22.5[ab]	22.4[ab]	22.9[a]	21.6[b]	1.2	0.0003
Wings (g)		232	244	233	233	10.7	0.0780
Wings[6] (%)		11.6[ab]	11.6[ab]	11.7[a]	11.4[b]	0.2	0.0222
Liver[6] (%)		2.91[a]	2.81[b]	2.91[ab]	2.94[b]	0.11	0.0199
Intestine[6] (%)		6.28[ab]	5.71[c]	6.35[a]	6.07[bc]	0.46	0.0303

[a,b,c]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[3]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[4]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[5]Breast filet: Pectoralis major.

[6]Yield as the percent of eviscerated carcass weight per bird.

Table 3.7 Significant interaction effects between strain (ST), dietary MaxiChick® (MC)[1] and dietary marigold extract (MG)[2] on thigh weights and yield[3], spleen yield skin colour[4], and bone breaking strength[5] at processing age (PA)[6]. Dependent variables adjusted to a common heat stress index (HSI)[7] and a common PA with analysis of covariance.

ST	A				B				Pooled SEM	P-values
MC	0		250		0		250			
MG	0	1.0	0	1.0	0	1.0	0	1.0		
Thigh (g)	377 ^{b}	393^{a}	380^{ab}	390^{ab}	379^{ab}	390^{ab}	344^{c}	394^{a}	15.6	0.0024
Thigh[3] (%)	18.1^{c}	18.6^{ab}	18.3^{bc}	18.7^{abc}	18.7^{abc}	18.7^{abc}	17.3^{d}	19.2^{a}	0.41	< 0.0001
Spleen[3] (%)	0.132^{c}	0.132^{c}	0.175^{a}	0.160^{b}	0.185^{a}	0.169^{ab}	0.148^{bc}	0.164^{ab}	0.027	0.0031
Skin redness[4] (a*)	3.20^{ab}	0.24^{d}	3.30^{ab}	1.07^{c}	2.39^{b}	0.11^{cd}	3.46^{c}	0.01^{d}	0.89	< 0.0001
Skin yellowness[4] (b*)	1.69^{c}	21.26^{a}	2.33^{c}	16.64^{b}	3.97^{c}	20.87^{ab}	0.99^{c}	21.03^{ab}	4.38	< 0.0001
Bone breaking strength[5] (KgF)	25.9^{a}	22.0^{b}	24.3^{ab}	25.8^{a}	21.4^{b}	24.0^{ab}	24.7^{ab}	26.7^{a}	1.3	0.0473

[a,-d]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg. Supplier: DSM Nutritional Products Colombia.

[3]Yield as the percent of eviscerated carcass weight per bird.

[4]Measurements were taken on the right medial breast surface after chilling.

[5]Measurements taken from right tarsometatarsus (shank) with flesh and skin intact.

[6]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[7]Heat stress index = [daily maximum house temperature − daily recommended temperature (^{o}C)]/ processing age – 21 (d).

Table 3.8 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on relative weight of organs[3] at processing age (PA)[4]. Dependent variables adjusted to a common heat stress index (HSI)[5] and a common PA with analysis of covariance.

Effect			Heart	Liver	Spleen	Intestine	Fat pad[6]
		n[7]	% of Eviscerated Carcass Weight				
S	Female	1609	0.85[b]	3.09	0.17	6.83[b]	2.51[a]
	Male	893	0.89[a]	3.14	0.17	7.18[a]	2.04[b]
	Pooled SEM		0.01	0.06	0.01	0.4	0.09
ST	A	1143	0.85[b]	3.01[b]	0.16	6.52[b]	2.25
	B	1359	0.88[a]	3.22[a]	0.17	7.49[a]	2.30
	Pooled SEM		0.01	0.06	0.01	0.4	0.09
MC	0	674	0.86	3.12	0.17	7.03	2.25
	250	685	0.87	3.11	0.17	6.99	2.30
	Pooled SEM		0.01	0.06	0.01	0.4	0.09
MG	0	451	0.81[a]	2.91	0.16	6.32[a]	2.07
	100	569	0.76[b]	2.87	0.16	5.89[b]	2.13
	Pooled SEM		0.02	0.11	0.02	0.5	0.14
Covariates	HSI [%/(°C x d)]		-0.0001	0.0002	-0.0004	-0.0001	0.0001
	PA (%/d)		-0.005	-0.059	-0.001	-0.178	0.024
Sources of variation			P-values				
	HSI		0.3465	0.2903	0.3236	0.3084	0.1002
	PA		0.0525	< 0.0001	0.2718	< 0.0001	0.0099
	S		0.0014	0.0969	0.3515	< 0.0001	< 0.0001
	ST		0.0080	0.0001	0.2964	0.0002	0.2198
	MC		0.4186	0.8305	0.2930	0.8358	0.2673
	MG		0.0011	0.1184	0.3211	< 0.0001	0.0616
	S x ST		*	NS	NS	NS	**
	S x MC		NS	NS	NS	NS	NS
	ST x MC		NS	NS	*	NS	*
	ST x MG		*	NS	NS	NS	**
	MC x MG		NS	*	NS	*	NS
	S x ST x MC		***	*	*	***	NS
	ST x MC x MG		NS	NS	*	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[3]Calculated as the percent of eviscerated carcass weight per bird.

[4]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[5]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[6]Fat pad: abdominal fat excluding gizzard fat.

[7]Bird as experimental unit.

Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant: P > 0.05.

Table 3.9 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on skin colour[3] at processing age (PA)[4]. Dependent variables adjusted to a common heat stress index (HSI)[5] and a common PA with analysis of covariance.

Effect			Lightness	Redness	Yellowness
		n[6]	L	a*	b*
S	Female	1609	73.17[b]	2.42[b]	6.45[a]
	Male	893	73.88[a]	2.82[a]	4.53[b]
	Pooled SEM		0.34	0.16	0.96
ST	A	1143	73.17	2.74	6.17[a]
	B	1359	73.48	2.50	4.16[b]
	Pooled SEM		0.35	0.16	0.98
MC	0	674	73.45	2.62	4.94
	250	685	73.20	2.63	5.19
	Pooled SEM		0.35	0.16	0.98
MG	0	451	73.32[a]	3.09[a]	2.24[b]
	100	569	71.32[b]	0.35[b]	19.95[a]
	Pooled SEM		0.39	0.85	3.91
Covariates	HSI [colour unit/(°C x d)]		-0.002	-0.0001	0.005
	PA (colour unit/d)		0.038	-0.109	0.099
Sources of variation			P-values		
	HSI		0.2276	0.6163	0.0236
	PA		0.2825	< 0.0001	0.0208
	S		0.0446	0.0009	< 0.0001
	ST		0.2811	0.1602	0.0289
	MC		0.2992	0.9847	0.5692
	MG		< 0.0001	< 0.0001	< 0.0001
	S x ST		NS	NS	< 0.0001
	S x MC		***	*	NS
	ST x MC		NS	*	***
	ST x MG		NS	NS	*
	MC x MG		NS	NS	NS
	S x ST x MC		NS	***	***
	ST x MC x MG		**	***	***

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[3]Measurements were taken on the right medial breast surface after chilling.

[4]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[5]Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d).

[6]Bird as experimental unit.

Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant: P > 0.05.

Table 3.10 Main effects of sex (S), strain (ST), MaxiChick® (MC)[1], and marigold extract (MG)[2] on bone breaking strength[3] and bone breaking stress[3] at processing age (PA)[4]. Dependent variables adjusted to a common heat stress index (HSI)[5] and a common PA with analysis of covariance.

Effect			Bone breaking strength	Bone breaking stress
		n[6]	KgF	g/mm^2
S	Female	360	21.1[b]	229.8[b]
	Male	209	23.2[a]	254.2[a]
	Pooled SEM		1.2	12.7
ST	A	271	22.5	246.1
	B	298	21.7	238.0
	Pooled SEM		1.2	12.6
MC	0	287	21.4	231.8[b]
	250	282	22.8	257.2[a]
	Pooled SEM		1.2	12.6
MG	0	209	24.1	247.5
	100	258	24.6	235.1
	Pooled SEM		0.75	7.57
	HSI [KgF/(ºC x d)]		-0.0001	-0.017
	PA (g/mm^2/d)		0.440	-0.189
Sources of variation			P-values	
	HSI		0.8619	0.7896
	PA		0.0118	0.9252
	S		0.0118	0.0097
	ST		0.3836	0.4033
	MC		0.0768	0.0493
	MG		0.4783	0.0856
	S x ST		NS	NS
	S x MC		NS	NS
	ST x MC		***	NS
	ST x MG		NS	NS
	MC x MG		NS	NS
	S x ST x MC		*	NS
	ST x MC x MG		*	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]MC: 0 or 250 g/tonne, containing 69 µg of 25-OH-D_3 and 6 mg of CX per kg of complete feed. DSM Nutritional Products Colombia, Tocancipá, Cundinamarca, Colombia.

[2]MG: 0 or 1.0 kg/tonne. Supplemented in 53% of males of both strains. Nutrezcol Colombia, Bogotá D.C., Colombia.

[3]Measurements were taken from right tarsometatarsus (shank) with flesh and skin intact.

[4]The overall average PA was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[5]Heat stress index = [daily maximum house temperature − daily recommended temperature (ºC)]/ processing age – 21 (d).

[6]Bird as experimental unit.

Probabilities of interactions: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

3.7 FIGURES

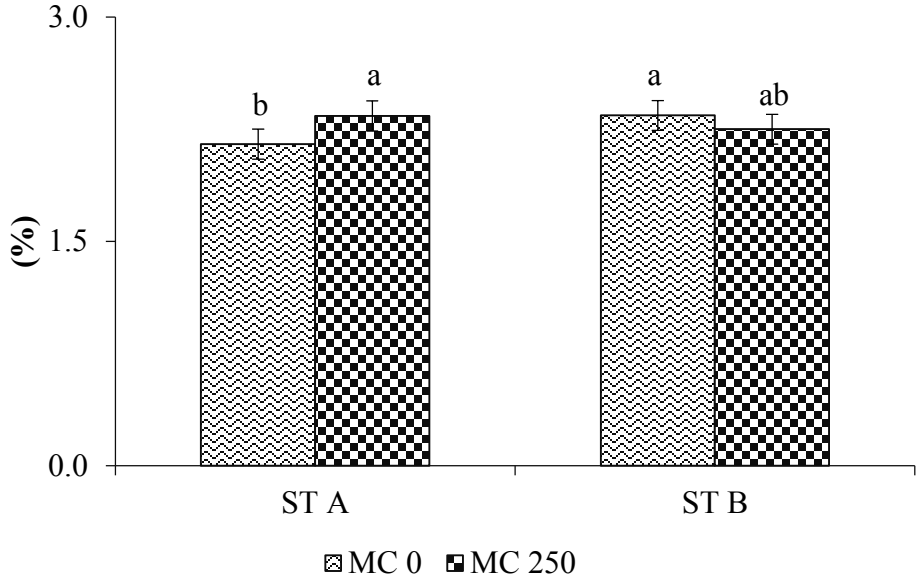

Figure 3.1. MaxiChick® (MC) x strain (ST) interaction for fat pad relative weight adjusted to a common heat stress index and a common processing age with analysis of covariance (P = 0.0005). Broilers of ST A and B were fed with one of two levels of dietary MC (0 or 250 g/tonne) in pre-starter and starter diets (from 0 to approximately d 21). Heat stress index = [daily maximum house temperature − daily recommended temperature (°C)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

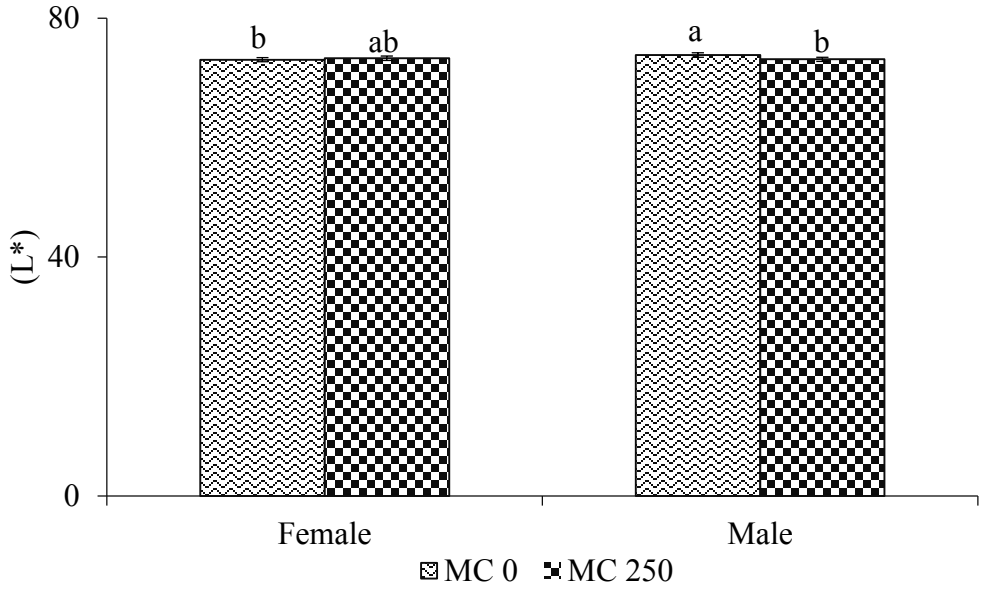

Figure 3.2. MaxiChick® (MC) x sex interaction for carcass skin lightness adjusted to a common heat stress index and a common processing age with analysis of covariance (P = < 0.0001). Broilers were fed with one of two levels of dietary MC (0 or 250 g/tonne) in pre-starter and starter diets (from 0 to approximately d 21). Heat stress index = [daily maximum house temperature − daily recommended temperature (ºC)]/ processing age – 21 (d). The overall average age at processing was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

4. EFFECT OF GEOGRAPHICAL REGION AND HOUSING ON ENVIRONMENTAL TEMPERATURE, PRODUCTIVITY, YIELD, AND BONE QUALITY OF BROILERS IN COMMERCIAL PRODUCTION CONDITIONS IN COLOMBIA

ABSTRACT

This study was conducted to determine regional and housing effects on house ambient temperature, performance, meat yield and bone characteristics of broiler chickens. A total of 4,922,130 broilers of two commercial broiler strains, reared sex-separately were followed from placement to processing in each of 372 houses located in 58 farms in Colombia. Broiler chicks were randomly allocated to farms in two geographical regions: Coffee region (CR; n = 191), and Valle region (VR; n = 181). There were 4 house types: Open-sided (OS; n = 43), open-sided with fans (OF; n = 290), tunnel-ventilated with foggers (TVF; n = 7), and tunnel-ventilated with cooling pad (TVP; n = 32). Houses had two types of floor: Concrete (CF; n = 175), or dirt (DF; n = 195). There were 274 single-storey houses (OL) and 53 two-storey houses. Birds were placed in the first level (FL) of all of the two-storey houses (n = 53), and in the second level (SL) of 45 two-storey houses. Processing age and stocking density were included as covariates. VR had higher and more stable ambient temperature than CR. VR birds had a higher feed intake, FCR and abdominal fat, but lower carcass yield of females, and carcass water absorption than CR birds. OS had higher ambient temperature than OF houses. Tunnel-ventilated houses had a more stable ambient temperature than OS and OF. TVF increased BW, and increased carcass weight and yield in males relative to other houses. Bone breaking strength was higher in birds from tunnel-ventilated houses than open-sided. Houses with CF had higher and more stable ambient temperatures than DF. CF increased BW but reduced mortality in comparison to DF. Birds in FL had lower BW and mortality than OL and SL. Carcass traits had a tendency to be lower in SL than OL and FL ($P < 0.1$). Most

differences in broiler traits were related to differences in ambient temperature. Stable environmental temperatures close to the thermoneutral zone may increase broiler performance, meat yield and bone strength through a lower energy expenditure and lower panting.

Key words: broiler chicken, Colombia, environmental temperature, broiler house, performance, bones quality.

4.1 INTRODUCTION

Housing conditions must provide a suitable environment that allows broilers to express their genetic potential for rapid growth and meat yield (Reece and Lott, 1982; Feddes et al., 2002; Cangar et al., 2008). Poultry houses vary around the world according to environmental conditions, bird type, processing age, handling systems, and cost of investment (Miragliotta et al., 2006; Tirawattanawanich et al., 2011).

Ambient temperature in a broiler house is the result of several factors such as heat production by the bird, floor temperature, floor material, litter material, heating of litter by micro-biological production, moisture loss from litter, natural convection around birds, stocking density, stratification and radiation and environmental temperature (VanBeek and Beeking, 1995; Reiter and Bessei, 2000). Environmental conditions differ between geographical regions due to deviations in altitude, winds, humidity, seasons and vegetation (Bernal et al., 1989; Gates et al., 1995). Stratification and radiation are influenced by outside temperature, house construction material, solar radiation, and air circulation (VanBeek and Beeking, 1995). Ascites in broilers can be caused by housing at high altitude above sea level due to the low partial pressure of oxygen that causes hypoxemia (Arce-Menocal et al., 2009).

Due to genetic selection for rapid growth of broiler chickens, unwanted outcomes such as skeletal disorders (Shim et al., 2012), and increased susceptibility to heat stress (Deeb and

Cahaner, 2001) have emerged. Heat stress reduces livability, weight gain and meat yield (May et al., 1998; Olanrewaju et al., 2010). Optimal ventilation reduce heat stress (Yahav et al., 2004; 2008; Feddes et al., 2003). Conventional poultry houses are being replaced by artificial-ventilated poultry houses because broiler performance is increased (Lacy and Czarick, 1992). Artificial ventilation provides fresh air, and removes heat, moisture, and gases added to the micro environment by the birds and the litter (Weaver and Meijerhof, 1991; Bucklin et al., 2009). Increasing air velocity through artificial ventilation systems improves broiler performance and allows a better convection heat exchange between birds and the air, which reduces heat stress (Lott et al., 1998; Yahav et al., 2001). As a result, artificial ventilation systems such as tunnel ventilation with cooling pads, and forced ventilation with fogging systems are becoming more common in broiler operations. In some developing countries not all broiler producers can implement these systems due to high cost (Tirawattanawanich et al., 2011). For example, in Colombia, the majority of poultry houses are open-sided and naturally ventilated without climate control systems. There are no published comparisons of artificially ventilated and naturally-ventilated, open-sided housing systems.

There is a lack of studies on the effect of floor type on broiler performance and house thermal environment. Concrete floor (CF) has been considered as the most effective floor type for poultry houses because it allows better disinfection between flocks (Payne et al., 2005), facilitates handling and improves comfort (Abreu et al., 2011). Live performance was not influenced by floor type, but total mortality was higher in broilers raised on dirt floor (DF; 2.6 %) than on CF (1.5 %; Abreu et al., 2011). Nevertheless, DF are used to reduce building cost.

Producers increase stocking density in order to maximize meat production within the house (Feddes et al., 2002; Verspecht et al., 2011). In an environmentally controlled house (cool cells) a

density of 17 and 19 bird/m^2 for males and females respectively, increased net profit compared to a stocking density of 10 to 15 bird/m^2 (Puron et al., 1995). However, increased stocking densities may reduce BW and feed intake (Simsek et al., 2011), increase FCR (Dozier et al., 2005; Guardia et al., 2011; Tong et al., 2012), reduce bone quality and development (Simsek et al., 2011; Buijs et al., 2012), gait scores and welfare (Thomas et al., 2004; Sun et al., 2013). To reduce fixed costs and increase efficiency per area of land, two-storey broiler houses are becoming more common in Colombia. There are no scientific comparisons between these housing systems and conventional poultry houses. Thus, broiler performance can be influenced depending where the poultry operations are located, building type, and by the housing conditions (Miragliotta et al., 2006). The aim of this study was to determinate if house type, house floor type, and house floor level influence broiler performance, meat yield, internal organ yield, skin colour and bone strength of broilers in commercial conditions of production in Colombia. It was hypothesized that tunnel-ventilated houses, CF, houses at lower altitude, and houses of one level would enhance production traits, livability, and bone characteristics of broilers.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design.

To assess the effect of geographic region, house type, floor type, and floor level on ambient temperature, broiler performance, meat yield, internal organ yield, bone strength and skin colour, a whole broiler cycle was followed in a commercial boiler integration in Colombia (Avidesa, MacPollo de Occidente). This production cycle included 4,922,130 broilers of both sexes (reared separately) that were placed in 58 farms (n = 372 houses). The experiment was conducted as a completely randomized design. This analysis is part of the a field study where the effects and interactions on broiler performance and processing traits of two broiler strains (A, B), two sexes,

two dietary MaxiChick® levels (0 and 250g/tonne), and two levels of inclusion of Marigold extract (0 and 1.0 kg/tonne) were analyzed as an incomplete factorial design (Chapter 3; Chapter 3).

Farms were situated in two geographical regions: Coffee region, which is located in the departments of Quindío, Caldas, Risaralda and Valle del Cauca, Colombia (CR; n = 191), and Valle region, located in the department of Valle del Cauca, Colombia (VR; n = 181). In each region, broilers were placed in four house types: Open-sided (OS; n = 43; Figure 4.1), open-sided with fans (OF; n = 290; Figure 4.2), tunnel-ventilated with foggers (TVF; n = 7; Figure 4.3), and tunnel-ventilated with cooling pad (TVP; n = 32). Houses had two types of floor: CF (n = 175), and DF (n = 195). From the total of the houses, 274 were single-storey houses at ground level or with one level (OL), and 53 were two-storey houses (Figure 4.4). Birds were placed in the first level (FL) of all of the houses with two storeys (n = 53), and in the second level (SL) of 45 two-storey houses. For treatment distribution, animals, housing, broiler management and data recorded refer to materials and methods in Chapter 2 and Chapter 3.

Heat stress index (HSI) was calculated each day after d 21, and the difference between the maximum temperature and the recommended temperature determined (Ryder et al., 2004; Chapter 2). The recommended temperature average from d 21 to d 42 was 21.3ºC (Aviagen 2009; Cobb-Vantress, 2012). The HSI indicated how much the temperature was above or below the recommended temperature.

4.2.2 Statistical Analyses.

Differences between geographical regions, house type, floor type, and floor level were independently evaluated by ANCOVA using the procedure for linear mixed models (PROC MIXED) of SAS 9.3© for Windows (SAS Institute Inc., Cary, NC). For broiler performance analysis, the experimental unit was the individual house. For processing traits and bone

characteristic analysis, the experimental unit was the individual bird. Due to sales demand and logistics, this broiler integration has a different BW objective for males and females, and processing ages varied among houses. The overall processing age average was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d. The overall stocking density average was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2. For this variation, processing age (PA) and stocking density (D) were considered as covariates. Covariate is a variable that influences the dependent variables. To reduce error (residual) and increase precision, the least square means were adjusted to those common covariates.

Means were separated using pairwise tests, and differences between treatment means were reported at $P < 0.05$. The analysis was in accordance with the following model:

$$Y_{ij} = \mu + V_i + S_j + (VS)_{ij} + \beta\ (PA_{ijkl} - PAa) + \beta\ (D_{ijkl} - Da) + E_{ij},$$

where μ was the population mean; V_i was the effect of each variable analyzed such as geographical region (i = 1 to 2), house type (i = 1 to 4), floor type (i = 1 to 2), floor level (i = 1 to 3); S_j was the effect of bird sex (j = 1 to 2); $(VS)_{ij}$ were the interactions of each variable with sex; $\beta\ (HSI_{ijkl} - HSIa)$ was a covariate coefficient multiplied by the difference between individual PA (PA_{ijkl}) and average PA (PAa); $\beta\ (D_{ijkl} - Da)$ was a covariate coefficient multiplied by the difference between individual D (D_{ijkl}) and average D (HSIa); and E_{ijk1} was the residual error. In the main study, treatment effects were minimal; therefore, they were ignored in this analysis.

The coefficient of variation for the HSI was calculated for each geographical region, house type, floor type, and floor level according to this formula:

$$CV_{HSI} = (s_{HSI} / HSIa) \times 100$$

where CV_{HSI} was coefficient of variation for HSI, s_{HSI} was the HSI standard deviation, HSIa was the HSI average.

4.3 RESULTS AND DISCUSSION

4.3.1 Regional effects

4.3.1.1 Ambient temperature

Poultry houses located in VR had a higher HSI than those located in CR (Table 4.1). In VR, the HSI ranged from 4.6ºC to 12.4ºC, and in CR from 0.1ºC to 10.9ºC. The coefficient of variation of the HSI in VR was lower than in CR (25.2 % vs. 41.9 % respectively). According to the HSI and the recommended ambient temperature, the average environmental temperature from d 21 to processing age in VR was 29.3ºC and 26.9ºC in CR. Therefore, poultry houses in VR had warmer and more stable environmental temperature than CR after brooding. The warmer ambient temperature in VR is likely related to the differences in vegetation, topography, rain fall, and altitude that exist between those regions (Bernal et al., 1989). The variation in ambient temperature and humidity between geographical regions are a determinant in management decisions and poultry housing design (Gates et al., 1995).

4.3.1.2 Final performance

Broilers housed in VR had higher FCR compared to CR because of greater feed intake, and similar final BW (Table 4.1). Males in VR had a higher feed intake in comparison to CR, but there was no difference between females (Table 4.2). Feed intake and weight gain was reduced when birds were kept at 32ºC from 4 to 7 wk in comparison to birds kept at 20ºC (Baziz et al., 1996). The higher feed intake in males in VR is in contrast to the higher HSI in that region, since at higher temperatures in broilers reduce feed intake to maintain homeothermy (Baziz et al., 1996; Lu and Zhang, 2007). Weight gain is reduced in broilers raised at temperatures beyond their thermoneutral

zone, because latent heat loss via panting increases energy expenditure (Dozier et al., 2005; Yahav et al., 2008); this may be the reason for the lack of differences in BW and FCR between males grown in VR and CR.

4.3.1.3 Processing traits

Carcass weight, carcass yield, breast and breast filet weights were significantly lower in females housed in VR compared to CR. The same trend was seen in thigh and wing weights (P = 0.0652; P = 0.0763, respectively). In contrast, those traits and breast yield in males were significantly higher in VR than CR (Table 4.2). Females of VR had higher drum yield than females of CR, but males were similar. Males of VR had lower wing yield than males of CR, but females were similar. Chronic high temperature reduces meat yield in broilers. Weights and yield of breast and thigh were reduced in broilers raised at 34ºC from d 29 to d 42 relative to birds at 23ºC (Zhang et al., 2012). Breast yield was reduced by 1.5% in broilers kept at 34ºC from 3 to 7 wk in comparison to those at 22ºC (Akşit et al., 2006). Carcass yield was reduced in broilers maintained at 28ºC from 1000h to 1700h and at 22°C from 1700 to 1000 h in comparison to those maintained at 23ºC constantly (Akşit et al., 2006). Since VR had a higher HSI than CR during the trial, our results align with those previous reports. High environmental temperature decreased rate of protein synthesis, resulting in a reduction in protein deposition (Baziz et al., 1996; Zhang et al., 2012).

Carcass water absorption is crucial because retention and gains or losses of water may affect the weight and the economic value of chicken meat. Carcass water absorption was higher in CR than VR (Table 4.4). Ambient temperature was higher in VR during the field trial. Akşit et al. (2006) reported a lower breast meat moisture from carcasses of broilers exposed to a constant temperature of 34°C from 3 to 7 wk than those from birds maintained between 22 and 28 °C, and the control group maintained at 22ºC.

4.3.1.4 Relative Weight of Organs

Relative heart weight was lower in females in CR than in VR, but there was no difference between males (Table 4.2). A greater heart relative weight might suggest an increased growth rate (Bouyeh, 2012); however, there were no differences in final BW between VR and CR. The VR farms were located between 900 to 1,000 meters above sea level whereas the CR farms were located between 1,200 to 1,500 meters above sea level. Polycythaemia and blood viscosity are increased as altitude increases, which may result in ascites through a higher cardiac output (Özkan et al., 2010). In CR, 11.0% of the houses had reports of ascites. In VR only 1.7% of the houses had reports of hydro-pericardium, which is preliminary stage of ascites. This is likely related to the lower altitude in VR relative to CR. However, there were no differences in heart size in males between the two geographical regions, and females of CR had lower heart yield. Other situations such as cold ambient temperature, mycotoxins and reduced ventilation can result in ascites (Julian, 2000; Özkan et al., 2010); however, there was also no evidence of this during the trial in either region.

Relative liver weight was lower in males housed in VR than CR; but there were no differences in females (Table 4.2; Table 4.5). Changes in liver size may be directly related to metabolic activity (Palo et al., 1995).

Relative spleen weights were lower in CR than VR (Table 4.5). Spleen weight has been used to evaluate immune and heat stress status in chickens (Zhou et al., 2009; Quinteiro-Filho et al., 2010). During this trial, there was no evidence of pathological events in VR that would result in inflammation and an increased spleen yield relative to CR. Birds raised at 36^{o}C from 35 to 42 d had decreased spleen yield in comparison to those at 21^{o}C (Quinteiro-Filho et al. (2010). This is

in contrast to our findings since VR had higher HSI than CR; however, the temperatures in both regions were well below that reported by Quinteiro-Filho et al. (2010).

Relative intestine weights were higher in females in VR than in CR, but there was no difference in males (Table 4.2). Compared with other organs, gut maintenance nutrient requirement is greater, and any additional gut tissue turnover might increase nutrient requirements for maintenance, thereby reducing feed efficiency (Moghaddam et al., 2012). Hence, lighter intestines may lower the dietary energy need for gut maintenance, and liberate more energy and protein for other functions like growth or muscle deposition (Moghaddam et al., 2012). The reduced carcass traits in VR relative to CR may be related to an increased maintenance energy due to higher intestine weights.

Relative fat pad weight was significantly greater in females housed in VR than CR; but it was similar among males (Table 4.2; Table 4.5). Fat pad yield was increased in broilers raised at 34^{o}C from d 29 to d 42 relative to birds at 23^{o}C (Zhang et al., 2012). At high ambient temperatures, as was seen in VR, there is an imbalance between energy ingested and heat dissipation which resulting in more fatness in broiler chickens (Geraert et al., 1996). Females accumulate fat faster and in higher quantities than males (Plavnik and Hurwitz, 1982; Zuidhof, 2005; Murawska et al., 2011).

4.3.1.5 Bone Characteristics

Bone breaking stress had a trend to be reduced in females in VR in comparison to those in CR (P = 0.0664; Table 4.2); in contrast the same trait tended to be increased in males in VR (P = 0.0664; Table 4.2). Environmental rearing temperatures above the thermoneutral zone reduced bone length and diameter (Bruno et al., 2000; Pelicano et al., 2005). Heat stress induces respiratory alkalosis by increasing respiration rate (Teeter et al., 1985), which increases calcium fixation by

blood proteins, and reduces retention of calcium, phosphorus, potassium, sodium, magnesium, sulphur and manganese, making them less available for bone formation (Belay and Teeter, 1996). Moreover, during alkalosis, blood carbon dioxide concentration is reduced, which increases dissolution of apatite crystals in bones that reduces bone development process. (Bushinsky and Sessler, 1992).

4.3.2 Barn Type effects

4.3.2.1 Ambient temperature

The OF houses had the lowest HSI among the different house types but not statistically different from TVF and TVP houses (Table 4.1). The HSI in OS was greater than in OF, but was not different from TVF or TVP. The combined effect of ambient temperature and RH are crucial in determining the bird's comfort and susceptibility to heat stress (Xin et al., 1994), but RH was not evaluated in this trial. The HSI took into account only the ambient temperature (maximum temperature on a daily basis) and its deviation from the recommended temperature (Aviagen 2009; Cobb-Vantress, 2012).

All TVF houses were located in VR; therefore they were exposed to a higher ambient temperature. In contrast, all TVP were located in CR, which has a cooler ambient temperature. However, these two types of houses were not statistically different in HSI. Temperatures under the surface of the litter, at litter surface and at 10 cm above litter surface increased as stocking density increased from 5, to 10 and 20 bird/m^2; the difference between the lowest and highest stocking density had a range of 6 to 8ºC (Reiter and Bessei, 2000). The stocking density average in TVF (14.5 bird/m^2) and TVP (15.4 bird/m^2) were higher than those in OS (12.0 bird/m^2) and OF (12.1 bird/m^2) that is likely the reason for the higher HSI in the tunnel-ventilated houses. However, taking into account the variation in HSI, environmental temperatures were more stable in TVF

(CV = 9.1%) and TVP (CV = 13.3%) than in OS (CV = 19.9%) and OF (CV = 40.8%), indicating a lower temperature fluctuation inside the house.

4.3.2.2 Final performance

Broilers in TVF houses had higher BW than broilers housed in the other house types (Table 4.1). This house type had also the highest HSI, and the higher temperatures have been associated with a reduction in feed intake and slower growth rates (Lu and Zhang, 2007; Quinteiro-Filho et al., 2010). However, there were no differences in feed intake and FCR between house types. In a previous study, no differences were found in weight gain between broilers housed in a tunnel-ventilated and fogging house and in an open-sided house with fogging system (Aradas et al., 2005). These authors stated that tunnel-ventilation and fogging reduced heat stress more efficiently than other type of house, because mortality was lower (2.7 % vs. 3.0 %). They also observed less panting and prostration of birds in tunnel-ventilated with foggers houses. In the present study, TVF had a high HSI, but a less fluctuating environment than other house types. A warmer and stable environment reduces energy requirements in comparison to colder environments or chronic high temperatures (Hurwitz et al., 1980; Dozier et al., 2005; Yahav et al., 2008), which therefore might allow the birds housed in TVF to utilize more energy for growth and meat yield.

4.3.2.3 Processing traits

In females, carcass weight was lower in OS and OF in comparison to TVF, and TVP; carcass and breast filet yield were similar between females of all house types (Table 4.6). In males, carcass weight and yield were higher in TVF relative to the other houses, but those traits were reduced in OF in comparison to the other houses. In females, breast and thigh absolute weights were lower in OF relative to other house types. Females in TVF had higher thigh weight and yield than the other houses. In males, breast and thigh weights were lower in OF relative to other houses;

with the exception of OS, TVF increased breast weight in comparison to the other house types. Females in TVF had higher breast filet weight than the other houses. In males, OF reduced breast filet weight in comparison OS and TVP. Males in TVF had lower breast filet yield than OS and TVP. In males, thigh yield was lower in OF than OS. Females in OS had lower drum weight than the other house types. Females in OF had higher drum yield than OS and TVF. In males, TVF reduced drum weight and yield relative to the other houses; males in OF had lower drum weight than OS and TVP. Females in TVP hag higher wing weights than OS and OF. In females, wing yield was reduced in TVF and increased in OF relative to the other houses. Males in OS had higher wing weights and yield than the other houses.

Both heat stress and chronic heat exposure can reduce meat yield, muscle protein deposition and turnover by reducing protein synthesis (Yunianto et al., 1997; Temim et al., 2000; Zhang et al., 2012). Despite having the lowest HSI, OF birds were exposed to the highest fluctuations in temperature (HSI CV = 40.8%). In contrast, birds housed in TVF had the highest HSI but the most stable environment (HSI CV = 9.1%). Therefore, the greater fluctuation in ambient temperature might be associated with reduced carcass portion weights and yields. Artificial ventilation systems with misting help to cool the bird environment, reduce heat stress, and increase carcass quality (Ryder et al., 2004).

Weights and yields of carcass and breast were negatively correlated with yields of drums and wings (Table 4.7). This correlation was also observed in that males TVF had increased weights of carcass and breast, and carcass yield, but at the same time reduced drum yield. Chronic heat stress decreased the proportion of breast but increased thigh yield, which has been associated with different rates of protein deposition, proteolysis and protein turnover among different muscles (Baziz et al., 1996; Temim et al., 2000; Zhang et al., 2012). Breast muscles are more responsive

to nutritional changes and stressful conditions than other muscles, and they are a major source of protein compared to other more functional muscles such as legs in situations of amino acid restriction (Tesseraud et al., 1996). Therefore, the increases in breast weight and yield may allow us to determine what house type offered a more suitable environment. Birds in TVF houses had a warmer and more stable environment in comparison to the other houses; therefore, broilers in these houses may had been able to utilize more energy for growth and meat yield than for maintenance.

Females in OF and TVP had higher carcass water absorption relative that in the other houses, but there was no difference between males (Table 4.6). These two types of houses also had lower HSI, meaning a lower ambient temperature during the grow-out period. Constant environmental temperature of 34ºC from 3 to 7 wk reduced moisture in meat breast at processing in comparison to birds kept between 22 and 28°C, and at 22ºC (Akşit et al., 2006).

4.3.2.4 Relative Weight of Organs

In females, relative heart weight was reduced when housed in TVP, but was increased in OS and TVF. In males, relative heart weight was reduced in TVF but increased in TVP (Table 4.6). Therefore, the effect of tunnel ventilation on relative heart weight depended on bird sex. There are no previous reports about this effect, therefore further studies are needed to clarify the reasons. Liver weight was lower in TVF birds in comparison to other house types (Table 4.5); this may indicate a reduced metabolic activity (Palo et al., 1995). Relative spleen weight was also lower in tunnel-ventilated houses than in open-sided houses (Table 4.5). This may indicate that birds housed in tunnel-ventilated houses had no an increased immune response or greater heat stress relative to birds housed in open-sided houses. In broilers, an increased spleen size has been used as an indicator of heat stress (Quinteiro-Filho et al., 2010) and of an increased immune response (Sajadifar et al., 2012).

In males, relative intestine weight was increased in OF, but reduced in TVF. In females, this trait was reduced in OS, but was similar between other type of houses (Table 4.6). Intestine is one of the most energy consuming organs (Moghaddam et al., 2012), therefore the reductions in intestine weight may have resulted in lower carcass traits in birds housed in OF, and higher meat yield in TVF. Relative fat pad weight was higher in OS but not different than TVF (Table 4.5). These two types of houses had also the highest HSI values (Table 4.1); higher ambient temperature facilitates fat deposition (Kubena et al., 1974; Baziz et al., 1996; Yunianto et al., 1997; Zhang et al., 2012).

4.3.2.5 Bone Characteristics

Bone breaking strength was greater in birds housed in TVF and TVP relative to OS and OF. Birds in TVF and OF had higher bone breaking stress than OS and TVP (Table 4.8). Higher environmental rearing temperatures outside the thermoneutral zone and heat stress reduce bone development (Bushinky and Sessler, 1992; Belay and Teeter, 1996; Pelicano et al., 2005). Birds housed in tunnel-ventilated houses may have had a more suitable environment resulting in an increased bone quality due to less respiratory alkalosis.

4.3.3 Floor Type Effects

4.3.3.1 Ambient temperature.

Poultry houses with CF had a higher HSI than those with DF during the grow-out period (Table 4.1), despite having a lower stocking density (11.9 vs. 12.7 bird/m^2 respectively). In addition, CF had a more stable temperature than DF (HSI CV = 34.0% and 37.5% respectively). Similarly, Abreu et al. (2011) reported that at 6 weeks of age poultry houses with CF had significantly higher air and litter temperatures than houses with DF. Therefore, CF keeps the air

and litter temperature in poultry houses warmer, in contrast with a DF that may reduce ambient temperature of the house by direct convection to the surface of the soil. The thermal conductivity of soil is higher than concrete (VanBeek and Beeking, 1995).

4.3.3.2 Final performance and mortality

Broilers in CF houses had higher BW than DF broilers (Table 4.1). In contrast, Abreu et al. (2011) did not find differences in broiler performance between CF and DF houses. Despite feed intake being similar between floor types, FCR was higher in DF broilers in than CF broilers because of a lower BW (Table 4.1). The warmer environment observed with CF may have reduced energy requirement in comparison to the cooler environment in the DF houses (Hurwitz et al., 1980) and therefore increased feed efficiency. The ambient temperature of these two type of houses were not high enough to cause heat stress or reduce feed intake. Cooler temperatures (below the thermoneutral zone) increased oxygen demand and affected energy balance (Akşit et al., 2013). This broiler vertical integration try to maintain the ambient temperature in all poultry houses at approximately 29°C during the first two weeks, and then at approximately 21°C which is close to the thermoneutral zone (Donkoh, 1989).

Mortality was higher in DF broilers than CF broilers (Table 4.1). Similarly, Abreu et al. (2011) found a higher mortality, especially sudden death in broilers in houses with DF than CF, with no explanation of the causes. In the current study, there was no pathological evidence that could explain that difference between CF and DF.

4.3.3.3 Processing traits

In comparison to DF, CF increased absolute weights of carcass and drums in males, and thigh weight and yield, and wing yield in females (Table 4.9). Compared to CF, DF increased carcass yield in females, and breast weight in males; however, birds in CF houses had higher breast

filet yield than those in DF. Birds in CF may have had more energy available for growth and meat yield since they had a more stable ambient temperature than DF. There are no published reports related to these effects; however, these findings may indicate a more favorable environment for broilers in CF than DF. In comparison to DF, CF increased carcass water absorption in females (Table 4.9). Floor type effect on carcass water absorption depended on bird sex. Carcass water absorption is related to differences in ambient temperature. Rearing broilers at 34°C from 3 to 7 wk reduced breast moisture content at processing in comparison to birds at 22ºC, and those housed between 22 and 28 °C, perhaps due to higher dehydration or changes in protein levels (Akşit et al., 2006). However, in the present study the ambient temperature was not maintained at those high levels.

4.3.3.4 Relative Weight of Organs

Birds in CF houses had lower relative heart and spleen weights than DF (Table 4.5). There are no previous reports about this effect, therefore further studies are needed to clarify the reasons. However, it may indicate that birds in CF had lower immune activity than birds in DF, or that a warmer environment can reduce organ size (Quinteiro-Filho et al., 2012). In comparison to DF, CF increased relative fat pad weight of females, but reduced it in males. Females had a greater and faster fat deposition than males (Murawska et al., 2011). In this study, floor type effect on fat deposition depended on bird sex; however, the differences in ambient temperature may also be involved. It has been reported that fat deposition is increased when broilers are maintained at ambient temperature higher than the thermoneutral zone (Baziz et al., 1996; Yunianto et al., 1997; Zhang et al., 2012).

4.3.4. Floor Level effects

4.3.4.1 Final performance and mortality

Birds in OL houses, and at SL had higher BW than birds at FL (Table 4.1). Feed intake was lower when males were housed at FL compared to those at SL and OL; but there were no differences between floor levels in females (Table 4.1). There was a similar trend in FCR (P = 0.0560; Table 4.1). There are no similar comparisons in the literature, and since there were no differences in HSI among the various levels, it is difficult to explain these results. In this study RH was not evaluated, however Abreu at al. (2011) found that CF increased RH in comparison to DF. A RH of 70 to 75% combined with an ambient temperature of 28ºC reduced BW and feed intake of broilers in comparison to a RH of 60 to 65% and the same ambient temperature (Yahav et al., 2000). As FL houses had floor and ceiling built of concrete, that characteristic may have reduced ventilation and increased RH, which in turn reduced BW and feed intake.

Birds in OL and SL had higher mortality than birds place at FL, which may be related to higher BW in those houses since higher growth rates have been correlated to higher mortality rates (Gonzales et al., 1998; Havenstein et al., 2003; Shim et al., 2012). Moreover, there was no evidence of pathological events in the current study that would provide an explanation.

4.3.4.2 Final processing traits

Carcass weight was higher in OL than SL, but not different from FL (Table 4.3). Absolute weights of drums and wings were lower in SL than FL and OL (Table 4.3). Breast filet yield was increased in SL relative to FL and OL (Table 4.4). In comparison to FL and OL, SL reduced carcass yield of females; in comparison to OL, SL reduced carcass yield of males (Table 4.10). In females, absolute weight of breast filet and thighs were nearly significantly lower (P = 0.0800) in FL in comparison to OL but not different from SL. In males, breast filet weights were higher in SL in

comparison to OL but not different from FL (Table 4.10). In males, thigh weights and yield were higher in FL in comparison to SL and OL, but there were no differences in female thigh yield between floor levels (Table 4.10). In females, drum weights were lower in SL in comparison to FL and OL, but they were similar in males among floor levels (Table 4.10). Females housed at FL had a trend (P = 0.0829; Table 4.10) to have higher wing yield than those at SL and OL, but this was not observed in males. There is a lack of data in the literature about different floor levels and their effect on broiler performance and meat yield; therefore further studies are needed to clarify these effects. However, with the exception of breast filet weights and yield, it seems that carcass traits tend to be reduced when broiler are housed at SL. Micro-environment and comfort in those houses may change as a result of having another house or group of birds situated directly below them. Furthermore, in two-storey houses with no environmental control systems, the floor temperature and RH may be higher as a result of bird respiration and CF, thus reducing performance and meat yield.

4.3.4.3 Relative Weight of Organs

Relative heart size was increased in females housed at SL in comparison to FL and OL, but was similar between males (Table 4.10). This may indicate a higher heart output in in those birds, but since there were no differences in HSI among the floor levels, it is difficult to explain these results. Relative liver and intestine weights were lower in OL than FL and SL (Table 4.5). A lower intestine weight may allow more available energy and protein for growth and meat yield in those birds, because the intestine is a highly energy demanding organ (Moghaddam et al., 2012). Fat pad deposition was reduced in females when housed at FL in comparison to SL and OL, but was increased in males at OL compared to FL and SL (Table 4.5; Table 4.10). There were no differences in ambient temperature between floor levels; therefore, this finding is difficult to

explain. However, an increased availability of energy to the OL birds due to lower liver and intestine weights could allow for a higher fat deposition in males.

4.3.5 Conclusion

Most of the differences observed in the present study were related to differences in HSI. Environmental temperature has major effects on the broiler productivity. Acute or chronic heat stress reduces performance and meat yield in broiler chickens (Lu and Zhang, 2007; Zhang et al., 2012). Relative weights of internal organs such as heart, liver, spleen, and intestine, and fat pad may be used as indicators of heat stress in broilers (Quinteiro-Filho et al., 2010; Zhang et al., 2012). The combined effect of ambient temperature and RH determine bird comfort and the severity of heat stress (Xin et al., 1994). Therefore, reducing harsh environmental conditions in broiler houses has the potential to increase performance through a more stable metabolism and reduced heat stress. Housing conditions that provide a stable ambient temperature close to the thermoneutral zone, may allow the birds to utilize more energy for growth and meat yield. Increased performance was observed in broilers housed in TVF houses, which has also been reported by Ryder et al. (2004). Excessive fat deposition in broilers is a worldwide concern (Zhou et al., 2009). Therefore, in VR, and in open poultry houses it would be useful to reduce environment temperatures and RH, increase ventilation rate, or adjust the dietary energy in order to produce leaner carcasses. Poultry houses with CF provided a warmer environment in comparison to DF; CF has the potential to increase performance and reduce mortality. The implementation of CF and artificial ventilation systems such as tunnel ventilation with cooling pads or fogging systems sometimes are perceived as a costly investment by some producers. However, it is important to evaluate the return of the investment through increased productivity when those housing systems are established.

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4.5 TABLES

Table 4.1 Main effects and covariate coefficients of geographical region (GR), house type (HT), floor type (FT), floor level (FL), and sex (S) on broiler performance at processing age (PA)[1]. Dependent variables adjusted to a common PA and stocking density (D)[2] with analysis of covariance.

Effect			HSI[3]	SEM	BW	SEM	Feed Intake	SEM	FCR	SEM	Mortality	SEM
		n[4]	°C x d		g				g:g		%	
GR	Coffee region	191	5.6[b]	0.2	2,512	6.9	4,403[b]	10.4	1.753[b]	0.005	4.80	0.1
	Valle region	181	8.0[a]	0.2	2,497	7.1	4,456[a]	10.6	1.785[a]	0.005	4.60	0.1
	PA (Unit/d)		0.17		72.4		185.2		0.024		-0.001	
	D (Unit/m^2)		0.18		11.3		9.2		-0.004		-0.124	
HT	Open	43	7.7[a]	0.4	2,525[b]	14.4	4,425	22.2	1.751	0.011	4.40	0.3
	Open + Fans	290	6.6[b]	0.1	2,499[b]	5.7	4,427	8.8	1.772	0.004	4.80	0.1
	Tunnel ventilated and foggers	7	7.8[ab]	0.9	2,609[a]	37.0	4,480	57.0	1.722	0.028	4.20	0.7
	Tunnel ventilated and cooling pad	32	7.0[ab]	0.5	2,506[b]	20.9	4,437	32.2	1.775	0.016	4.60	0.4
	PA (Unit/d)		0.2		71.7		186.3		0.245		-0.147	
	D (Unit/m^2)		-0.1		10.7		3.7		-0.006		-0.069	
FT	Concrete	177	7.3[a]	0.2	2,523[a]	7.1	4,438	10.9	1.758[b]	0.005	4.16[b]	0.1
	Dirt	195	6.2[b]	0.2	2,487[b]	6.8	4,419	10.5	1.778[a]	0.005	5.21[a]	0.1
	PA (Unit/d)		0.2		71.6		185.3		0.024		-0.004	
	D (Unit/m^2)		0.06		15.3		7.4		-0.008		-0.186	
FL	First level	53	6.4	0.3	2,471[b]	13.3	4,347[b]	20.0	1.761	0.010	3.80[b]	0.4
	Second level	45	6.6	0.4	2,526[a]	15.5	4,434[a]	23.4	1.754	0.011	4.61[ab]	0.3
	One level[5]	274	6.8	0.1	2,509[a]	5.6	4,442[a]	8.6	1.771	0.004	4.80[a]	0.1
	PA (Unit/d)		0.2		72.3		186.1		0.024		0.002	
	D (Unit/m^2)		-0.03		12.6		4.1		-0.007		-0.124	
S	Females	178	6.9	0.2	2,318[b]	8.9	4,260[b]	13.6	1.833[a]	0.006	3.9[b]	0.2
	Males	194	6.6	0.2	2,691[a]	8.4	4,579[a]	12.9	1.704[b]	0.006	5.4[a]	0.2
	PA (Unit/d)		11.1		75.0		185.6		0.024		-0.011	
	D (Unit/m^2)		-1.6		12.8		6.0		-0.007		-0.107	
Sources of variation			P-values									
	PA		< 0.0001		< 0.0001		< 0.0001		< 0.0001		0.8755	
	D		0.3817		0.0089		0.0542		0.0326		0.0480	
	GR		< 0.0001		0.1657		0.0005		< 0.0001		0.3497	
	HT		0.0327		0.0153		0.8347		0.0905		0.5378	
	FT		< 0.0001		0.0004		0.2195		0.0074		< 0.0001	
	FL		0.5381		0.0119		0.0001		0.3148		0.0234	
	S		0.1872		< 0.0001		< 0.0001		< 0.0001		< 0.0001	
	GR x S		NS		NS		*		NS		NS	
	HT x S		NS		NS		NS		NS		NS	
	FT x S		NS		NS		NS		NS		NS	

FL x S	NS	NS	*	**	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.
[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2
[3]Heat stress index = [daily maximum house temperature − daily recommended temperature (^oC)]/ PA – 21 (d).
[4]House as experimental unit.
[5]Single-storey house
Probabilities of interactions: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant ($P > 0.05$).

Table 4.2 Significant and nearly significant interaction effects between geographical region (GR), and sex (S) at processing age[1]. Dependent variables adjusted to a common processing age and stocking density[2] with analysis of covariance.

GRS	Valle region		Coffee Region		Pooled SEM	P-values
	Female	Male	Female	Male		
Feed Intake (g)	4,256[c]	4,657[a]	4,257[c]	4,595[b]	16.7	0.0002
Carcass (g)	1,622[d]	1,930[a]	1,653[c]	1,903[b]	7.3	< 0.0001
Carcass (%)[3]	69.8[b]	69.9[ab]	70.1[a]	69.3[c]	0.09	< 0.0001
Breast (g)[4]	643[d]	755[a]	657[c]	736[b]	3.6	< 0.0001
Breast (%)[5]	39.7[a]	39.0[b]	39.9[a]	38.6[c]	0.07	0.0017
Breast filet (g)[5,6]	370[d]	428[a]	380[c]	421[b]	3.6	0.0004
Thighs (g)	301	354	306	353	2.0	0.0652
Drums (%)[5]	14.8[b]	15.0[a]	14.4[c]	15.0[a]	0.06	< 0.0001
Wings (g)	193	224	197	224	1.0	0.0763
Wing (%)[5]	12.0[a]	11.7[b]	11.9[a]	11.9[a]	0.03	0.0076
Heart (%)[5]	0.818[a]	0.819[a]	0.783[b]	0.819[a]	0.036	0.0009
Liver (%)[5]	3.05[a]	2.98[b]	3.01[ab]	3.04[a]	0.031	0.0013
Intestine (%)[5]	7.05[a]	6.72[b]	6.62[b]	6.73[b]	0.054	< 0.0001
Fat pad (%)[5]	2.58[a]	2.19[c]	2.46[b]	2.19[c]	0.023	0.0033
Bone breaking stress (g/mm^2)[7]	225.5	241.7	236.2	234.3	5.8	0.0664

[a-c]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2

[3]Yield as the percent of live BW per bird.

[4]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[5]Yield as the percent of eviscerated carcass weight per bird.

[6]Breast filet: Pectoralis major.

[7]Measurements taken from right tarsometatarsus (shank) with flesh and skin intact.

Table 4.3 Main effects and covariate coefficients of geographical region (GR), house type (HT), house floor type (FT), house floor level (FL), and sex (S) on absolute weights of carcass traits at processing age (PA)[1]. Dependent variables adjusted to a common PA and stocking density (D)[2] with analysis of covariance.

Effect			BW	SEM	Carcass	SEM	Breast[3]	SEM	BF[4]	SEM	Thighs	SEM	Drums	SEM	Wings	SEM
		n[5]	g													
GR	Coffee Region	1,741	2,544	5.7	1,778	4.5	696.5	2.3	400.5	1.7	329.5	1.2	262.8	0.9	210.5	0.6
	Valle	1,868	2,536	5.4	1,776	4.3	698.9	2.1	398.8	1.6	327.4	1.2	264.1	0.8	208.8	0.6
	PA (Unit/d)		72.9		59.6		24.3		15.8		9.3		8.3		5.3	
	D (Unit/m^2)		-1.45		3.3		3.6		1.2		-0.4		0.4		-0.2	
HT	Open	364	2,578^{b}	12.3	1,806^{b}	9.7	714.3^{b}	4.9	408.5^{a}	3.7	341.1^{b}	2.7	264.0^{ab}	1.9	213.5^{a}	1.4
	Open + Fans	2,912	2,528^{c}	4.4	1,766^{c}	3.4	692.4^{c}	1.7	396.9^{b}	1.3	325.0^{c}	0.9	262.9^{b}	0.7	208.9^{b}	0.5
	Tunnel ventilated and foggers	57	2,664^{a}	34.3	1,908^{a}	26.8	745.9^{a}	13.2	424.5^{a}	10.2	375.5^{a}	7.2	259.2^{b}	5.0	209.8^{ab}	3.8
	Tunnel ventilated and cooling pad	276	2,591^{b}	17.4	1,824^{b}	13.9	722.6^{ab}	6.9	411.1^{a}	5.3	340.9^{b}	3.8	270.2^{a}	2.6	211.2^{ab}	2.0
	PA (Unit/d)		72.8		59.6		24.4		15.6		9.3		8.4		5.3	
	D (Unit/m^2)		-8.01		-3.7		-0.1		-0.3		-2.5		-0.2		-0.2	
FT	Concrete	1,829	2,553^{a}	5.6	1,782^{a}	4.4	701.3^{a}	2.2	404.2^{a}	1.7	331.6^{a}	1.2	265.2^{a}	0.8	211.4^{a}	0.6
	Dirt	1,780	2,522^{b}	5.6	1,767^{b}	4.4	692.2^{b}	2.2	394.1^{b}	1.7	325.3^{b}	1.2	261.4^{b}	0.8	207.6^{b}	0.6
	PA (Unit/d)		71.6		58.3		23.8		15.5		9.2		8.2		5.2	
	D (Unit/m^2)		2.25		5.5		4.5		2.2		0.1		0.6		0.2	
FL	First level	440	2,523^{ab}	13.0	1,762^{ab}	10.2	695.0	5.0	394.3	3.9	333.0^{a}	2.8	263.1^{a}	1.9	209.6^{a}	1.4
	Second level	530	2,513^{b}	13.8	1,740^{b}	11.0	687.6	5.5	404.0	4.2	320.3^{b}	3.0	257.1^{b}	2.1	204.0^{b}	1.6
	One level[6]	2,639	2,546^{a}	4.6	1,783^{a}	3.7	698.9	1.8	399.2	1.4	330.1^{a}	1.0	264.0^{a}	0.7	210.2^{a}	0.5
	PA (Unit/d)				59.7		24.2		15.6		9.4		8.4		5.4	
	D (Unit/m^2)				2.6		3.3		1.3		-0.4		0.2		-0.1	
S	Females	1,609	2,332^{b}	8.0	1,634^{b}	6.3	648.9^{b}	3.1	373.9^{b}	2.4	302.7^{b}	1.7	239.2^{b}	1.2	194.6^{b}	0.9
	Males	2,000	2,746^{a}	6.8	1,918^{a}	5.3	745.8^{a}	2.7	425.0^{a}	2.0	353.9^{a}	1.5	287.8^{a}	1.0	224.4^{a}	0.8
	PA (Unit/d)		72.2		58.8		24.0		15.6		9.2		8.3		5.3	
	D (Unit/m^2)		-0.44		3.9		3.6		1.5		-0.2		0.3		-0.02	
Sources of variation			P-values													
	PA		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001	
	D		0.5895		0.1309		0.5309		0.1323		0.5184		0.3245		0.5992	
	GR		0.2618		0.7083		0.4330		0.4943		0.2316		0.2791		0.0633	
	HT		< 0.0001		< 0 .0001		< 0.0001		0.0004		< 0.0001		0.0361		0.0163	
	FT		< 0.0001		0.0121		0.0038		< 0.0001		0.0002		0.0015		< 0.0001	
	FL		0.0345		0.0004		0.1336		0.2293		0.0032		0.0078		0.0009	
	S		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001	
	GR x S		*		***		***		*		**		NS		**	
	HT x S		*		*		*		*		*		***		***	
	FT x S		*		*		*		NS		*		NS		NS	
	FL x S		NS		NS		NS		**		*		NS		NS	

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2
[3]Breast: Pectoralis major, Pectoralis minor, bone and skin included.
[4]Breast filet: Pectoralis major.
[5]Bird as experimental unit.
[6]Single-storey house
Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant (P > 0.05).

Table 4.4 Main effects and covariate coefficients of geographical region (GR), house type (HT), house floor type (FT), house floor level (FL), and sex (S) on water absorption (WA)[1], and carcass[2] and parts yield[3] at processing age (PA)[4]. Dependent variables adjusted to a common PA and stocking density (D)[5] with analysis of covariance.

Effect			WA	SEM	Carcass	SEM	Breast[6]	SEM	BF[7]	SEM	Thighs	SEM	Drums	SEM	Wings	SEM
		n[8]	%		% of Live BW		% of Eviscerated Carcass Weight									
GR	Coffee Region	1,741	8.6[a]	0.08	69.7[b]	0.05	39.2	0.07	22.5	0.07	18.6	0.05	14.8[b]	0.03	11.9	0.03
	Valle	1,868	8.2[b]	0.07	69.8[a]	0.04	39.3	0.07	22.4	0.07	18.5	0.05	14.9[a]	0.04	11.8	0.03
	PA (Unit/d)		-0.003		0.36		0.07		0.16		-0.1		-0.04		-0.12	
	D (Unit/m^2)		-0.002		0.15		0.09		0.004		-0.05		0.001		0.02	
HT	Open	364	8.0[bc]	0.17	69.9[b]	0.10	39.5	0.15	22.5	0.15	18.8[b]	0.11	14.6[b]	0.08	11.9[a]	0.07
	Open + Fans	2,912	8.5[a]	0.06	69.7[b]	0.04	39.2	0.05	22.4	0.05	18.5[c]	0.04	14.9[a]	0.03	11.9[a]	0.02
	Tunnel ventilated and foggers	57	7.3[c]	0.46	71.4[a]	0.28	39.2	0.39	22.5	0.42	19.9[a]	0.31	13.6[c]	0.22	11.0[c]	0.17
	Tunnel ventilated and cooling pad	276	8.4[ab]	0.24	69.9[b]	0.15	39.6	0.21	22.5	0.22	18.7[bc]	0.16	14.7[ab]	0.11	11.6[b]	0.09
	PA (Unit/d)		-0.002		0.37		0.07		0.15		-0.11		-0.03		-0.12	
	D (Unit/m^2)		-0.004		0.09		0.05		0.001		-0.09		0.03		0.03	
FT	Concrete	1,829	8.6[a]	0.08	69.9[a]	0.07	39.3	0.06	22.6[a]	0.07	18.7[a]	0.05	14.9	0.04	12.0[a]	0.03
	Dirt	1,780	8.3[b]	0.08	69.6[b]	0.06	39.2	0.06	22.3[b]	0.07	18.4[b]	0.05	14.8	0.04	11.8[b]	0.03
	PA (Unit/d)		-0.003		0.36		0.07		0.15		-0.1		-0.03		-0.11	
	D (Unit/m^2)		0.001		0.14		0.09		0.03		-0.04		-0.01		-0.02	
FL	First level	440	8.2	0.17	69.6[b]	0.11	39.4	0.15	22.3[b]	0.16	18.9[a]	0.12	15.0	0.08	12.0	0.07
	Second level	530	8.3	0.19	69.0[c]	0.12	39.4	0.16	23.1[a]	0.17	18.4[b]	0.13	14.8	0.09	11.8	0.07
	One level[9]	2,639	8.5	0.06	69.9[a]	0.04	39.3	0.05	22.4[b]	0.06	18.6[b]	0.04	14.8	0.03	11.9	0.02
	PA (Unit/d)		-0.003		0.37		0.07		0.14		-0.1		-0.03		-0.12	
	D (Unit/m^2)		0.001		0.13		0.09		0.02		-0.04		-0.01		-0.01	
S	Females	1,609	9.6[a]	0.07	69.9[a]	0.07	39.8[a]	0.09	22.9[a]	0.10	18.5	0.07	14.6[b]	0.05	12.0[a]	0.04
	Males	2,000	7.3[b]	0.07	69.6[b]	0.06	38.8[b]	0.08	22.1[b]	0.08	18.5	0.06	15.0[a]	0.04	11.8[b]	0.03
	PA (Unit/d)		-0.002		0.36		0.07		0.15		-0.1		-0.03		-0.12	
	D (Unit/m^2)		0.001		0.15		0.09		0.01		-0.004		-0.01		-0.02	
Sources of variation			P-values													
	PA		< 0.0001		< 0.0001		0.0018		< 0.0001		< 0.0001		0.0083		< 0.0001	
	D		0.5611		< 0.0001		0.0044		0.9153		0.0638		0.9924		0.1075	
	GR		0.0002		0.0442		0.2978		0.2946		0.2033		0.0147		0.0589	
	HT		0.0041		< 0.0001		0.0898		0.8943		< 0.0001		< 0.0001		< 0.0001	
	FT		0.0119		0.0007		0.2856		0.0005		0.0011		0.1162		0.0004	
	FL		0.2691		< 0.0001		0.4883		< 0.0001		0.0070		0.1327		0.0737	
	S		< 0 .0001		0.0016		< 0.0001		< 0.0001		0.7574		< 0.0001		0.0167	
	GR x S		NS		***		*		NS		NS		***		*	
	HT x S		*		***		NS		*		*		*		*	
	FT x S		***		*		NS		NS		***		NS		*	
	FL x S		NS		*		NS		NS		***		NS		**	

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]Water absorption: Percent of increase in the carcass weight by moisture retention after pre-chilling and chilling

[2]Yield as the percent of live BW per bird.
[3]Yield as the percent of eviscerated carcass weight per bird.
[4]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.
[5]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2
[6]Breast: Pectoralis major, Pectoralis minor, bone and skin included.
[7]Breast filet: Pectoralis major.
[8]House as experimental unit.
[9]Single-storey house
Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant (P > 0.05).

Table 4.5 Main effects and covariate coefficients of geographical region (GR), house type (HT), house floor type (FT), house floor level (FL), and sex (S) on relative weight of organs[1] at processing age (PA)[2]. Dependent variables adjusted to a common PA and stocking density (D)[3] with analysis of covariance.

Effect			Heart	SEM	Liver	SEM	Spleen	SEM	Intestine	SEM	Fat pad[4]	SEM
		n[5]	% of Eviscerated Carcass Weight									
GR	Coffee Region	1,741	0.800[b]	0.004	3.02	0.011	0.161[b]	0.002	6.67[b]	0.033	2.32[b]	0.015
	Valle	1,868	0.818[a]	0.004	3.01	0.010	0.169[a]	0.001	6.89[a]	0.031	2.38[a]	0.014
	PA (Unit/d)		0.0004		-0.05		-0.003		-0.2		0.01	
	D (Unit/m^2)		0.011		-0.02		0.002		-0.12		0.04	
HT	Open	364	0.846[a]	0.008	3.00[a]	0.023	0.178[a]	0.003	6.50[b]	0.072	2.46[a]	0.032
	Open + Fans	2,912	0.806[b]	0.003	3.03[a]	0.008	0.166[b]	0.001	6.86[a]	0.025	2.35[b]	0.011
	Tunnel ventilated and foggers	57	0.853[a]	0.023	2.75[b]	0.063	0.147[c]	0.009	5.82[c]	0.198	2.46[ab]	0.089
	Tunnel ventilated and cooling pad	276	0.804[b]	0.012	2.98[a]	0.033	0.157[bc]	0.005	6.73[ab]	0.103	2.29[b]	0.046
	PA (Unit/d)		0.0001		-0.05		-0.003		-0.2		0.01	
	D (Unit/m^2)		0.001		-0.01		0.003		-0.11		0.04	
FT	Concrete	1,829	0.803[b]	0.004	3.02	0.010	0.163[b]	0.002	6.81	0.033	2.41	0.145
	Dirt	1,780	0.818[a]	0.004	3.02	0.010	0.168[a]	0.002	6.80	0.033	2.40	0.145
	PA (Unit/d)		0.0001		-0.05		-0.003		-0.2		0.01	
	D (Unit/m^2)		0.001		-0.02		0.001		-0.11		0.03	
FL	First level	440	0.802[b]	0.009	3.09[a]	0.024	0.162	0.004	7.23[a]	0.075	2.15[b]	0.033
	Second level	530	0.850[a]	0.009	3.12[a]	0.026	0.164	0.004	7.19[a]	0.080	2.33[a]	0.036
	One level[6]	2,639	0.808[b]	0.003	2.98[b]	0.009	0.167	0.001	6.65[b]	0.027	2.40[a]	0.012
	PA (Unit/d)		0.0004		-0.05		-0.003		-0.2		0.02	
	D (Unit/m^2)		0.01		-0.02		0.001		-0.12		0.03	
S	Females	1,609	0.804	0.005	3.03	0.015	0.163	0.002	6.88[a]	0.047	2.53[a]	0.021
	Males	2,000	0.817	0.004	3.01	0.013	0.169	0.002	6.70[b]	0.040	2.18[b]	0.018
	PA (Unit/d)		0.001		-0.05		-0.002		-0.2		0.02	
	D (Unit/m^2)		0.01		-0.02		0.001		-0.14		0.03	
Sources of variation			P-values									
	PA		0.7273		< 0.0001		< 0.0001		< 0.0001		0.0122	
	D		< 0.0001		< 0.0001		0.0071		< 0.0001		< 0.0001	
	GR		0.0008		0.5477		0.0004		< 0.0001		0.0048	
	HT		< 0.0001		0.0003		0.0003		< 0.0001		0.0023	
	FT		0.0038		0.8485		0.0285		0.2072		0.2039	
	FL		< 0.0001		< 0.0001		0.2850		< 0.0001		< 0.0001	
	S		0.1280		0.3180		0.0504		0.0100		< 0.0001	
	GR x S		*		*		NS		***		*	
	HT x S		***		NS		NS		*		NS	
	FT x S		NS		NS		NS		NS		***	
	FL x S		*		NS		NS		NS		*	

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different (P < 0.05).

[1]Yield as the percent of eviscerated carcass weight per bird.

[2]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.
[3]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2
[4]Fat pad: abdominal fat excluding gizzard fat.
[5]House as experimental unit.
[6]Single-storey house
Probabilities of interactions: *P < 0.05; **P < 0.1; ***P < 0.0001; [NS]Not significant (P > 0.05).

Table 4.6 Significant interaction effects between house type (HT), and sex (S) at processing age[1]. Dependent variables adjusted to a common processing age and stocking density[2] with analysis of covariance.

HT	Open-sided		Open + Fans		Tunnel ventilated and foggers		Tunnel ventilated and cooling pad		Pooled SEM	P-values
S	Female	Male	Female	Male	Female	Male	Female	Male		
Carcass (g)	1,650^{e}	1,964^{b}	1,634^{e}	1,898^{c}	1,782^{d}	2,034^{a}	1,710^{d}	1,938^{b}	26.4	0.0314
Carcass (%)[3]	70.0^{b}	69.7^{b}	70.0^{b}	69.4^{c}	70.4^{b}	72.4^{a}	70.0^{b}	70.0^{b}	0.3	< 0.0001
Breast (g)[4]	683^{d}	771^{ab}	649^{e}	734^{c}	695^{d}	797^{a}	684^{d}	760^{b}	12.5	0.0298
Breast filet (g)[5]	375^{c}	441^{a}	374^{c}	420^{b}	421^{ab}	428^{ab}	384^{c}	438^{a}	9.6	0.0088
Breast filet (%)[5,6]	22.8^{ab}	22.2^{bc}	22.9^{a}	22.0^{cd}	23.1^{a}	21.4^{d}	22.5^{abc}	22.6^{ab}	0.6	0.0065
Thighs (g)	312^{c}	370^{a}	302^{d}	348^{b}	373^{a}	378^{a}	320^{c}	372^{a}	6.8	0.0025
Thigh (%)[6]	18.8^{b}	18.7^{b}	18.5^{bc}	18.3^{c}	21.1^{a}	18.7^{bc}	18.8^{bc}	18.7^{bc}	0.3	0.0025
Drums (g)	232^{f}	296^{a}	241^{e}	286^{b}	246de	272^{c}	248^{d}	292^{a}	5.0	< 0.0001
Drum (%)[6]	14.1^{c}	15.1^{a}	14.7^{b}	15.1^{a}	13.8^{cd}	13.5^{d}	14.4^{bc}	15.1^{a}	0.3	0.0009
Wings (g)	192^{d}	235^{a}	194^{d}	223^{b}	197^{cd}	222^{b}	200^{c}	222^{b}	3.6	< 0.0001
Wing (%)[6]	11.6^{bc}	12.0^{a}	12.0^{a}	11.9^{ab}	11.2^{de}	11.0^{e}	11.8^{ab}	11.4^{cd}	0.2	0.0375
Water absorption (%)[7]	8.7^{b}	7.3^{c}	9.6^{a}	7.4^{c}	8.0^{bc}	6.6^{c}	9.7^{a}	7.0^{c}	0.7	0.0208
Heart (%)[6]	0.876^{a}	0.837^{bc}	0.802^{d}	0.814^{cd}	0.901^{a}	0.797^{cde}	0.772^{e}	0.855^{ab}	0.021	< 0.0001
Intestine (%)[6]	6.52^{b}	6.48^{b}	6.90^{a}	6.81^{a}	6.33^{ab}	5.32^{c}	6.96^{a}	6.49^{b}	0.186	0.0128

[a-e]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2

[5]Yield as the percent of live BW per bird.

[4]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[5]Breast filet: Pectoralis major.

[6]Yield as the percent of eviscerated carcass weight per bird.

[7]Percent of increase in the carcass weight by moisture retention after pre-chilling and chilling.

Table 4.7 Pearson correlation coefficients between carcass parts at processing age[1] of broilers.

		Breast[2]		Carcass		Breast filet[3]		Thighs		Drums		Wings	
		Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield	Weight	Yield
Breast	Weight	1.000	-	-	-	-	-	-	-	-	-	-	-
	Yield	0.295**	1.000	-	-	-	-	-	-	-	-	-	-
Carcass	Weight	0.938**	-0.048*	1.000	-	-	-	-	-	-	-	-	-
	Yield	0.563**	0.158**	0.537**	1.000	-	-	-	-	-	-	-	-
Breast filet	Weight	0.880**	0.213**	0.841**	0.559**	1.000	-	-	-	-	-	-	-
	Yield	0.232**	0.467**	0.072**	0.249**	0.591**	1.000	-	-	-	-	-	-
Thigh	Weight	0.799**	-0.019^{NS}	0.841**	0.428**	0.725**	0.090**	1.000	-	-	-	-	-
	Yield	-0.110**	0.054*	-0.137**	-0.116**	-0.084**	0.041*	0.411**	1.000	-	-	-	-
Drum	Weight	0.803**	-0.103**	0.876**	0.351**	0.693**	-0.016^{NS}	0.777**	-0.052*	1.000	-	-	-
	Yield	-0.084**	-0.126**	-0.051*	-0.273**	-0.129**	-0.175**	0.037*	0.145**	0.431**	1.000	-	-
Wings	Weight	0.779**	-0.117**	0.853**	0.383**	0.701**	0.018^{NS}	0.757**	-0.049*	0.835**	0.139**	1.000	-
	Yield	-0.399**	-0.127**	-0.383**	-0.352**	-0.361**	-0.122**	-0.259**	0.173**	-0.184**	0.346**	0.144**	1.000

*P < 0.05; **P < 0.0001

^{NS}Not significant (P > 0.05).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[1]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[2]Breast filet: Pectoralis major.

Table 4.8 Main effects and covariate coefficients of geographical region (GR), house type (HT), house floor type (FT), house floor level (FL), and sex (S) on bone breaking strength and bone breaking stress at processing age (PA)[1]. Dependent variables adjusted to a common PA and stocking density (D)[2] with analysis of covariance.

Effect			Bone Breaking Strength[3]	SEM	Bone Breaking Stress[3]	SEM
		n[4]	KgF		g/mm^2	
GR	Coffee Region	395	22.4	0.35	235.3	5.1
	Valle	432	22.2	0.32	233.6	4.2
	PA (Unit/d)		0.872		0.843	
	D (Unit/m^2)		-0.0005		0.391	
HT	Open	86	22.7^{ab}	0.73	207.1^{b}	7.6
	Open + Fans	661	22.0^{b}	0.26	235.3^{a}	2.8
	Tunnel ventilated and foggers	15	26.1^{a}	1.88	266.1^{a}	19.6
	Tunnel ventilated and cooling pad	65	24.8^{a}	1.04	207.1^{b}	10.8
	PA (Unit/d)		0.899		1.412	
	D (Unit/m^2)		-0.34		-2.235	
FT	Concrete	415	22.4	0.34	231.6	3.5
	Dirt	412	22.2	0.33	236.4	3.5
	PA (Unit/d)		0.873		0.698	
	D (Unit/m^2)		0.003		0.405	
FL	First level	99	20.9	0.77	250.5	8.0
	Second level	126	21.7	0.78	230.4	8.1
	One level[5]	602	22.4	0.28	230.2	2.9
	PA (Unit/d)		0.884		0.469	
	D (Unit/m^2)		-0.016		1.775	
S	Females	360	21.8	0.48	229.7	5.0
	Males	467	22.8	0.40	238.5	4.2
	PA (Unit/d)		0.866		0.665	
	D (Unit/m^2)		0.013		0.64	
Sources of variation			P-values			
	PA		< 0.0001		0.5098	
	D		0.9927		0.8182	
	GR		0.7449		0.7419	
	HT		0.0210		0.0002	
	FT		0.7360		0.3367	
	FL		0.1378		0.0573	
	S		0.1841		0.2626	
	GR x S		NS		**	
	HT x S		NS		NS	
	FT x S		NS		NS	
	FL x S		NS		NS	

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.
[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2
[3]Measurements taken from right tarsometatarsus (shank) with flesh and skin intact.
[4]House as experimental unit.
[5]Single-storey house
Probabilities of interactions: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant ($P > 0.05$).

Table 4.9 Significant interaction effects between house floor type (FT), and sex (S) at processing age[1]. Dependent variables adjusted to a common processing age and stocking density[2] with analysis of covariance.

FT	Concrete		Dirt		Pooled SEM	P-values
S	Female	Male	Female	Male		
Carcass (g)	1,630[c]	1,935[a]	1,634[c]	1,900[b]	7.3	0.0018
Carcass (%)[3]	69.7[b]	69.5[b]	70.1[a]	69.6[b]	0.08	0.0122
Breast (g)[4]	648[c]	755[c]	648[c]	736[b]	3.6	0.0015
Thigh (g)	308[b]	355[a]	298[c]	352[a]	2.0	0.0467
Thigh (%)[5]	18.9[a]	18.4[bc]	18.3[c]	18.6[b]	0.08	< 0.0001
Drum (g)	239[c]	291[a]	238[c]	285[b]	1.3	0.0314
Wing (%)[5]	12.1[a]	11.8[b]	11.8[b]	11.8[b]	0.05	0.0001
Water absorption (%)[6]	10.0[a]	7.2[c]	9.2[b]	7.4[c]	0.1	< 0.0001
Fat Pad yield (%)[5,7]	2.60[a]	2.15[d]	2.48[b]	2.21[c]	0.023	< 0.0001

[a-c]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2

[3]Yield as the percent of live BW per bird.

[4]Breast: Pectoralis major, Pectoralis minor, bone and skin included.

[5]Yield as the percent of eviscerated carcass weight per bird.

[6]Percent of increase in the carcass weight by moisture retention after pre-chilling and chilling.

[7]Fat pad: abdominal fat excluding gizzard fat.

Table 4.10 Significant interaction effects between house floor level (FL), and sex (S) at processing age[1]. Dependent variables adjusted to a common processing age and stocking density[2] with analysis of covariance.

	FL	First level		Second level		One level		Pooled SEM	P-values
	S	Female	Male	Female	Male	Female	Male		
Feed Intake (g)		4235^{c}	4460^{b}	4281^{c}	4589^{a}	4268^{c}	4616^{a}	26.6	0.0177
FCR (g:g)		1.845^{a}	1.677^{b}	1.801^{a}	1.706^{b}	1.843^{a}	1.707^{b}	0.043	0.0560
Carcass (%)[3]		69.9^{ab}	69.4^{cd}	68.7^{e}	69.2^{d}	70.1^{a}	69.6^{bc}	0.012	0.0008
Breast filet (g)[4]		366^{d}	422^{ab}	372^{cd}	435^{a}	376^{c}	422^{b}	6.9	0.0800
Thighs (g)		293^{e}	368^{a}	295de	345^{c}	300^{d}	356^{b}	4.9	0.0038
Thigh (%)[5]		18.4^{bc}	19.4^{a}	18.6^{bc}	18.3^{c}	18.6^{bc}	18.5^{b}	0.13	< 0.0001
Drums (g)		241^{b}	284^{a}	229^{c}	286^{a}	240^{b}	288^{a}	2.24	0.0491
Wing (%)[5]		12.2^{a}	11.8^{b}	11.9^{b}	11.7^{b}	11.9^{b}	11.8^{b}	0.13	0.0829
Heart (%)[5]		0.798^{cd}	0.806^{bcd}	0.875^{a}	0.826^{b}	0.787^{d}	0.818^{bc}	0.019	0.0019
Fat pad (%)[5,6]		2.39^{b}	1.91^{e}	2.60^{a}	2.07^{d}	2.58^{a}	2.22^{c}	0.057	0.0261

[a-e]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The overall average processing age was 40.8 d for females with a range of 37 to 47 d, and 45.0 d for males with a range of 40 to 48 d.

[2]The overall average stocking density was 13.2 bird/m^2 for females with a range of 9.2 to 19.8 bird/m^2, and 11.6 bird/m^2 for males with a range of 7.9 to 16.6 bird/m^2

[3]Yield as the percent of live BW per bird.

[4]Breast filet: Pectoralis major.

[5]Yield as the percent of eviscerated carcass weight per bird.

[6]Fat pad: abdominal fat excluding gizzard fat.

4.6 FIGURES

Figure 4.1. Open-sided house.

Figure 4.2. Open-sided house with fans.

Figure 4.3. Tunnel-ventilated house with foggers.

Figure 4.4. Two-storey house.

5. EFFECTS OF DIETARY 25-OH-D_3, CANTHAXANTHIN AND ITS COMBINATION IN BROILERS

ABSTRACT

The effects of 25-hydroxy vitamin D_3 (25-OH-D_3), canthaxanthin (CX), alone and in combination on performance, yield, oxidative status, skin colour and bone characteristics of broiler chickens were studied. A total of 657 broiler chicks were randomly allocated to 63 floor pens. Each pen was randomly assigned to 1 of 7 dietary treatments: Control (2,760 IU of vitamin D_3/kg of feed from d 0 to 40); 25D (69 μg/kg 25-OH-D_3 from d 0 to 40); CX (Control + 6 mg/kg CX from d 0 to 40); 25DCX (25D diet + 6 mg/kg CX, from d 0 to 40); 25D-Early (25D diet from 0 to 19 d; Control diet thereafter); CX-Early (CX diet from 0 to 19 d; Control diet thereafter); 25DCX-Early (25DCX diet from 0 to 19 d; Control diet thereafter). BW, feed intake, FCR, breast weight, skin and meat colour, and bone breaking strength were assessed at d 11, 19 and 39. At those same ages, mineral density and cross sectional areas were measured from the right femur using quantitative computed tomography. Oxidative status, measured as malondialdehyde (MDA) concentration was determined from liver samples at d 11 and 19, and from meat at d 39. Birds were processed at d 40 and carcass and portion yields measured. Data were analyzed as a one way analysis of variance. For bone characteristics BW was included as covariate. Diets containing CX increased BW and reduced FCR at d 11. At 19 and 39 d, diets containing CX had a tendency to increase Pectoralis major weights ($P < 0.1$). CX increased redness and yellowness of shank, breast skin and breast muscle; this effect was most pronounced when CX was fed for the entire trial. At 11 and 19 d, MDA was lower in birds fed diets containing CX relative to the other treatments. At d 19, trabecular bone cross-sectional area (at 30% of total femur length from the proximal epiphysis) of 25DCX and 25DCX-Early treatments was greater than 25D, CX and 25D-Early,

possibly indicating a synergy between 25-OH-D_3 and CX. At d 39, 25D-Early and 25DCX-Early increased bone breaking strength relative to the other treatments, likely due to the inclusion of 25-OH-D_3. Increased productivity at early ages in the treatments containing CX may have been due to increased antioxidant status. Dietary CX did not have a residual effect on skin or meat colour 20 d after withdrawal.

Key words: 25-hydroxy vitamin D_3, canthaxanthin, performance, bones, TBARS.

5.1 INTRODUCTION

Vitamin D_3 is absorbed in the upper intestine and metabolized into 25-OH-D_3 (calcidiol) by the action of 25-hydroxylase in the liver, and subsequently in the kidneys into its active form 1,25-$(OH)_2$-D_3 (calcitriol) by the action of 1α-hydroxylase, a step that is highly regulated (Bar et al., 1980; Soares et al., 1995). The most predominant circulating form of vitamin D_3 is 25-OH-D_3 (Bar et al., 1980). In comparison to vitamin D_3, 25-OH-D_3 is more biologically active per molecule (Ledwaba and Roberson, 2003), has a greater retention (Bar et al., 1980), lower excretion (Chou et al., 2009), and is absorbed by the intestine more efficiently (Phadnis and Nemere, 2003). At early ages, due to immaturity of the digestive tract and enzymatic systems of chicks (Noy and Sklan, 1995), vitamin D_3 absorption can be reduced. Situations where liver function is affected such as heat stress (Thaxton and Puvadolpirod, 2000), mycotoxicosis (Yarru et al., 2009), and bacterial infection (Peighambari et al., 2000) may reduce endogenous production of 1,25-$(OH)_2$-D_3. The effect of these situations on the bird may be reduced when 25-OH-D_3 is supplied in the diet, because the initial conversion in the liver is bypassed (Soares et al., 1995), and its absorption is less dependent on bile salt secretion and fat absorption (Compston et al., 1981, Borel, 2003). Dietary 25-OH-D_3 increased broiler performance (Yarger et al., 1995; Fritts and Waldroup, 2003), and meat yield (Saunders-Blades, 2008; Brito et al., 2010; Vignale et al., 2013) when used as a

partial or sole source of vitamin D_3 during the grow-out period or at early ages. Supplemental 25-OH-D_3 increased bone ash (Fritts and Waldroup, 2003), bone breaking strength (Saunders-Blades, 2008), and reduced severity of tibial dyschondroplasia (Parkinson and Cransberg, 2004) when compared to dietary vitamin D_3 at the same level of inclusion.

Canthaxanthin (CX) is an oxycarotenoid that increases serum total antioxidant capacity, reduces lipid peroxidation (Surai et al., 2003; Rosa et al., 2012; Rocha et al., 2013), is a potent free radical scavenger, and increases chick livability through enhanced antioxidant status (Zhang et al., 2011). Carotenoids have been used in poultry for egg and skin pigmentation; but no reports show effects on meat yield. Dietary lutein, another oxycarotenoid, increased BW (Rajput et al., 2012), and reduced inflammatory response as a result of a higher antioxidant status (Shanmugasundaram and Selvaraj, 2011). In vitro, differentiation of mouse osteoblastic cells was increased by CX (Park et al., 1997); therefore carotenoids ca be involved in bone metabolism.

A combination of 6 mg CX and 69 μg 25-OH-D_3 per kg of complete feed (MaxiChick® (MC); DSM Nutritional Products; (DSM Nutritional Products, Basel, Switzerland) has been studied in broiler breeders (Rosa et al., 2010a; 2010b). Hatchability was increased, and embryo mortality and oxidative status were reduced in hatching eggs from hens supplemented with MC relative to hatching eggs from the control group (Rosa et al., 2010b). This combination increased sperm quality in roosters (Rosa et al., 2010a), and phagocytic capacity in chicks (Cho et al., 2013). To date, there are no reports about the effect of the dietary combination of CX and 25-OH-D_3 on broiler performance. Therefore, the aim of the present research was to determine the effect of dietary 25-OH-D_3, CX and its combination on performance, meat yield, skin colour, oxidative status and bone characteristics of broilers under controlled environmental conditions. It was

hypothesized that those traits would increase with dietary supplementation of 25-OH-D_3 and CX together and that there would be a synergy or additive effect between them.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Diets

Corn-soy diets were formulated to meet or exceed current NRC recommendations (National Research Council, 1994) and were based on the primary breeder recommendations for Ross 308 mixed-sex broilers. A three phase feeding program with starter (0 to 11 d), grower (12 to19 d) and finisher (20 to 39 d) phases was used (Table 5.1). For each of the 3 phases, a basal diet without supplemental vitamin D activity was mixed. Each of the seven dietary treatments was randomly distributed among the 63 pens. The Control treatment received a diet containing 2,760 IU of vitamin D_3 per kg of feed from 0 to 39 d. The 25D treatment received 69 μg of 25-OH-D_3 per kg of feed, which is the equivalent of 2,760 IU of vitamin D_3 activity (NRC, 1994), from 0 to 39 d. The CX treatment received Control diet plus 6 mg of CX per kg of feed from 0 to 39 d. The 25DCX treatment received the 25D diet plus 6 mg of CX per kg of feed from 0 to 39 d. The 25D-Early treatment received the 25D diet during the starter and grower phases (from 0 to 19 d) and then the Control diet during the finisher phase (from 20 to 39 d). The CX-Early treatment, received the CX diet during the starter and grower phases (from 0 to 19 d) and then the Control diet during the finisher phase (from 20 to 39 d). The 25DCX-Early treatment received the 25DCX diet during the starter and grower phases (from 0 to 19 d), and then the Control diet during the finisher phase (from 20 to 39 d). Each dietary treatment was replicated 9 times. The CX and 25-OH-D_3 were supplied by DSM Nutritional Products, Parsippany, NJ.

5.2.2 Experimental Conditions

All experimental procedures were approved by the University of Alberta Animal Care and Use Committee in accordance with the Canadian Council of Animal Care (1993) guide.

The experiment was conducted as a completely randomized design. A total of 657 broiler chicks (Ross 308) were obtained from a commercial hatchery, manually feather-sexed, individually tagged, and randomly allocated to 63 floor pens at a rate of 10 to 11 chicks per pen (2.8 to 3.1 bird/m^2) and grown for 39 d.

All pens were bedded with clean wood shavings before the start of the experiment. Birds were raised in a light-tight barn with incandescent lighting, providing 20L:4D during the entire growing period. Temperature was managed according to commercial primary breeder recommendations for Ross 308 (Aviagen, 2009). Chicks had *ad libitum* access to feed (mash form) and water (nipple drinkers).

5.2.3 Data Recorded

5.2.3.1 Performance and carcass measures

At days 11, 19 and 39, BW on a pen basis was obtained and feed consumption measured for the starter, grower and finisher phases, respectively. Feed conversion ratio (g feed/g gain) was calculated and corrected for mortality and culled birds. At the same ages, two birds (one female, and one male) from each pen were randomly selected, euthanized and individual weights of Pectoralis major (P. major), and Pectoralis minor (P. minor) were obtained. Breast meat was calculated as the sum of the two muscles; yield was calculated as a proportion of live BW [(breast portion / live BW) × 100 %]. Bird sex was visually confirmed at dissection. Liver and right P. major, were stored at -20 ^{o}C until analysis for antioxidant status.

At 40 d of age, the remaining birds (n = 249) were processed. Feed and water were withdrawn for 12 h prior to slaughter. The birds were electrically stunned and then bled for 2 min. After scalding for 45 s (scald temperature of 52 to 53°C to allow retention of the epidermis), carcasses were mechanically defeathered, manually eviscerated, and carcass traits assessed. Individual weight of total carcass, P. major, P. minor, wings, thighs and drums were obtained. Carcass yield was calculated as the percent of live BW [(carcass weight / live BW) × 100 %]. Percent of yield for each carcass component was calculated as the percent of eviscerated carcass [(carcass portion / eviscerated carcass weight) × 100 %],

5.2.4 Skin and meat colour measures

At days 11, 19, and 39, skin colour was measured at the right lateral shank surface and the right medial breast surface using a Minolta Chroma meter CR-300 and according to the method described by Castañeda et al. (2005), and Huezo et al. (2007; Chapter 3). At those same ages, meat colour measurements were conducted on the right medial surface of the P. major as described by Petracci et al. (2001). At processing, breast skin and breast meat surface colour was measured after evisceration. The colorimeter was calibrated throughout the trial using a standard white ceramic tile. The colour was expressed in terms of CIE colour system values (Commission Internationale de l'Eclairage) for lightness (L*), redness (a*), and yellowness (b*).

5.2.5 Bone characteristics

5.2.5.1 Femur Cross-sectional Area and Bone Mineral Density

At days 11, 19, and 39, the right femur (with no flesh) was collected from the sampled birds and stored at -20 ^{o}C until analysis for bone characteristics. Femur samples were thawed at 4 to 6 ^{o}C for 24 hours, and bone mineral density (BMD) and cross-sectional area (CSA) were measured (Riczu et al., 2004) using Quantitative Computed Tomography with a Stratec Norland

XCT Research scanner (Norland Medical Systems, Inc., Fort Atkinson, WI) having a 50 kV x-ray tube. The scans were performed at 30% and 50% of the total bone length from the proximal epiphysis. Total, cortical, and trabecular bone densities and areas were determined using Norland XMENU software version 5.40C (Norland Medical Systems, Inc., Fort Atkinson, WI). Total, cortical and trabecular bone mineral content (BMC; mg/cm; the amount of bone mineral in each 1 mm scan, extrapolated to a 1 cm length of bone) were calculated by multiplying the bone density (mg/cm^3) by the cross sectional area (cm^2). The bones were then stored at -20 ^oC until analysis for femur breaking strength.

5.2.5.2 Femur Breaking Strength

Bone breaking strength (BBS) was determined for the same bones using an Instron Materials Tester (Model 4411, Instron Corp., Canton, Ma, USA) with Automated Materials Test System software version 8.09, a standard 2 kN load cell, and a modified sheer plate (8 cm in length and 1 mm in width). The method was based on the 3-point bending previously described by Patterson et al. (1986). Before breaking, the bones were thawed at 4 to 6 ^oC for 24 hours and marked at the midpoint (determined with a digital caliper). Supports were positioned at bone epiphysis and a force was applied at the midpoint of each bone with a crosshead speed of 100 mm/min. The distance between supports was 14 mm for bones of broilers at 11 d of age, 22 mm at 19 d of age, and 32 mm at 39 d of age.

5.2.6 Antioxidant status

Oxidative status in the liver and breast meat was determined by measuring malondialdehyde (MDA) concentrations, through the thiobarbituric acid reactive substances (TBARS) assay. Briefly, 8 livers per treatment from the birds sampled at d 11 and d 19, and 8 breast meat samples per treatment from birds sampled at d 39 were thawed at 4 to 6 ^oC for 24

hours. From each sample, 3 g were weighed into a 50 mL plastic tube and homogenized in 25 mL of 1.15% KCl for 30 s at 9,500 rpm using a PowerGen™ 1000 homogenizer (Fisher Scientific™, Pittsburgh, PA, USA). A 200 µL aliquot of the homogenate was mixed with 1,000 µL of 80 mM Tris/maleate buffer (pH 7.4), 400 µL of 2.5 mM ascorbic acid and 400 µL of 50 µM ferrous sulphate and incubated for 150 min in a 37°C water bath. Then, a total of 4 mL of 26 mM TBA, 0.92 M TCA and 0.8 mM HCl were added; this mixture was then incubated in a water bath at 94 °C for 15 min. After cooling down, the supernatant was collected by centrifugation at 2,000 x g for 5 min. The absorbance of the final solution was determined at 532 nm against a blank that contained all the reagents except the homogenate using UV/VIS Spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan) and the TBARS were expressed as nmol malonaldehyde/g of tissue (liver or meat).

5.2.7. Statistical Analysis

The pen was the experimental unit for production data. The individual bird was the experimental unit for skin and breast colour, bone breaking strength, BD and area, TBARS, and processing data. All data were analyzed as a one way analysis of variance with 7 dietary treatments using the procedure for linear mixed models (PROC MIXED) of SAS (SAS 9.3© for Windows; SAS Institute Inc., Cary, NC). Bone trait data were analyzed including BW as the covariate. Differences between means were classified by pairwise comparisons, and unless otherwise noted, differences were considered significant at $P < 0.05$. For production performance, carcass traits, skin and meat colour, and lipid oxidation the ANOVA was in accordance with the following model:

$$Y_{ij} = \mu + DT_i + S_j + (DTS)_{ij} + E_{ij},$$

where μ was the population mean; DT_i was the effect of each dietary treatments (i = 1 to 7); S_j was the effect of bird sex (j = 1 to 2); $(DTS)_{ij}$ was the interactions of each dietary treatment with sex; and E_{ij} was the residual error.

For bone characteristics BW was included as covariate and the ANCOVA was in accordance with the following model:

$$Y_{ij} = \mu + DT_i + S_j + (DTS)_{ij} + \beta\ (BW_{ij} - BWa) + E_{ij},$$

where μ was the population mean; DT_i was the effect of each dietary treatments (i = 1 to 7); S_j was the effect of bird sex (j = 1 to 2); $(DTS)_{ij}$ was the interactions of each dietary treatment with sex; $\beta\ (BW_{ij} - BWa)$ was a covariate coefficient multiplied by the difference between individual BW (BW_{ij}) and average BW (BWa); and E_{ij} was the residual error. Pearson correlation coefficients and regression were analyzed for BMD, CSA and BMC using the CORR and REG procedures of SAS (SAS 9.3© for Windows; SAS Institute Inc., Cary, NC).

5.3 RESULTS AND DISCUSSION

5.3.1 Production performance

Broiler BW was similar among dietary treatments at d 0 (Table 5.2). At d 11, CX, CX-Early, and 25DCX-Early had higher BW than all other treatments except 25DCX. Therefore, at early ages birds fed any diet containing CX were heavier than other treatments. Similarly, BW gain was greater in birds fed with diets that contained CX alone or in addition to 25-OH-D_3. At the end of the starter phase (d 11), the lowest FCR was in 25DCX and 25DCX-Early; in contrast the Control group had the highest FCR, but it was not different from 25D-Early (Table 5.2).

In this study, dietary 25-OH-D_3 alone, either for the first 21 days, or through the entire grow-out period did not affect performance relative to the Control diet, which contained the same level of vitamin D_3 activity. Previous studies did not show differences between dietary 25-OH-D_3

and vitamin D_3 compared at the same level of activity in broiler performance, including at levels similar to those used in this study (2,760 IU/kg of feed; Bar et al., 2003; Angel et al., 2006; Roberson et al., 2005; and Fritts and Waldroup, 2005). In contrast, other authors have reported an increased broiler performance in birds fed dietary 25-OH-D_3 relative to birds fed vitamin D_3 especially when included at early ages (Yarger et al, 1995; Fritts and Waldroup, 2003; Parkinson and Cransberg, 2004; Saunders-Blades, 2008), when birds are considered more sensitive to the sources and levels of vitamin D_3; or when Ca and P are restricted in the diet (Bar et al., 2003; Ledwaba and Roberson, 2003).

At early ages, CX increased BW gain and feed efficiency in this study. However, broiler performance was not affected by dietary CX supplementation at 2 mg/kg (Jensen, 1998), at 2 or 5 mg/kg of feed (Perez-Vendrell et al., 2001), at 6 mg/kg of feed (Chapter 2), nor even at 25, 50 and 100 mg/kg of feed (Tunio et al., 2013). The dissimilarity in the results of supplemental CX among the different studies may be explained by differences in CX source and in the contents of other ingredients such as vitamins in basal feeds used. Dietary lutein (oxycarotenoid) from 4 to 42 d at 200 mg/kg of feed, increased BW as compared to the control and other levels of inclusion (100 or 150 mg/kg of feed), and it was proposed that that this effect might be related to a higher antioxidant status (Rajput et al., 2012). In 50-d-old turkeys injected with lipopolysaccharide, dietary lutein (50 mg/kg, from 1 d of age) reduced MDA concentration (700 nmol/g) of liver samples in comparison to the control group (1,400 nmol/g; Shanmugasundaram and Selvaraj, 2011). The same study showed that after lipopolysaccharide injection, Interleukin-1ß mRNA amount was lower and BW was not reduced in birds fed lutein; these authors related those effects to the higher antioxidant status of the birds fed lutein. Therefore, the increase in the early broiler performance found in the current study was likely related to a higher antioxidant by supplemental CX.

5.3.2 Carcass characteristics

From the birds sampled at d 11, the lowest BW was found in the Control treatment, which was not different from 25D and 25D-Early (Table 5.3). This finding is similar to what was seen in the production performance analysis where the treatments that contained CX had higher BW during the starter phase than the other treatments with no CX. Within each dietary phase and at processing age, weights and yields of breast, P. major, and P. minor of sampled birds were similar between all treatments (Table 5.4; Table 5.5; Table 5.6; Table 5.7). However, there was a trend in treatments that contained CX either alone or in addition to dietary 25-OH-D_3, to have higher P. major weights at d 19 and d 39 than the other treatments ($P < 0.10$; Table 5.4).

The only treatment differences in meat yield were observed in legs. At processing, birds fed 25DCX-Early had the lowest leg yield but not different to 25DCX; the highest leg yield was seen in CX, which was not different from the 25D, 25D-Early, and CX-Early treatments (Table 5.7). Dietary 25DCX inclusion during the first 3 weeks of age reduced carcass and drum weights of males, and drum yield of both sexes in strain A, but had no effect in strain B (Chapter 3). Previous publications have concluded that carotenoids, including CX, added to basal broiler diets did not increase meat yield (Pérez-Vendrell et al., 2001; Martínez-Peña et al., 2004; Castañeda et al., 2005; Muñoz-Díaz et al., 2012). From our data, it seems that the combination of CX and 25-OH-D_3 reduces meat yield in the posterior half of the carcass. As this combination has been tested mainly in broiler breeders, further studies are needed to confirm and clarify the reason of this effect.

Previous reports have shown a higher meat yield in broiler fed 25-OH-D_3 throughout the grow-out period in comparison to vitamin D_3 at the same levels of inclusion (Yarger et al., 1995; Saunders-Blades, 2008; Brito et al., 2010; Vignale et al., 2013). However, similar to the present

study, other authors have also not found any difference in meat yield in birds fed 25-OH-D_3 as compared to vitamin D_3 at several dietary levels, including the same level as used in the present study (Angel et al., 2006; Michalczuk et al., 2010). It has been proposed that 25-OH-D_3 has a higher biopotency than vitamin D_3 when other nutrients such as Ca and P are restricted (Bar et al., 2003; Ledwaba and Roberson, 2003). Therefore, the lack of effect of 25-OH-D_3 in the present study may indicate that nutrients, housing and handling conditions did not cause any pressure on the metabolism of the bird, which did not allow us to confirm the greater biopotency of 25-OH-D_3 through a higher meat yield when used at 2,760 IU/kg of vitamin D_3 activity.

At d 19, d 39 and at processing age, males had heavier BW, breast, P. major, leg, wing and liver weights compared to females (Table 5.3; Table 5.4; Table 5.5). At d 39, P. minor was significantly heavier in males than females, and nearly significantly at d 19 ($P < 0.10$). At d 11 there were no differences between sexes in these traits. At d 19, females had a nearly significantly higher P. minor yield than males (Table 5.6). In females, at d 39 breast and P. minor yield was greater in comparison to males (Table 5.6). At processing age, females had higher yield of breast, P. major and P. minor, but lower leg yield than males (Table 5.7). Others have also reported similar differences between sexes in these traits (Abdullah et al., 2010; López et al., 2011; Brewer et al., 2012; Shim et al., 2012b). Those differences have been related to the sexual dimorphism and the different growth dynamics between sexes (Fanatico et al., 2005; Zuidhof, 2005; Murawska et al., 2011).

At d 19 and d 39, absolute liver weight was higher in males than females (Table 5.4). Similarly, Murawska et al., 2011 reported an average higher liver size in males than females during the first 10 weeks of life. However, at d 39 females had higher relative liver weight than males. These differences were attributed to the sexual dimorphism in broilers.

5.3.3 Skin and Meat Colour

Values of lightness (L*), and yellowness (b*) from the shank skin were numerically higher than those taken from breast skin and breast meat; similar observations have been reported by others (Pérez-Vendrell et al., 2001; Liu et al., 2008).

At d 11, shank skin lightness was not different between treatments (Table 5.8). At d 19, the highest shank skin lightness was seen in the 25D-Early, which was not different to the Control and 25D treatments. Diets that contained CX resulted in lower shank skin lightness than other treatments. At d 39, the lowest shank skin lightness was seen in 25DCX, which was not different than the CX treatment. Dietary carotenoid supplementation for broilers reduced shank skin lightness, meaning that the shanks look darker (Pérez-Vendrell et al., 2001). In all the evaluations of breast skin and breast meat, lightness was similar among treatments. (Table 5.8; Table 5.9; Table 5.10; Table 5.11). Similarly, no differences in breast skin lightness were seen between different sources and levels of dietary carotenoids in the past (Martínez-Peña et al., 2004). Therefore, in the present study, neither breast skin nor breast meat was affected by dietary treatments.

Carotenoid CX is a red xanthophyll (Pérez-Vendrell et al., 2001) that can be synthetically produced (EFSA, 2010). After absorption, CX is rapidly deposited in broiler tissues such as shank and breast skin, principally in the subcutaneous adipose layer (Pérez-Vendrell et al., 2001; Liu et al., 2008). At d 11, and d 19 d a higher shank skin redness was found in diets that contained CX alone or in combination with 25-OH-D_3 (Table 5.8). At d 39, the highest shank redness was seen in CX and 25DCX (Table 5.8). In breast skin and breast meat, there were differences in redness between dietary treatments only at d 40 (Table 5.11). At that age, diets that included CX alone or in addition to 25-OH-D_3 had higher redness of breast skin than other treatments; CX and 25DCX

had the higher redness of breast meat. Frequently, consumers base their food selection, including poultry products, on its appearance. Consumer preference for broiler skin colour varies from white, to pale yellow, to deeply pigmented, it depending on traditional regional supplies (Fletcher, 2002). For that reason, yellowness is the parameter in skin and meat colour evaluations that best illustrates visual differences in broilers (Sirri et al., 2010). At d 11, the higher yellowness of shank and breast skin, and breast meat was found in diets that contained CX either alone or in combination with 25-OH-D_3 (Table 5.8; Table 5.9; Table 5.10). This finding is similar to what was observed in shank skin redness at the same age. At d 19, shanks and breast skin were less pale (lower yellowness) in Control and 25D relative to the other treatments (Table 5.8; Table 5.9). In addition, at this age breast meat was more pale (higher yellowness) in birds fed diets that contained CX either alone or in addition to 25-OH-D_3 (Table 5.10). At d 39 and at processing age, CX and 25DCX had higher yellowness as compared to other treatments (Table 5.10; Table 5.11). A similar situation was found in breast skin at processing (d 40). However, at d 39, 25D had similar yellowness values to CX and 25DCX (Table 5.9). It is likely, that breast skin yellowness at d 39 were not repeated at d 40 (Table 5.11), because the scalding and plucking procedures have the potential to increase epidermis removal, and affect skin pigmentation (Heath and Thomas, 1973; Petracci and Fletcher, 2002).

Despite being a red pigment, in this trail CX increased shank and breast skin yellowness, and breast muscle yellowness. The CX used in this trial had concentration of 10% in a form of beadlet of cornstarch-coated vegetable matrix; however, the effect of the carrier on pigmentation is not considerable. As corn has yellow pigments, this situation may have been involved in this effect. Previous reports have shown that CX in combination with natural sources of yellow pigments (e.g. yellow corn as in this trial) increased pigment intensity and yellowness producing

a more orange colour tonality (Perez-Vendrell et al., 2001; Castañeda et al., 2005). The effect of CX on yellowness and redness was mainly detected when was supplemented during the whole production cycle; therefore, in the current study at 6 mg/kg of feed, CX did not have a residual effect after 20 d withdrawal.

At d 11, shanks, breast skin and breast meat were darker (lower lightness) in females than males; the same effect was seen in breast skin at d 19 and d 39, and in breast meat at d 39 and at processing age (Table; 5.8; Table 5.9; Table 5.10; Table 5.11). There were no differences in redness and yellowness of shank skin between sexes (Table 5.8). At d 11, breast skin and breast meat redness were higher in females (Table 5.9; Table 5.10); but at processing redness was higher in males than females (Table 5.11). Similar differences in lightness and redness were reported by Sirri et al. (2010) in yellow-skinned broilers at processing, but were not considered of industry relevance because those differences are not be perceived by the consumer if they are not quantified by a special tools.. At d 19, d 39 and at processing age, breast skin and breast meat were paler in females than males (Table 5.9; Table 5.10; Table 5.11); that is in agreement with Sirri et al. (2010) in yellow-skinned broilers. That characteristic has been related to the greater capacity of females to deposit fat, especially sub-cutaneously (Murawska et al., 2011), since skin pigmentation is linked to subcutaneous deposition of fat (Petracci and Fletcher, 2002).

5.3.4 Effect on Lipid Oxidation in Liver and Breast meat

Excessive levels of oxidants such as free radicals can induce tissue damage (Iqbal et al., 2002), oxidize lipids (Smet et al., 2008; Tavárez et al., 2011), protein (Iqbal et al., 2004), DNA (Voljč et al., 2011), and reduce broiler performance and meat stability (Mujahid et al., 2005). The MDA concentration has been used as a biomarker of lipid peroxidation (Mateos and Bravo, 2007; Zhang et al., 2011). In the present study, the liver was chosen to evaluate lipid oxidation through

MDA levels because carotenoids are found in relatively high concentrations and because it is highly susceptible of lipid peroxidation due to due to its higher fat content in comparison to other tissues (Woodall et al., 1996; Surai and Speak, 1998; Surai et al., 1998; Karadas et al., 2005; 2011). At d 11 and d 19, an increased antioxidative status was reflected in a decreased MDA concentration of liver samples of diets that contained CX either alone or in combination with 25-OH-D_3 (Table 5.12). Several reports have shown the CX antioxidant effect. Total TBARS of liver samples was reduced in 35-day old White Leghorn chicks fed a diet deficient in vitamin E and selenium that included 0.5 g CX/kg in comparison to chicks fed the control diet without CX (Mayne and Parker, 1989). In vitro, MDA level was reduced in rat liver microsomal membranes incubated in the presence of CX compared to the control and β-carotene groups, but not different to the astaxanthin group (Palozza and Krinsky, 1992). Maternal supplementation of 6 mg CX/kg of feed reduced TBARS in liver, egg yolks and plasma of chicks at hatch (Surai et al., 2003; Zhang, et al., 2011; Rosa et al., 2012). These effects are likely related to the ability of CX to quench singlet molecular oxygen, and scavenge other free radicals (Mortensen et al., 1997; Böhm et al., 2012).

There were no differences in breast meat MDA level among dietary treatments (Table 5.12). Therefore, dietary supplementation of 25-OH- D_3, CX or the combination of this two additives did not influence lipid peroxidation or meat stability of breast meat. However, this effect was not evaluated after storage. Additionally, the breast fat content is lower than other tissues (Cahaner et al., 1986; Zhao et al., 2011), meaning that is less susceptible to lipid peroxidation. Similar results were previously reported by Jensen et al. (1998) with a dietary CX inclusion of 2 mg/kg of feed; however, an increased storage stability of meat was seen in chickens fed 3.6 mg/kg CX (Barroeta and King, 1991). Jensen et al. (1998) proposed that these contradictory results can be due to different contents of vitamin E in basal diets, and different methods to measure MDA between

studies. After 72 h of slaughter as well as after 56 days of storage at -18°C, the TBARS of abdominal fat and fat of leg muscle were reduced in broilers fed the combination of 25-OH-D_3 and vitamin D_3 at 4,000 IU/kg of total vitamin D_3 activity as compared to broilers fed only vitamin D_3 at the same level; however, the mechanism was not clearly established (Michalczuk et al.; 2010). A high oxidative stability of poultry meat is crucial to avoid or delay development of oxidation that may deteriorate meat quality, and considerably shorten its shelf life (Cortinas et al., 2005; Narciso-Gaytán et al., 2010).

5.3.5 Effect on Bone Characteristics

5.3.5.1 Bone mineral density

Cortical BMD increased with age when was measured at 30% and 50% of femur length from the proximal epiphysis (Table 5.13; Table 5.14). In contrast, total and trabecular BMD was lower at 39 d of age in comparison to 19 d. The same pattern was previously reported by Leslie et al. (2006), and it was related to the decreasing rate of mineral deposition (Williams et al., 2000), and femur growth after the fourth wk of age (Applegate and Lilburn, 2002; Talaty et al., 2009).

There were no main effects of dietary treatments on BMD. At d 11, total BMD measured at 30% of femur length from the proximal epiphysis was lower in females in the 25DCX and CX-Early treatments as compared to the females of the Control. In contrast, those diets and 25DCX-Early increased that trait in males relative to the Control; the other treatments did not affect total BMD regardless of bird sex (Table 5.15). At d 19, all treatments had a tendency to reduced total BMD at 30% in females as compared to the Control group ($P = 0.0803$; Table 5.15). In males, that tendency was also seen in 25DCX, CX-Early and 25DCX-Early, but total BMD tended to be higher in males with 25D, CX and 25D-Early relative to the other treatments ($P = 0.0803$; Table 5.15). At 39 d, 25DCX reduced total BMD at 30% in males relative to females, but was not different to

the Control group. At this age, within other treatments, there were no differences between sexes in total BMD measured at 30% of femur length from the proximal epiphysis, nor relative to the Control group (Table 5.15). At 50% of total femur length, total BMD was higher in females than males at d 11, and nearly higher at d 39 (P = 0.0877; Table 5.14).

At d 11, in females all the experimental diets had a tendency to reduce cortical BMD at 30% of femur length relative to the Control group; but in males, 25DCX tended to increase that trait as compared to the other treatments (P = 0.0568; Table 5.15). At d 19, females of 25D, CX-Early and 25DCX-Early treatments tended to have lower cortical BMD at 30% of femur length than females of other treatments; but in males, CX and 25D-Early tended to increase this trait as compared to the other diets (P = 0.0864; Table 5.15). At d 19, CX-Early had a tendency to have lower cortical BMD at 30% of femur length than the other treatments (P = 0.0575; Table 5.13). At 30% of femur length, cortical BMD was higher in females than males in all ages analyzed (Table 5.13). When measured at 50% of femur length, females had higher cortical BMD at d 19 than males, and nearly higher at d 39 (P = 0.0880; Table 5.14).

At d 11, in females with the exception of 25DCX-Early, all experimental treatments tended to reduce trabecular BMD at 30% of femur length relative to the Control (P = 0.0684; Table 5.15). In males, all experimental treatments tended to increase trabecular BMD at 30% of femur length especially CX as compared to the Control (P = 0.0684; Table 5.15). At d 19, trabecular BMD at 50% of femur length was nearly increased in females of CX and CX-Early as compared to the other treatments (P = 0.0800; Table 5.15). In contrast, those same diets in males had a tendency to reduce trabecular BMD at 50% in comparison to the other treatments (P = 0.0800; Table 5.15). At d 39, females had higher trabecular BMD at 30% of femur length than males (Table 5.13).

5.3.5.2 Bone cross sectional areas

Total, cortical and trabecular CSA increased with age when measured at 30% and 50% of femur length from the proximal epiphysis (Table 5.16; Table 5.17). Dietary treatments did not affect CSA. At d 19, females of all treatments had similar total CSA at 30% of femur length (Table 5.18). Simultaneously, males of 25D, 25D-Early, and CX treatments had smaller total CSA than males in the 25DCX, CX-Early, 25DCX-Early treatments, but all diets were similar to the Control (Table 5.18). At d 39, males had greater total CSA either at 30% or 50% of femur length than females (Table 5.16; Table 5.17). At d 39, females of 25D-Early and CX-Early had smaller cortical CSA at 30% of femur length relative to females of the 25D and Control groups; the other treatments did not differ from the Control (Table 5.18). In males at d 39, CX-Early reduced cortical CSA relative to the other treatments that were also similar to the Control; and 25D-Early had greater cortical CSA at 30% of femur length than 25DCX and CX-Early (Table 5.18). At d 19, males had greater cortical CSA than females at the two scan locations (Table 5.16; Table 5.17). At d 19, females of 25D, 25D-Early, CX and CX-Early had greater trabecular CSA at 30% of femur length relative to the Control group; 25DCX and 25DCX-Early did not differ from the other treatments (Table 5.18). In males at d 19, with the exception of 25D-Early, all treatments had similar trabecular CSA at 30% of femur length to the Control group. 25D-Early reduced trabecular CSA at 30% of femur length as compared to 25DCX, 25DCX-Early, CX-Early and Control. In addition, 25DCX and 25DCX-Early increased trabecular CSA at 30% of femur length in males relative to 25D, CX and 25D-Early (Table 5.18). At d 11 and d 39, males had greater trabecular CSA at the two scan locations than females (Table 5.16; Table 5.17).

5.3.5.3 Bone mineral content

In both scan locations (30 and 50% of femur length), total, cortical and trabecular BMC increased with age (Table 5.19; Table 5.20). Dietary treatments did not affect BMC. At d 19, males had higher total BMC at 30% of femur length than females (Table 5.19). At d 39, in males and females, all treatments had similar total and cortical BMC at 30% of femur length relative to the Control; however, these bone characteristics were lower in females of 25D-Early than females of 25D (Table 5.18). In males at this age, 25DCX and CX-Early had lower total and cortical BMC at 30% of femur length relative to 25D, 25D-Early, and 25DCX-Early (Table 5.18). Males had higher trabecular BMC at 30% of femur length than females at d 39 (Table 5.19).

5.3.5.4 Bone breaking strength

There were no main effects of dietary treatments on BBS (Table 5.21). At d 19, 25D and 25D-Early nearly reduced BBS in females as compared to the Control ($P = 0.0868$; Table 5.22); similar tendency was seen in males of 25D and 25DCX ($P = 0.0868$; Table 5.22). At d 39, females of all treatments had similar BBS to the Control group; however, this trait was lower in females of 25D-Early compared to females of 25D, CX, 25DCX and 25DCX-Early. At the same age, males of 25D-Early and 25DCX-Early had the highest BBS relative to the other diets; males of 25D and CX had higher BBS relative to the Control, and 25DCX and CX-Early were similar to the Control (Table 5.22).

The role of vitamin D_3 and its metabolites in mineral metabolism and bone formation has been clearly established (Rama-Rao et al., 2006; 2009; Khan et al., 2010). Previous studies have shown that dietary 25-OH-D_3 increased bone ash (Bar et al., 2003), reduced the incidence and severity of tibial dyschondroplasia in broilers (Fritts and Waldroup, 2003; Roberson et al., 2005), and increased femur cortical area and total BMD (Saunders-Blades, 2008). In the present study

there were no main effects on bone quantitative computed tomography traits and BBS regarding to the source of vitamin D_3. The dissimilarity of these results with previous studies may be related to different dietary levels of vitamin D_3, 25-OH-D_3, Ca and P. The effects of 25-OH-D_3 on bone ash and severity of TD were more pronounced when Ca and P were restricted in the diet (Bar et al., 2003; Ledwaba and Roberson, 2003). In this trial, housing, handling, environmental and nutritional conditions were established, controlled, and supervised according to the management guides; therefore, the potential advantages of 25-OH-D_3 over the vitamin D_3 at the same level of inclusion under harsh conditions, nutritional deficiencies or metabolic stress were not expressed.

The effects of dietary treatments containing CX on quantitative computed tomography traits depended on bird sex. As it was seen for instance at d 19, where CX and CX-Early as compared to the other treatments tended to increase trabecular BMD at 50% of femur length in females; but in males the same diets tended to reduce it ($P = 0.0800$; Table 5.15).

Carotenoids are not considered to be directly involved in bone metabolism. However, in an ovariectomized rat model, CX increased bone quality and mitigated osteoporosis (Lin-Peng et al., 2009); moreover, in vitro retinol and several carotenoids (including CX) stimulated differentiation of osteoblasts (Park et al., 1997). As CX is considered a potent antioxidant (Surai, 2001), a free radical scavenger (Zhang et al., 2011), and increased phagocytic capacity (Cho et al., 2013), other process like bone formation might be affected positively and indirectly with its dietary inclusion since oxidative processes and inflammation during stress or diseases might be reduced.

Over the whole trial, males tended to have greater CSA and BMC than females, but at the same time females had higher bone densities. Greater CSA, length and width of bones are related to greater growth rates (Williams, et al., 2000; 2004; Venäläinen et al., 2006; Shim et al., 2012a). In the present study, total CSA and cortical BD at 30% were negatively correlated at 11 d ($r = -$

0.58; $P < 0.0001$), 19 d ($r = -0.36$; $P < 0.0001$), and 39 d ($r = -0.69$; $P < 0.0001$). The rapid growth causes birds to increase their bone width faster than they can mineralize bone; therefore, the osteons are not completely filled by bone apposition, which reduces bone density resulting in a more porous bone (Williams et al., 2004). For this reason, females tended to have higher BMD than males because they had lower growth rate and smaller CSA which may have resulted in a less porous bone. The differences between sexes in total BMC indicate a greater mineral deposition in males; previously other authors have shown similar differences related to the sexual dimorphism in that males have larger and more mineralized bones than females (Yalcin et al., 2001; Venäläinen et al., 2006; Shim et al., 2012a; Charuta et al., 2013). Despite total BMC being associated with BBS at d 11 ($R^2 = 0.48$; $P < 0.0001$), d 19 ($R^2 = 0.28$; $P < 0.0001$), and d 39 ($R^2 = 0.51$; $P < 0.0001$) at 30% of total bone length, both sexes had similar BBS at each of the sampling days. Cortical CSA and BMC were related with BBS at d 39 ($R^2 = 0.53$; $R^2 = 0.63$; respectively; $P < 0.0001$). A correlation between averages of tibia ash and BBS was reported in the past ($r = +0.63$; $P < 0.0001$; Rath et al., 2000). Therefore, the increased cortical CSA and BMC led to a higher BBS in males with dietary 25DCX-Early, which is likely related to the presence of 25-OH-D_3. At d 19, in males a synergy may exist between 25-OH-D3 and CX for trabecular CSA at 30% of femur length since 25DCX and 25DCX-Early had significant greater areas than 25D, CX and 25D-Early; however, its implications are not well understood yet.

5.3.6 Conclusion

Overall, treatments that contained CX have the potential to increase performance at early ages, possibly due to the increased antioxidant status indicated by the reduced TBARS of liver at 11 and 19 d. This finding confirms the antioxidant capacity of carotenoids in broilers and its potential to increase productivity (Shanmugasundaram and Selvaraj, 2011; Rajput et al., 2012).

The increased BBS at the end of the trial in males of 25D-Early and 25DCX-Early was likely due to the presence of 25-OH-D_3. A synergy between 25-OH-D_3 and CX was seen at d 19 in the trabecular CSA; however further studies may be needed to confirm this effect. Dietary 25-OH-D_3 and CX can affect bone metabolism through different metabolic pathways (Park et al., 1997; Fritts and Waldroup, 2003). Dietary CX may affect bone metabolism, probably through a higher antioxidant status (Shanmugasundaram and Selvaraj, 2011; Rajput et al., 2012), and through an increased osteoblastic differentiation (Park et al., 1997). There was no evidence that CX, 25-OH-D_3 or its combination reduced lipid oxidation in breast meat at processing, however, this effect was not evaluated after long-term storage which would be necessary to clearly determine that effect.

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5.5 TABLES

Table 5.1 Basal composition of the experimental diets

Item	Starter 0 to 11 d	Grower 12 to 19 d	Finisher 20 to 39 d
Ingredient (%)			
Corn, Yellow	59.17	61.58	66.50
Soybean meal	34.12	30.93	26.20
Calcium Carbonate	1.30	1.05	1.01
Dicalcium phosphate	1.30	1.06	0.93
Salt	0.26	0.26	0.26
L-Lysine	0.22	0.13	0.12
DL - Methionine	0.32	0.25	0.21
L-Threonine	0.01	-	-
Vitamin & mineral premix[1]	1.00	1.00	1.00
Enzyme[2]	0.01	0.01	0.01
Choline Chloride premix[3]	0.50	0.50	0.50
Vitamin E premix[4]	0.50	0.50	0.50
Canola Oil	1.18	2.58	2.60
Amprolium	0.05	0.05	0.05
Antibiotic growth promoter[5]	0.05	0.05	0.05
Calculated nutrients			
CP (%)	22.5	21.0	19.0
ME (kcal/kg)	3,025	3,150	3,200
Ca (%)	1.05	0.90	0.85
Available P (%)	0.50	0.45	0.42
Total methionine + cysteine (%)	1.04	0.94	0.86
Total methionine (%)	0.66	0.58	0.52
Total Lysine (%)	1.37	1.19	1.06

[1]The Broiler premix contained per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; vitamin E (DL-α-tocopheryl acetate), 35 IU; vitamin K, 4.0 mg; pantothenic acid, 15 mg; riboflavin, 10 mg; folic acid, 0.2 mg; niacin, 65 mg; thiamine, 4.0 mg; pyridoxine, 5.0 mg; vitamin B12, 0.02 mg; biotin, 0.2 mg; iodine, 1.65 mg; Mn, 120 mg; Cu, 20 mg; Zn, 100 mg, Se, 0.3 mg; Fe, 800 mg, and choline chloride, 100 mg.
[2]Enzyme: Phyzyme XP, Phytase enzyme, Danisco Animal Nutrition, Marlborough, Wiltshire, UK.
[3]Provided 100 mg choline per kg of diet
[4]Provided 15 IU vitamin E per kg of diet
[5]Bacitracin Methylene Disalicylate 55 mg per kg.

Table 5.2 Effect of dietary treatments on broiler growth and production traits during starter (0 to 11 d), grower (12 to 19 d) and finisher (20 to 39 d) periods.

		BW				Gain			Feed Intake			FCR		
		0 d	11 d	19 d	39 d	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter	Grower	Finisher
Diet	n[1]	g/bird				g/bird/d						g:g		
Control[2]	9	41.7	329.4^{c}	849.1	2,896	26.1^{c}	54.9	87.6	33.3	89.0	177.6	1.276^{a}	1.627	2.037
25D[3]	9	42.0	338.6^{bc}	857.5	2,896	26.9^{bc}	54.5	88.6	32.6	89.4	172.3	1.214^{bc}	1.649	1.946
CX[4]	9	42.2	350.3^{a}	862.9	2,949	27.8^{ab}	53.3	86.9	33.8	89.4	177.5	1.215^{bc}	1.679	2.058
25DCX[5]	9	42.4	347.6^{ab}	865.2	2,955	27.6^{ab}	52.4	89.5	32.4	88.2	176.2	1.174^{d}	1.693	1.974
25D-Early[6]	9	42.5	335.6^{c}	843.6	2,870	26.3^{c}	53.0	86.8	33.3	90.1	174.4	1.251^{ab}	1.700	2.021
CX-Early[7]	9	43.1	350.6^{a}	869.9	2,936	28.0^{a}	54.2	88.5	34.2	90.3	173.5	1.203^{c}	1.670	1.965
25DCX-Early[8]	9	42.7	356.5^{a}	894.1	3,076	28.4^{a}	56.3	95.7	33.2	92.1	179.8	1.174^{d}	1.647	1.922
Pooled SEM		0.4	4.0	11.5	47.0	0.4	1.2	2.3	0.5	1.7	3.2	0.014	0.026	0.004
Sources of variation	DF	P-values												
Diet	6	0.1969	< 0.0001	0.0777	0.1330	< 0.0001	0.3334	0.2243	0.2215	0.8129	0.6712	< 0.0001	0.3097	0.2440

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.3 Effect of dietary treatments on BW of sampled birds at 11, 19 and 39 d.

		BW		
		11 d	19 d	39 d
Diet	n[1]	g/bird		
Control[2]	18	319.3[b]	862.2	2,901
25D[3]	18	338.4[ab]	868.7	2,857
CX[4]	18	349.6[a]	889.7	3,031
25DCX[5]	18	346.3[a]	877.5	2,891
25D-Early[6]	18	334.5[ab]	873.8	2,905
CX-Early[7]	18	348.5[a]	896.5	2,937
25DCX-Early[8]	18	345.2[a]	921.1	3,100
Pooled SEM		7.01	19.4	70.9
Sex				
Female	63	340.0	849.2[b]	2,719[b]
Male	63	340.5	919.3[a]	3,174[a]
Pooled SEM		3.8	10.1	37.9
Sources of variation	DF	P-values		
Diet	6	0.0357	0.3941	0.1902
Sex	1	0.9182	< 0.0001	< 0.0001
Diet x Sex	6	0.5215	0.4814	0.8697

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.4 Effect of dietary treatments on absolute weights of breast, Pectoralis major, Pectoralis minor and liver of sampled birds at 11, 19 and 39 d.

		Breast[1]			Pectoralis major			Pectoralis minor			Liver		
		11 d	19 d	39 d	39 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[2]	Absolute weight (g/bird)											
Control[3]	18	37.1	126.9	615.2	30.0	104.1	502.8	7.0	22.8	112.5	11.7	26.2	67.1
25D[4]	18	38.9	129.3	579.3	31.8	107.1	476.1	7.1	22.1	103.1	12.0	28.8	69.9
CX[5]	18	40.3	132.4	634.6	33.4	108.8	523.5	7.1	23.7	111.1	13.3	27.8	67.4
25DCX[6]	18	40.7	133.7	620.1	33.2	109.5	510.0	7.5	24.2	110.2	13.1	27.0	64.3
25D-Early[7]	18	38.6	126.0	610.0	31.7	102.6	500.8	7.4	23.4	109.2	11.9	27.6	64.1
CX-Early[8]	18	40.0	137.4	628.3	33.3	114.0	515.5	6.6	23.3	112.8	12.6	27.3	65.4
25DCX-Early[9]	18	39.2	140.2	663.9	32.4	115.1	549.7	6.8	25.1	114.2	12.8	28.7	69.5
Pooled SEM		1.3	3.9	19.5	1.1	3.3	16.4	0.3	0.8	3.5	0.5	1.1	2.3
Sex													
Female	63	39.3	128.1[b]	581.6[b]	32.1	105.1[b]	475.8[b]	7.2	23.0	105.8[b]	12.35	26.8[b]	65.0[b]
Male	63	39.2	136.5[a]	661.7[a]	32.4	112.4[a]	546.6[a]	7.0	24.1	115.1[a]	12.62	28.7[a]	68.6[a]
Pooled SEM		0.7	2.1	10.2	0.6	1.7	8.4	0.2	0.4	1.7	0.2	0.5	1.2
Sources of variation	DF	P-values											
Diet	6	0.5144	0.1032	0.1299	0.2626	0.0686	0.0980	0.5972	0.2137	0.4030	0.1730	0.6937	0.4402
Sex	1	0.9541	0.0058	< 0.0001	0.7781	0.0043	< 0.0001	0.5198	0.0545	0.0002	0.3823	0.0070	0.0438
Diet x Sex	6	0.4022	0.3270	0.8316	0.2937	0.3034	0.8878	0.7167	0.3188	0.4857	0.9540	0.8397	0.7705

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Breast: *Pectoralis major* and *Pectoralis minor*, bone and skin not included.

[2]Bird was the experimental unit.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.5 Effect of dietary treatments on live BW, and absolute weights of carcass parts at processing at 40 d.

		BW	Carcass	Breast[2]	Pectoralis major	Pectoralis minor	Legs[3]	Wings
Diet	n[1]	g/bird						
Control[4]	36	2,984	1,938	628.2	518.3	109.9	584.5	217.5
25D[5]	37	2,919	1,918	614.6	505.0	109.7	580.2	218.0
CX[6]	35	2,888	1,884	600.7	494.7	106.0	578.4	209.6
MC[7]	35	3,016	1,957	625.5	514.6	110.9	582.2	218.0
25D-Early[8]	34	2,852	1,874	600.1	492.4	107.8	568.0	213.1
CX Early[9]	37	2,925	1,896	608.8	501.4	107.4	575.2	213.8
MC Early[10]	34	2,984	1,960	640.5	528.4	112.2	577.2	218.6
Pooled SEM		57.5	37.5	14.8	12.5	2.6	10.1	4.3
Sex								
Female	126	2,656[b]	1,732[b]	568.1[b]	464.4[b]	103.8[b]	515.4[b]	195.4[b]
Male	122	3,221[a]	2,105[a]	665.7[a]	551.3[a]	114.5[a]	640.4[a]	235.6[a]
Pooled SEM		26.4	18.1	7.2	6.1	1.2	5.2	2.0
Sources of variation	DF	P-values						
Diet	6	0.4192	0.5489	0.4165	0.3819	0.6579	0.9472	0.7173
Sex	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Diet x Sex	6	0.3147	0.5973	0.7403	0.7579	0.7375	0.2700	0.2730

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1] Bird was the experimental unit.

[2]Breast: Pectoralis major and Pectoralis minor.

[3]Legs: Drums and thighs bone and skin included.

[4]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[6]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[8]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[9]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[10]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.6 Effect of dietary treatments on relative weight of breast, Pectoralis major, Pectoralis minor and liver of sampled birds at 11, 19 and 39 d.

		Breast[2]			Pectoralis major			Pectoralis minor			Liver		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	% of live BW											
Control[3]	18	11.6	14.7	21.2	9.4	12.1	17.4	2.2	2.6	3.9	3.7	3.0	2.3
25D[4]	18	11.5	14.9	20.3	9.4	12.3	16.7	2.1	2.5	3.6	3.5	3.3	2.4
CX[5]	18	11.6	14.9	20.9	9.5	12.2	17.2	2.0	2.7	3.7	3.8	3.1	2.2
25DCX[6]	18	11.8	15.2	21.4	9.6	12.4	17.6	2.2	2.8	3.8	3.8	3.2	2.3
25D-Early[7]	18	11.7	14.4	21.0	9.5	11.7	17.2	2.2	2.7	3.8	3.6	3.2	2.2
CX-Early[8]	18	11.5	15.3	21.4	9.6	12.7	17.5	1.9	2.6	3.8	3.6	3.0	2.2
25DCX-Early[9]	18	11.3	15.2	21.4	9.3	12.5	17.7	2.0	2.7	3.7	3.7	3.1	2.2
Pooled SEM		0.3	0.3	0.3	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Sex													
Female	63	11.6	15.1	21.4[a]	9.5	12.4	17.5	2.1	2.7	3.9[a]	3.6	3.2	2.4[a]
Male	63	11.6	14.8	20.8[b]	9.5	12.2	17.2	2.1	2.6	3.6[b]	3.7	3.1	2.2[b]
Pooled SEM		0.2	0.1	0.2	0.3	0.1	0.2	0.04	0.03	0.03	0.05	0.04	0.03
Sources of variation	DF	P-values											
Diet	6	0.9545	0.2505	0.2035	0.9803	0.1172	0.2391	0.1214	0.2441	0.0726	0.6051	0.3989	0.3751
Sex	1	0.9921	0.2299	0.0084	0.7978	0.3586	0.1169	0.5168	0.0576	< 0.0001	0.3276	0.6708	< 0.0001
Diet x Sex	6	0.3421	0.3716	0.4139	0.2303	0.2779	0.6092	0.5739	0.5002	0.1745	0.8453	0.3002	0.5077

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Bird was the experimental unit.

[2]Breast: Pectoralis major and Pectoralis minor.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.7 Effect of dietary treatments on carcass and parts yield at processing at 40 d.

		Carcass	Breast[2]	Pectoralis major	Pectoralis minor	Legs	Wings
Diet	n[1]	% of Live BW	% of Eviscerated carcass weight				
Control[3]	36	65.0	32.5	26.8	5.7	30.1[abc]	11.2
25D[4]	37	65.6	32.0	26.3	5.7	30.3[ab]	11.3
CX[5]	35	65.2	31.9	26.2	5.6	30.8[a]	11.1
25DCX[6]	35	65.1	32.0	26.3	5.7	29.7[bc]	11.2
25D-Early[7]	34	65.6	32.0	26.2	5.8	30.3[ab]	11.4
CX-Early[8]	37	64.6	32.1	26.4	5.7	30.4[ab]	11.3
25DCX-Early[9]	34	65.6	32.7	27.0	5.8	29.4[c]	11.2
Pooled SEM		0.4	0.3	0.3	0.1	0.3	0.1
Sex							
Female	126	65.2	32.7[a]	26.8[a]	6.0[a]	29.8[b]	11.3
Male	122	65.3	31.6[b]	26.2[b]	5.4[b]	30.5[a]	11.2
Pooled SEM		0.2	0.2	0.1	0.03	0.1	0.07
Sources of variation	DF	P-values					
Diet	6	0.3411	0.3243	0.2779	0.8757	0.0157	0.8023
Sex	1	0.7029	< 0.0001	0.0018	< 0.0001	0.0002	0.7370
Diet x Sex	6	0.2414	0.8996	0.9447	0.7849	0.5165	0.8954

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Bird was the experimental unit.

[2]Breast: Pectoralis major and Pectoralis minor.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.8 Effect of dietary treatments on shank skin lightness, redness, and yellowness at 11, 19, and 39 d.

		Lightness[2]			Redness[2]			Yellowness[2]		
Diet	n[1]	11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Control[3]	18	61.34	65.73^{ab}	75.67^{a}	1.59^{b}	-0.01^{b}	-2.53^{d}	14.34^{b}	26.13^{c}	43.38
25D[4]	18	60.54	66.50^{ab}	75.86^{a}	1.87^{b}	-0.88^{c}	-1.85^{cd}	14.52^{b}	27.85^{bc}	43.67
CX[5]	18	59.32	65.09^{bc}	73.62^{bc}	2.99^{a}	1.91^{a}	2.76^{a}	18.87^{a}	31.77^{a}	45.15
25DCX[6]	18	58.61	64.17^{c}	72.95^{c}	3.25^{a}	2.15^{a}	3.20^{a}	19.71^{a}	30.23^{ab}	45.66
25D-Early[7]	18	59.60	67.89^{a}	75.58^{a}	1.96^{b}	-0.33^{bc}	-2.28^{d}	14.98^{b}	29.82^{ab}	43.19
CX-Early[8]	18	59.43	64.09^{c}	74.88^{ab}	3.08^{a}	1.58^{a}	-0.62^{b}	20.26^{a}	30.78^{a}	44.43
25DCX-Early[9]	18	57.87	65.47^{bc}	75.06^{ab}	3.08^{a}	1.91^{a}	-0.97^{bc}	19.43^{a}	31.35^{a}	41.95
Pooled SEM		1.04	0.77	0.57	0.31	0.29	0.37	0.83	0.96	1.27
Sex										
Female	63	58.55^{b}	65.29	74.62	2.67	0.95	-0.55	17.28	29.20	43.39
Male	63	60.51^{a}	65.83	74.99	2.42	0.87	-0.11	17.61	30.21	44.45
Pooled SEM		0.54	0.41	0.30	0.15	0.14	0.19	0.44	0.51	0.67
Sources of variation	DF	P-values								
Diet	6	0.3103	0.0133	0.0014	0.0004	< 0.0001	< 0.0001	< 0.0001	0.0004	0.4155
Sex	1	0.0095	0.3533	0.3726	0.1930	0.6478	0.0824	0.6086	0.1632	0.2664
Diet x Sex	6	0.3863	0.5764	0.7200	0.6529	0.2073	0.7632	0.4553	0.9591	0.1074

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Bird was the experimental unit.

[2]Measurements were taken on the right lateral shank surface.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.9 Effect of dietary treatments on breast skin lightness, redness, and yellowness at 11, 19, and 39 d.

		Lightness[2]			Redness[2]			Yellowness[2]		
Diet	n[1]	11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Control[3]	18	58.56	60.58	63.02	2.40	1.14	1.01	1.31^{bc}	4.51^{bc}	8.66^{c}
25D[4]	18	57.33	60.99	64.75	2.64	1.07	1.15	0.96^{c}	4.22^{c}	10.54^{ab}
CX[5]	18	57.37	60.65	62.63	2.72	1.06	0.77	2.08^{ab}	6.21^{a}	11.16^{a}
25DCX[6]	18	57.60	60.53	62.57	2.50	1.06	1.68	2.51^{a}	5.66^{ab}	11.57^{a}
25D-Early[7]	18	58.21	61.14	63.69	2.43	1.07	1.61	1.06^{c}	5.27^{ab}	9.07^{c}
CX-Early[8]	18	57.15	60.36	64.33	2.25	0.98	1.67	2.36^{a}	5.57^{ab}	9.41^{bc}
25DCX-Early[9]	18	57.36	60.26	63.49	2.45	1.35	2.09	2.06^{ab}	6.50^{a}	8.96^{c}
Pooled SEM		0.47	0.43	0.61	0.22	0.17	0.46	0.28	0.47	0.58
Sex										
Female	63	57.24^{b}	59.97^{b}	63.02^{b}	2.65^{a}	1.14	1.23	1.81	5.74^{a}	10.38^{a}
Male	63	58.07^{a}	61.31^{a}	63.97^{a}	2.32^{a}	1.05	1.63	1.72	5.10^{b}	9.53^{b}
Pooled SEM		0.25	0.23	0.32	0.11	0.09	0.22	0.16	0..23	0.30
Sources of variation	DF	P-values								
Diet	6	0.2741	0.7691	0.1061	0.8029	0.7533	0.4598	0.0003	0.0109	0.0011
Sex	1	0.0189	< 0.0001	0.0391	0.0167	0.4395	0.1486	0.7013	0.0457	0.0434
Diet x Sex	6	0.4833	0.8651	0.3721	0.4392	0.4753	0.7112	0.3156	0.4796	0.0555

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Bird was the experimental unit.

[2]Measurements were taken on the right medial breast surface of Pectoralis major..

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.10 Effect of dietary treatments on breast meat lightness, redness, and yellowness at 11, 19, and 39 d.

		Lightness[2]			Redness[2]			Yellowness[2]		
Diet	n[1]	11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Control[3]	18	49.14	47.28	47.28	4.07	2.61	2.33	4.67[b]	6.59[b]	4.99[c]
25D[4]	18	48.78	47.48	48.14	4.11	2.95	2.41	4.63[b]	6.36[b]	6.23[b]
CX[5]	18	48.73	46.78	46.08	3.89	3.32	3.05	5.84[a]	7.71[a]	7.27[a]
25DCX[6]	18	48.47	47.04	46.80	4.28	2.96	3.37	6.29[a]	7.69[a]	8.23[a]
25D-Early[7]	18	48.58	46.49	47.82	4.22	2.64	2.65	4.40[b]	6.65[b]	5.85[bc]
CX-Early[8]	18	47.66	47.20	47.44	4.03	2.85	2.77	6.62[a]	7.91[a]	5.99[b]
25DCX-Early[9]	18	47.27	46.67	47.84	4.46	3.41	3.30	5.92[a]	8.17[a]	5.67[bc]
Pooled SEM		0.46	0.38	0.54	0.20	0.21	0.34	0.31	0.33	0.33
Sex										
Female	63	47.61[b]	46.71	46.47[b]	4.50[a]	2.92	2.77	5.47	7.58[a]	6.88[a]
Male	63	49.15[a]	47.27	48.21[a]	3.81[b]	3.01	2.90	5.48	7.01[b]	5.75[b]
Pooled SEM		0.25	0.20	0.26	0.11	0.11	0.17	0.16	0.18	0.18
Sources of variation	DF	P-values								
Diet	6	0.0623	0.5269	0.1361	0.5622	0.0731	0.2105	< 0.0001	0.0002	< 0.0001
Sex	1	< 0.0001	0.0567	< 0.0001	< 0.0001	0.5684	0.5734	0.9549	0.0252	< 0.0001
Diet x Sex	6	0.3984	0.0201	0.3913	0.6762	0.1184	0.3705	0.7497	0.6388	0.1093

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Bird was the experimental unit.

[2]Measurements were taken on the right medial surface of Pectoralis major.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.11 Effect of dietary treatments on breast skin and breast meat lightness, redness, and yellowness at processing at 40 d.

		Breast skin			Breast meat		
Diet	n[1]	Lightness[2]	Redness[2]	Yellowness[2]	Lightness[3]	Redness[3]	Yellowness[3]
Control[4]	36	59.47	1.03[b]	9.97[c]	48.36	1.62[c]	7.02[b]
25D[5]	37	60.59	0.76[b]	12.13[b]	48.33	1.65[c]	7.56[b]
CX[6]	35	60.06	1.88[a]	15.11[a]	48.93	2.31[ab]	9.38[a]
25DCX[7]	35	59.15	2.07[a]	14.94[a]	48.26	2.68[a]	9.48[a]
25D-Early[8]	34	60.80	0.91[b]	11.42[bc]	48.24	1.67[c]	6.93[b]
CX-Early[9]	37	58.32	1.35[ab]	10.08[c]	48.14	1.96[bc]	6.95[b]
25DCX-Early[10]	34	59.53	1.96[a]	12.24[b]	49.17	1.99[bc]	7.57[b]
Pooled SEM		1.18	0.27	0.61	0.40	0.17	0.32
Sex							
Female	126	59.8	1.14[b]	12.77[a]	47.83[b]	1.68[b]	8.30[a]
Male	122	59.6	1.71[a]	11.77[b]	49.15[a]	2.28[a]	7.38[b]
Pooled SEM		0.51	0.13	0.30	0.21	0.09	0.16
Sources of variation	DF	P-values					
Diet	6	0.7842	0.0032	< 0.0001	0.4534	0.0002	< 0.0001
Sex	1	0.8203	0.0011	0.0120	< 0.0001	< 0.0001	< 0.0001
Diet x Sex	6	0.7229	0.4018	0.3869	0.7260	0.1878	0.1435

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]On a bird basis.

[2]Measurements were taken on the right medial breast surface.

[2]Measurements were taken on the right medial surface of Pectoralis major.

[3]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[5]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[7]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[8]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[9]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.12 Effect of dietary treatments on malonaldehyde (MDA) level of liver at 11 and 19 d, and breast meat at 39 d.

		Liver		Breast Meat
		11 d	19 d	39 d
Diet	n[1]	nmol MDA/g		
Control[2]	8	27.05[a]	30.91[a]	27.66
25D[3]	8	26.64[a]	32.12[a]	27.27
CX[4]	8	22.54[b]	23.73[b]	25.19
25DCX[5]	8	23.07[b]	24.33[b]	26.86
25D-Early[6]	8	26.99[a]	32.56[a]	27.57
CX-Early[7]	8	21.90[b]	24.17[b]	25.21
25DCX-Early[8]	8	22.30[b]	25.32[b]	26.13
Pooled SEM		1.00	0.92	1.97
Sex				
Female	28	24.28	27.88	26.38
Male	28	24.43	27.31	26.73
Pooled SEM		0.51	0.45	1.03
Sources of variation	DF	P-values		
Diet	6	0.0005	< 0.0001	0.9421
Sex	1	0.8289	0.3349	0.8077
Diet x Sex	6	0.9301	0.0510	0.9219

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.13 Effect of dietary treatments on bone mineral density as measured by quantitative computed tomography at 30% of femur length from the proximal epiphysis at 11, 19, and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mg/cm^3								
Control[2]	18	377.60	401.40	343.00	686.39	742.22	800.02	67.95	88.47	63.73
25D[3]	18	374.10	385.90	364.10	676.84	734.63	823.36	62.98	81.00	61.04
CX[4]	18	387.30	396.60	349.70	680.48	744.58	813.91	101.81	91.12	56.92
25DCX[5]	18	375.10	380.50	347.90	679.30	732.25	817.59	50.94	90.51	54.97
25D-Early[6]	18	377.80	389.90	362.40	673.06	743.9	808.55	65.62	72.95	61.87
CX-Early[7]	18	375.30	378.20	338.60	670.04	720.85	809.79	66.55	86.51	63.13
25DCX-Early[8]	18	391.60	376.70	356.60	674.74	731.56	815.01	81.41	78.65	58.24
Pooled SEM		8.9	9.9	11.1	6.83	5.8	8.3	12.37	10.7	5.5
Sex										
Female	63	387.60^a	384.30	349.2	684.52^a	743.46^a	822.10^a	73.84	78.60	50.93^b
Male	63	372.11^b	389.80	354.9	670.11^b	727.97^b	803.10^b	68.22	89.71	68.04^a
Pooled SEM		4.6	5.3	7.2	3.5	3.2	4.9	6.4	5.1	3.5
Covariate BW		0.115	0.078	0.028	0.016	0.059	-0.038	0.008	0.011	0.006
Sources of variation	DF	P-values								
Diet	6	0.7479	0.4963	0.6130	0.7301	0.0575	0.5851	0.2144	0.8022	0.8960
Sex	1	0.0266	0.4754	0.5122	0.0035	0.0018	0.0166	0.5466	0.1618	0.0063
BW	1	0.3419	0.1069	0.0959	0.8549	0.0404	0.0012	0.9615	0.8126	0.4708
Diet x Sex	6	*	**	*	**	**	NS	**	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.14 Effect of dietary treatments on bone mineral density as measured by quantitative computed tomography at 50% of femur length from the proximal epiphysis at 11, 19, and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mg/cm^3								
Control[2]	18	579.18	606.05	455.00	779.03	818.81	901.90	82.59	74.33	58.92
25D[3]	18	564.00	606.18	481.05	766.40	804.18	907.28	70.29	80.52	58.38
CX[4]	18	573.55	599.01	480.51	773.93	811.55	916.93	78.26	79.82	64.74
25DCX[5]	18	577.31	589.25	476.48	771.04	799.44	920.38	85.49	85.70	59.08
25D-Early[6]	18	577.71	613.53	476.40	768.82	823.10	908.58	96.29	77.55	60.86
CX-Early[7]	18	579.72	596.21	454.52	777.28	798.94	901.21	74.85	75.68	62.21
25DCX-Early[8]	18	571.88	607.81	475.69	766.57	808.07	922.36	91.70	82.48	52.29
Pooled SEM		7.2	9.3	15.4	7.5	8.2	9.7	13.7	10.7	5.9
Sex										
Female	63	580.76[a]	604.33	485.67	774.85	817.56[a]	919.51	77.65	80.31	59.34
Male	63	568.77[b]	600.82	457.08	768.60	800.75[b]	902.96	87.92	78.55	59.66
Pooled SEM		3.6	4.3	7.8	4.0	4.0	4.3	7.2	5.9	3.8
Covariate BW		0.263	0.067	0.038	0.097	0.041	-0.029	0.185	0.006	0.015
Sources of variation	DF	P-values								
Diet	6	0.6886	0.5697	0.7875	0.8283	0.2677	0.6123	0.8385	0.9915	0.8459
Sex	1	0.0251	0.6493	0.0877	0.2765	0.0154	0.0880	0.3095	0.8348	0.9586
BW	1	0.0070	0.1467	0.1068	0.3487	0.3197	0.0416	0.3217	0.9154	0.0731
Diet x Sex	6	NS	NS	NS	NS	NS	NS	NS	**	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.15 Significant and nearly significant interactions between diets and sex for bone mineral density as measured by quantitative computed tomography at 30% and 50% of femur length from the proximal epiphysis at 11, 19, and 39 d of broilers. Dependent variables adjusted to a common BW with analysis of covariance.

		At 30%						At 50%
		Total			Cortical		Trabecular	Trabecular
Diet	Age (d)	11 d	19 d	39 d	11 d	19 d	11 d	19 d
		mg/cm^3						
Control[1]	Female	409.5^{ab}	410.9	350.6^{ab}	703.9	759	104.3	64.3
	Male	345.6^{d}	392	355.4^{ab}	668.8	725.5	31.6	84.3
25D[2]	Female	373.4^{bcd}	371.2	373.0^{a}	688	750	55.7	74.5
	Male	374.6^{bcd}	400.6	355.1^{ab}	665.7	719.2	70.2	86.5
CX[3]	Female	410.7^{a}	389.3	355.7^{ab}	697.2	744.5	77.9	100.9
	Male	363.8^{cd}	403.9	343.7^{ab}	663.8	744.7	125.7	58.8
25DCX[4]	Female	369.8^{cd}	386.1	374.6^{a}	671.4	744.6	46.84	76.3
	Male	380.5^{abc}	374.9	321.2^{b}	687.2	719.9	55.1	95.1
25D-Early [5]	Female	387.7^{abc}	364.9	341.1^{ab}	678.3	739.3	66.9	71.9
	Male	367.8^{cd}	417.9	383.7^{a}	667.8	748.5	64.4	83.2
CX-Early[6]	Female	366.1^{cd}	384.5	355.9^{ab}	668.4	729.9	70.1	97.7
	Male	384.5^{ab}	372	321.2^{b}	671.7	711.9	62.9	53.6
25DCX-Early[7]	Female	395.5^{abc}	383	340.0^{ab}	684.2	736.9	95.14	76.5
	Male	387.7^{abc}	370.5	373.1^{a}	665.3	726.2	67.68	88.3
	Pooled SEM	12.3	12.3	16.2	9.2	8.3	17.3	14.0
	P-values	0.0125	0.0803	0.0354	0.0568	0.0864	0.0684	0.0800

[a,b,c,d]Treatment means within the same column with no common superscripts are significantly different ($P < 0.05$).

[1]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[2]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[4]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[6]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[7]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.16 Effect of dietary treatments on cross sectional area as measured by quantitative computed tomography at 30% of femur length from the proximal epiphysis at 11, 19, and 39 d, Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mm^2								
Control[2]	18	16.95	35.58	87.74	7.12	15.57	30.87	7.31	17.01	51.94
25D[3]	18	17.03	35.39	86.66	7.24	14.93	31.87	7.28	17.33	49.51
CX[4]	18	17.42	35.15	85.49	7.60	14.67	30.52	7.34	17.19	50.34
25DCX[5]	18	16.93	37.02	82.06	7.42	15.05	28.79	7.12	18.64	48.68
25D-Early[6]	18	17.83	35.78	82.12	7.74	15.11	30.69	7.55	17.30	46.21
CX-Early[7]	18	18.27	36.90	81.89	7.72	15.21	27.79	7.65	18.42	49.29
25DCX-Early[8]	18	17.63	37.23	84.97	7.91	15.13	31.51	7.12	18.66	48.55
Pooled SEM		0.4	0.7	2.1	0.2	0.4	1.1	0.2	0.6	1.9
Sex										
Female	63	17.07^{b}	34.71^{b}	80.70^{b}	7.49	14.74^{b}	26.69	7.14^{b}	17.70	46.86^{b}
Male	63	17.80^{a}	37.70^{a}	88.13^{a}	7.58	15.45^{a}	30.9	7.53^{a}	17.88	51.58^{a}
Pooled SEM		0.2	0.4	1.3	1.0	0.2	0.7	0.1	0.3	1.2
Covariate BW		0.037	0.023	0.022	0.017	0.011	0.011	0.011	0.007	0.008
Sources of variation	DF	P-values								
Diet	6	0.1736	0.1313	0.2854	0.2354	0.8895	0.1235	0.5941	0.1280	0.5295
Sex	1	0.0087	0.0100	< 0.0001	0.5741	0.0446	0.2377	0.0199	0.7032	0.0206
BW	1	< 0.0001	< 0.0001	0.0013	< 0.0001	< 0.0001	< 0.0001	0.0004	0.0127	0.0041
Diet x Sex	6	NS	*	NS	NS	NS	*	NS	*	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.17 Effect of dietary treatments on cross sectional area as measured by quantitative computed tomography at 50% of femur length from the proximal epiphysis at 11, 19, and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mm^2								
Control[2]	18	13.22	30.79	78.51	8.48	20.82	36.09	2.68	7.18	39.47
25D[3]	18	13.13	30.27	82.34	8.42	20.70	39.61	2.77	6.81	39.53
CX[4]	18	13.46	30.40	76.80	8.73	20.55	36.62	2.80	7.24	37.35
25DCX[5]	18	13.4	32.69	74.47	8.70	21.94	34.65	2.71	8.00	36.14
25D-Early[6]	18	13.92	31.38	75.34	9.05	21.33	36.14	2.84	7.16	35.88
CX-Early[7]	18	13.85	31.68	74.99	9.05	21.48	33.85	2.68	7.38	38.10
25DCX-Early[8]	18	13.65	32.00	76.01	8.93	22.02	35.25	2.74	7.05	36.31
Pooled SEM		0.4	0.7	2.6	0.3	0.5	1.6	0.1	0.3	1.7
Sex										
Female	63	13.38	30.83	72.90[b]	8.77	20.75[b]	34.91	2.65[b]	7.43	34.83[b]
Male	63	13.66	31.8	80.95[a]	8.86	21.77[a]	37.23	2.84[a]	7.09	40.25[a]
Pooled SEM		0.2	0.4	1.6	0.1	0.3	0.9	0.1	0.2	1.1
Covariate BW		0.036	0.027	0.022	0.024	0.019	0.012	0.003	0.005	0.007
Sources of variation	DF	P-values								
Diet	6	0.7724	0.1835	0.3690	0.5274	0.3003	0.2844	0.8298	0.1835	0.5563
Sex	1	0.3429	0.1132	0.0025	0.9555	0.0131	0.1115	0.0070	0.1762	0.0033
BW	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0202	0.0016	0.0105
Diet x Sex	6	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.18 Significant and nearly significant interactions between diets and sex for cross sectional area and bone mineral content as measured by quantitative computed tomography at 30% of femur length from the proximal epiphysis at 11, 19, and 39 d of broilers. Dependent variables adjusted to a common BW with analysis of covariance.

		Cross sectional area			Bone mineral content	
		Total	Cortical	Trabecular	Total	Cortical
Diet	Age (d)	19 d	39 d	19 d	39 d	39 d
		mm^2			mg/cm	
Control[1]	Female	34.1^{d}	31.2^{abc}	16.0^{de}	295.9^{bcd}	252.0^{abc}
	Male	37.1^{abc}	30.5^{abcd}	18.1^{abc}	309.1^{abc}	239.2^{abcd}
25D[2]	Female	35.4^{cd}	31.0^{abc}	18.2^{abc}	300.7^{abc}	256.7^{ab}
	Male	35.4^{cd}	32.7^{abc}	16.5^{cde}	325.3^{ab}	264.9^{ab}
CX[3]	Female	35.5^{bcd}	29.8^{bcd}	17.9^{bc}	288.7^{cd}	242.8^{abcd}
	Male	34.8^{cd}	31.2^{abc}	16.5^{cde}	305.2^{abc}	252.7^{abc}
25DCX[4]	Female	35.2^{cd}	29.6^{bcd}	17.4^{bcde}	284.2^{cd}	250.1^{abcd}
	Male	38.8^{a}	29.3^{bcd}	19.9^{a}	275.8^{cd}	223.9^{cd}
25D-Early [5]	Female	36.2^{bcd}	28.0^{de}	18.8^{ab}	267.5^{d}	226.3^{cd}
	Male	35.3^{bcd}	33.7^{a}	15.8^{e}	326.7^{ab}	269.9^{a}
CX-Early[6]	Female	36.1^{bcd}	28.2^{cde}	18.2^{abc}	280.6^{cd}	235.3^{bcd}
	Male	37.7^{ab}	26.7^{e}	18.7^{abc}	272.7^{cd}	213.2^{d}
25DCX-Early[7]	Female	35.6^{bcd}	29.6^{bcd}	17.5^{bcd}	280.2^{cd}	242.9^{abcd}
	Male	38.8^{a}	33.4^{ab}	19.8^{a}	333.9^{a}	268.0^{ab}
	Pooled SEM	0.91	1.44	0.80	12.6	11.7
	P-values	0.0485	0.0249	0.0018	0.0133	0.0221

[a,b,c,d,e]Treatment means within the same column with no common superscripts are significantly different ($P < 0.05$).

[1]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[2]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[4]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[6]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[7]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Table 5.19 Effect of dietary treatments on bone mineral content as measured by quantitative computed tomography at 30% of femur length from the proximal epiphysis at 11, 19, and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mg/cm								
Control[2]	18	63.88	142.59	302.48	48.88	115.77	245.57	4.54	15.39	33.58
25D[3]	18	63.79	137.01	313.00	48.99	109.57	260.80	4.29	13.89	30.98
CX[4]	18	67.39	139.21	295.91	51.73	109.42	247.71	5.45	15.81	28.10
25DCX[5]	18	63.53	141.37	279.99	50.35	110.20	236.99	3.29	16.93	26.79
25D-Early[6]	18	67.35	139.12	297.06	52.13	112.38	248.06	4.62	12.24	27.97
CX-Early[7]	18	68.64	139.54	276.63	51.76	109.70	224.26	4.86	15.87	31.70
25DCX-Early[8]	18	69.29	139.70	307.03	53.08	110.64	255.46	5.25	14.65	28.83
Pooled SEM		2.1	3.9	9.6	1.5	3.4	8.3	0.9	1.7	2.8
Sex										
Female	63	66.04	136.06[b]	285.09[b]	51.24	109.60	243.72	4.69	13.93	24.61[b]
Male	63	66.50	143.53[a]	306.94[a]	50.74	112.60	247.38	5.54	16.01	34.81[a]
Pooled SEM		1.0	2.1	5.6	0.8	1.8	5.2	0.5	0.9	1.70
Covariate BW		0.162	0.117	0.099	0.117	0.090	0.080	0.007	0.007	0.009
Sources of variation	DF	P-values								
Diet	6	0.1976	0.9729	0.0977	0.3529	0.8239	0.0817	0.7514	0.5629	0.6230
Sex	1	0.7611	0.0123	0.0149	0.6401	0.2600	0.6648	0.8311	0.1164	0.0008
BW	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5550	0.3351	0.0346
Diet x Sex	6	NS	NS	*	NS	NS	*	NS	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.20 Effect of dietary treatments on bone mineral content as measured by quantitative computed tomography at 50% of femur length from the proximal epiphysis at 11, 19, and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

		Total			Cortical			Trabecular		
		11 d	19 d	39 d	11 d	19 d	39 d	11 d	19 d	39 d
Diet	n[1]	mg/cm								
Control[2]	18	76.64	186.42	355.88	66.11	170.44	324.63	2.15	5.20	19.56
25D[3]	18	74.08	182.85	392.86	64.43	165.92	357.83	1.74	5.65	20.83
CX[4]	18	77.42	181.85	367.88	67.35	166.48	334.90	2.19	4.66	22.36
25DCX[5]	18	77.45	192.34	349.65	67.08	175.15	319.02	2.33	6.09	17.15
25D-Early[6]	18	80.36	192.21	360.19	69.49	175.33	328.22	2.65	5.33	21.80
CX-Early[7]	18	80.46	188.83	340.64	70.25	171.68	304.07	1.59	5.08	23.56
25DCX-Early[8]	18	78.13	194.67	364.26	67.91	177.44	325.80	2.21	5.33	16.10
Pooled SEM		2.1	4.2	14.5	1.7	3.8	14.1	0.4	0.9	2.5
Sex										
Female	63	77.07	186.15	350.99	67.87	169.51	319.56	1.88	5.63	18.96
Male	63	77.88	190.75	372.28	67.17	174.07	336.00	2.37	5.04	21.42
Pooled SEM		1.1	2.2	8.7	0.9	2.1	8.2	0.2	0.5	1.6
Covariate BW		0.242	0.180	0.131	0.194	0.160	0.093	0.009	0.008	0.012
Sources of variation	DF	P-values								
Diet	6	0.3380	0.2479	0.2622	0.2569	0.2578	0.2486	0.5516	0.9435	0.3278
Sex	1	0.9078	0.1734	0.1287	0.5690	0.1333	0.2001	0.0872	0.4054	0.3575
BW	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1100	0.0661	0.0022
Diet x Sex	6	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).

[1]Pen was the experimental unit.

[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.21 Effect of dietary treatments on femur breaking strength at 11, 19, and 39 d of age. Bone breaking strength adjusted to a common BW with analysis of covariance.

		Bone breaking strength		
		11d	19d	39d
Diet	n[1]	KgF		
Control[2]	18	10.88	21.66	32.23
25D[3]	18	10.88	20.08	35.32
CX[4]	18	11.27	21.28	34.69
25DCX[5]	18	10.79	19.27	33.2
25D-Early[6]	18	11.31	21.16	34.87
CX-Early[7]	18	11.95	21.03	31.43
25DCX-Early[8]	18	11.14	21.39	36.45
Pooled SEM		0.3	0.7	1.5
Sex				
Female	63	11.24	20.89	34.09
Male	63	11.10	20.78	33.96
Pooled SEM		0.2	0.4	0.4
Covariate BW		0.037	0.020	0.008
Sources of variation	DF	P-values		
Diet	6	0.2213	0.2392	0.1919
Sex	1	0.5185	0.8294	0.9217
BW	1	< 0.0001	< 0.0001	< 0.0001
Diet x Sex	6	NS	**	*

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
[1]Pen was the experimental unit.
[2]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.
[3]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.
[4]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.
[5]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.
[6]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.
[7]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.
[8]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.
Probabilities of interaction: *$P < 0.05$; **$P < 0.1$; ***$P < 0.0001$; [NS]Not significant: $P > 0.05$.

Table 5.22 Significant and nearly significant interactions between diet and sex for bone breaking strength of femur at 19 and 39 d. Dependent variables adjusted to a common BW with analysis of covariance.

Diet	— Control[1] —		— 25D[2] —		—— CX[3] ——		— 25DCX[4] —		— 25D-Early[5] —		— CX-Early[6] —		— 25DCX-Early[7] —		Pooled SEM	P-Values
	F[8]	M[9]	F	M	F	M	F	M	F	M	F	M	F	M		
19 d	21.7	21.6	20.1	20.1	22.0	20.6	20.6	17.9	20.2	22.2	21.1	20.9	20.6	22.2	1.0	0.0868
39 d	33.7^{bc}	30.1^{cd}	35.3^{b}	35.3^{b}	34.2^{b}	34.3^{b}	36.1^{b}	30.3^{cd}	30.6^{cd}	39.6^{a}	33.8^{bc}	29.5^{d}	34.8^{b}	39.4^{a}	2.0	0.0102

[a,b,c,d]Treatment means within the same row with no common superscripts are significantly different ($P < 0.05$).

[1]Control: Birds fed a diet containing 2,760 IU/kg complete feed of vitamin D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[2]Birds fed a diet containing 69 µg/kg complete feed of 25-OH- D_3 as the sole supplemental source of vitamin D_3 activity from 0 to 39 d.

[3]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 39 d.

[4]Birds fed the 25D diet, plus 6 mg/kg complete feed of CX from 0 to 39 d.

[5]Birds fed the 25D diet from 0 to 19 d, and the Control from 20 to 39 d.

[6]Birds fed the Control diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[7]Birds fed the 25D diet plus 6 mg/kg complete feed of CX from 0 to 19 d, and the Control diet from 20 to 39 d.

[8]F: Female.

[9]M: Male.

6. RESEARCH SYNTHESIS

6.1 INTRODUCTION

The broiler industry around the world is looking for innovations in management and nutrition that can increase growth performance, feed efficiency and yield, but at the same time improve welfare. Vitamin D_3 is well known for its role in Ca metabolism and bone formation (Fritts and Waldroup, 2003); however, it also participates in muscle metabolism (Boland, et al., 1985), immune response (Fritts et al., 2004), and reproduction (Coto et al., 2010). After absorption, vitamin D_3 is converted into 25-OH-D_3 in the liver, and then into 1,25-$(OH)_2$-D_3 in the kidney; which is the active form of vitamin D_3 (Bar et al., 1980; Soares et al., 1995). Dietary 25-OH-D_3 has previously been shown to increase broiler performance (Yarger et al., 1995; Fritts and Waldroup, 2003), meat yield (Yarger et al., 1995; Vignale et al., 2013), immune function (Chou et al., 2009), bone ash (Rennie and Whitehead, 1996), and bone breaking strength (Saunders-Blades, 2008) relative to vitamin D_3 at equal levels of supplementation. The carotenoid canthaxanthin (CX) is a red pigment that can increase the serum total antioxidant capacity in poultry (Surai et al., 2003; Rosa et al., 2012; Rocha et al., 2013) and has been mainly used for skin and egg pigmentation (Pérez-Vendrell et al., 2001; Cho et al., 2013a). There is no evidence that dietary CX increases broiler performance or meat yield; however, in rats CX reduced osteoporosis (Lin-Peng et al., 2009), and stimulated osteoblastic differentiation (Park et al., 1997). The dietary combination of 25-OH-D_3 and CX (MaxiChick®; MC) has been recently tested in broiler breeders and has shown to increase hatchability, fertility, sperm quality and phagocytic capacity, and reduce embryo mortality (Rosa et al., 2010a; 2010b; Cho et al., 2013b).

The study was conducted to investigate the effects of dietary MC on broiler performance through two approaches: from a field trial in Colombia and also under controlled environmental

conditions in the Poultry Research Centre of the University of Alberta in Canada. The main objective was to investigate the effect of MC on broiler performance (Chapter 2), meat yield, skin colour, antioxidant status, and bone characteristics (Chapter 3 and Chapter 5). Housing conditions and management influence broiler performance, health and welfare (Reece and Lott, 1982; Thomas et al., 2004). Because the first part of this research was conducted under commercial conditions of broiler production, a secondary objective was to analyze the effect of housing, geographical region, and environmental conditions on performance, yield and bone characteristics of broilers (Chapter 4). This study is the first research where the combination of 25-OH-D_3 and CX has been tested in broilers and investigated to determine the interactive effect between these two compounds.

6.2 SUMMARY

The objectives of this research were achieved by testing the following hypotheses:

1. We hypothesized that dietary MC would increase performance and livability of broilers under field conditions in Colombia. As 53% of males were supplemental marigold extract (MG) from approximately d 22 to processing age, the effect of MG was also analyzed. This hypothesis was addressed in Chapter 2 of the thesis. Although dietary MC from d 0 to approximately d 21 resulted in reduced mortality during the fifth wk of life relative to birds not fed MC, broiler production performance was not otherwise affected. Dietary MG increased feed intake at the end of the production cycle. During the fifth wk of age, dietary MG increased weight gain and reduced FCR in strain A males relative to strain B males. In the same period, MC reduced weight gain and increased FCR in males not fed MG, but had no effect in males fed MG. Dietary MG increased weight gain in males fed MC, but had no effect in males not fed MC. From 29 to 35 d, MG

increased mortality in males fed MC, but had no effect in males not fed MC. Supplemental MC reduced mortality in males not fed MG, but had no effect in males fed MG.

2. We hypothesized that the dietary MC would increase meat yield, skin colour and bone strength of broilers under commercial conditions in Colombia. This hypothesis was addressed in Chapter 3. In males, dietary MG was also included in the analysis. This hypothesis was partly supported, because supplemental MC at early ages increased bone quality especially in strain B. However, MC reduced most of the carcass traits especially in strain A and had no independent effect on visceral organ yield or skin colour. Dietary MG increased carcass yield in both strains, and all carcass traits of strain B except breast filet yield.

3. We hypothesized that placing broilers below 1,200 meters above sea level; or under controlled environmental conditions relative to open houses with or without fans; or that birds placed on concrete floor instead of dirt floor; or that birds placed at ground level in comparison to birds placed in a second floor would increase broiler performance, meat yield, and bone strength of broilers in Colombia. This hypothesis was addressed in Chapter 4 of the thesis. This hypothesis was partly supported, because tunnel-ventilated houses with foggers increased BW of broilers, and carcass weight and yield of males as compared to the other houses. Birds in tunnel-ventilated houses had higher bone breaking strength than in open-sided houses. Concrete floor increased BW and reduced mortality in comparison to dirt floor. Most of the differences were related to differences in environmental temperature.

4. We hypothesized that the dietary combination of 25-OH-D_3 and CX would increase broiler performance, meat yield, antioxidant status, skin colour and bone strength of broilers under controlled experimental conditions. This hypothesis was addressed in Chapter 5 of the thesis. This hypothesis was partly supported, because treatments containing CX increased performance at early

ages, and increased antioxidant status. In males, the dietary combination of 25-OH-D_3 and CX increased bone quality.

6.3 ANALYSIS AND IMPLICATIONS

Field experiments are used to evaluate the effect of factors that can influence animal health, nutrition, welfare, housing conditions, and performance. These types of studies allow assessment of the animal responses under commercial conditions of production where many factors interact; therefore, the information generated increases the confidence of producers who may want to implement alternatives in their current conditions of production. For instance, it was determined in a field trial that disinfectant type, application frequency, and organic matter influence microbial populations such as Salmonella on the surface of soil (Payne et al., 2005), which is valuable information for producers that have recurrent reports of Salmonellosis or have the necessity to recycle the litter. From a field test that involved 2.7 million broilers, it was possible to determine that environmental conditions have a stronger influence on welfare than stocking density (Dawkins et al., 2004), which allows producers to focus on the best solution for those concerns. On the other hand, data from field trials are difficult to analyze because many variables affect the results at the same time. However, by increasing sample size and standardizing procedures, the experimental precision can be increased (Steel et al., 1997). The field experiment was described in this thesis was conducted in a commercial broiler vertical integration in Colombia (Avidesa, MacPollo de Occidente). This company has standardized its procedures, handling, and also produce its own feed in a single feed-mill, which reduced variability. This field trial involved 4,922,130 broilers placed in 372 commercial barns, and very intensive sampling.

In the current field trial, broiler performance and meat yield were strain-dependent as previous studies have also shown in the past. (López et al., 2011; Shim et al., 2012). A reduction

in FCR has a big impact in economics, because 70% of the production cost comes from feed (Moosavi et al., 2011). Strain A needed 31 g of feed less per kg of gain than strain B. This difference can impact the net profit of vertical integrations and also that of the broiler industry. In Alberta which produces 126.76 million live kg chicken per year (Alberta Chicken Producers, 2013), this difference in FCR would represent 3,900 tonnes less feed in a year for producing the same amount of poultry meat, which is equivalent to a saving of approximately $1.5 million at a feed cost of $380/tonne.

In general, strain A produced more meat than stain B. One of the main objectives in the broiler industry is to maximize the extraction of saleable, and profitable meat produced per unit cost. Strain A females had 1.3% and 8.6% higher carcass yield and breast filet yield respectively than strain B. In males, strain A had 0.4% and 7.9 % higher carcass yield and breast filet yield respectively than strain B. The breast is the portion of the chicken carcass with the highest saleable price. For instance, the price for a eviscerated whole fresh chicken is $6.30 /kg in comparison with $10.50 /kg of bone-in breast with skin (Agriculture and Agri-Food Canada, 2014); therefore, small increases in breast yield significantly impact profitability. An increment of 1% in carcass yield may represent 1.2 million tonnes more of carcass meat per year in Alberta with a saleable price approximately of $7.6 million. Hence, the broiler strain choice may have a great impact on economics.

In the present study, dietary MC or dietary 25-OH-D_3 either for the first 21 days, or through the entire grow-out period did not increase broiler performance or meat yield. In contrast, MC reduced weight gain of males during the fifth wk of life, and also most of the carcass traits, especially in strain A. Previous studies have shown that dietary 25-OH-D_3 increased broiler performance and meat yield especially when included at early ages or during the entire grow-out

period at a dose of 2,760 IU/kg (Yarger et al., 1995; Saunders-Blades, 2008; Brito et al., 2010; Vignale et al., 2013). In the field trial, the MC group received a total of 6,760 IU/kg of vitamin D_3 activity compared to 4,000 IU/kg in the control group. From our data, an inclusion of 6,760 IU 25-OH-D_3 per kg of feed may reduce performance and meat yield with no signs of toxicity or increasing mortality. In addition, 2,760 IU 25-OH-D_3 per kg of feed also did not increase performance or yield in the present study.

It would seem, that the higher biopotency of 25-OH-D_3 in comparison to vitamin D_3 is more noticeable when the bird's liver function and absorption of fat-soluble compounds are reduced. None of those situations were established in the present study; it would be interesting consider them for further studies. In addition, the higher biopotency of 25-OH-D_3 than vitamin D3 allowed a reduction of Ca and P in the diet of broilers (Bar et al, 2003; Ledwaba and Robertson, 2003), this may be considered in future studies. Due to these characteristics of 25-OH-D_3, broiler producers could replace part of dietary vitamin D_3 with 25-OH-D_3, and use a vitamin D3 level of activity not greater than 6,760 IU/kg of complete feed.

As observed in Chapter 5, dietary CX has the potential to increase broiler performance at early ages, and also Pectoralis major weights when combined with dietary 25-OH-D_3. These effects are likely due to an enhanced antioxidant status as indicated by the reduced liver TBARS. Previous studies have shown that 25-OH-D_3 can also increase BW and meat yield; however, there was no evidence of an additive effect between these two compounds in these traits in this study. It would be interesting to evaluate the effects of MC and its individual components in controlled adverse conditions of housing such as chronic high temperatures, high stocking densities or bacterial contamination. These situations can increase oxidative stress and put more pressure on the birds'

metabolic systems. Sometimes those housing conditions can be found in commercial conditions of production.

Dietary MG did not increase BW, but increased FCR. Males fed MG required 35 g more feed/kg of gain at processing age than males not fed MG, which may have a large impact in economics of yellow-skinned chicken-producing integrations. However, MG increased carcass yield probably through an enhanced antioxidant status similar to CX, or also through an increased availability of metabolizable energy, since dietary MG reduced intestine yield. The intestine is a highly energy demanding organ (Moghaddam et al., 2012). The increase in BW in males fed MC plus MG during the fifth week suggests an additive effect between the carotenoids present in MG (lutein and zeaxanthin) and MC (CX) and may be related to an enhanced antioxidant status. Therefore, in markets where customers are willing to pay higher prices for pigmented chicken carcasses or parts, it would be beneficial to combine these two ingredients since an additive effect may exist between CX and the carotenoids present in MG extracts. An increment of 2.8 g/bird in weight gain during the fifth week may represent a difference of about 20 g/bird of live BW at processing which would represent 1.12 million tonnes more of live BW per year in Alberta with a saleable price approximately of $1.79 million.

Total mortality was similar between birds fed MC and birds not fed MC. However, during the fifth wk of age, dietary MC reduced mortality from 0.66% to 0.46 % relative to birds not fed MC. Production costs are increased by higher flock mortality (Korver et al., 2004), and this cost is even greater when mortality occurs at the end of the production cycle when several weeks of input costs have already been invested. Dietary 25-OH-D_3 and CX may influence livability through different mechanisms (Chou et al., 2009; Zhang et al., 2011). Because in the field trial the components were not compared separately, it was not possible determine if 25-OH-D_3, CX or their

combination caused the reduction in mortality. In Alberta, a reduction of 0.2 percentage units in mortality may represent 114,000 to 120,000 more saleable birds or 250,800 kg total live weight with a saleable price approximately of $0.4 million in one year.

Feed is the most critical factor that affects the broiler production cost (Moosavi et al., 2011). The cost of MC inclusion is approximately $6.50 per tonne. If MC is included during the first three weeks, the cost would be $0.4 million in a year for the Alberta broiler producers. It means that the cost of dietary MC would be covered by the increase in revenue associated with the increase in saleable birds. Mortality increases with age and leg problems are one of the principal causes (Goliomytis et al., 2003). Skeletal disorders are among the most costly issues in poultry industry (Lupo, et al., 2008). This problematic increases mortality and also reduces bird's walking ability (Bokkers and Koene, 2004; Sun et al., 2013). This is a bird welfare concerns due to they cannot get the feed and water, but also reduces performance and net profit.

The results in Chapter 2 showed that MC increased bone breaking stress, and also increased bone breaking strength, mainly in strain B. In Chapter 5, early 25-OH-D_3 increased cortical cross sectional area, and that early 25-OH-D_3 and early MC increased bone breaking strength. These affects are likely due to the presence of 25-OH-D_3 and its role in mineral metabolism and bone formation (Khan et al., 2010), and confirm the higher biopotency of 25-OH-D_3 than vitamin D_3 at early ages. There are no reports of CX effects on bone metabolism; however, in rats CX reduced osteoporosis (Lin-Peng et al., 2009) and increased differentiation of osteoblasts (Park et al., 1997). In Chapter 5 a synergy between 25-OH-D3 and CX was seen in for trabecular cross sectional area at 30% of the total femur length from the proximal epiphysis. The implications and mechanisms of this finding were not well understood. Further studies may be needed to conform that effect in other bones and areas. The positive influence in bone quality of dietary MC is likely related to the

presence of 25-OH-D_3; however, CX has the potential to positively influence bone metabolism. That characteristic could be the explanation of the synergy of CX and 25-OH-D_3 in the trabecular cross sectional area at 30% of the total femur length. From a survey that included 50% of the broiler production of United Kingdom it was determined that 27.6% of the broilers finished the cycle with locomotion problems, and 3.3% of those birds were unable to walk (Knowles et al., 2008). Therefore, dietary MC has the potential to reduce bird losses due to leg problems and increase meat yield through more saleable birds at processing, which would increase net profit.

Shelf life is very important for poultry meat because sometimes longer periods of storage are necessary, which can increase lipid oxidation (Narciso-Gaytán, et al., 2010). Today, consumers are interested to eat food that helps them to improve their health. For this reason, often poultry meat is enriched with dietary polyunsaturated fatty acids that increase susceptibility of oxidation (Cortinas et al., 2005; Betti et al., 2009). However, from our data there was no evidence that CX or 25-OH-D_3 alone or in combination reduced lipid oxidation in breast meat at processing.

Environmental temperature has major effects on broiler performance and meat yield (Lu and Zhang, 2007; Zhang et al., 2012), and welfare (Dawkins et al., 2004). In Chapter 4, most of the effects of geographical region, barn type, floor type and floor level on broiler performance, meat yield and bone characteristics were associated with differences in environmental temperatures. In the present study, broilers in tunnel-ventilated houses had higher BW, meat yield and bone quality in comparison to birds placed in open houses. Previous results have shown that tunnel-ventilated houses with misting systems positively influence meat quality (Ryder et al., 2004) and also bird welfare since they reduce heat stress, panting, prostration and mortality (Aradas et al., 2005). Houses with concrete floor increased BW and reduced mortality in comparison to dirt floor. These houses also had a more unstable stable environmental temperature than houses with dirt floor.

Therefore, housing conditions where environmental temperatures were more stable and closer to the thermoneutral zone tended to increase broiler productivity and bone quality. More stable environmental conditions may reduce bird energy expenditure due to lower respiratory frequency in comparison to birds in hotter and unstable environments, which leaves more available energy for growth and does not cause respiratory alkalosis.

Housing renovations including replacing curtain-sided with totally enclosed housing and cooling systems, have increased broiler BW, feed efficiency and livability (Liang et al., 2013). However, in some poultry markets there is still the misconception that implementing advances in housing design and cooling technologies are a costly investment.

Enclosed housing and cooling systems allow increased stocking densities (Aradas et al., 2005). This situation has the potential to increase the meat production per unit of area, thereby reducing fixed costs per kg of meat produced. Housing systems such as houses of two-storey houses are becoming more common in tropical countries such as Colombia. From our data, BW was increased in single-storey houses relative to birds housed in houses of two-storey. These type of houses increase the meat production per unit of land area and reduce fixed costs. However, further studies are needed to evaluate the impact of having such concentration of birds in a small area on the birds' micro-climate, welfare and health.

The results of the current studies indicate that providing the dietary combination of 25-OH-D_3 and CX did not increase broiler productivity; however, it increased livability, probably through enhanced antioxidant status and bone quality. Consumer concerns about farm animal welfare and production methods have increased during recent years (Hall and Sandilands, 2007; Pouta et al., 2010). Genetic selection has caused an imbalance between meat production and skeletal growth increasing broiler susceptibility to leg problems (Shim et al., 2012), higher culling and mortality

rates (Knowles et al., 2008), a reduced walking ability (Bokkers and Koene, 2004; Sun et al., 2013) that may reduce the bird's ability to reach the feeder and drinker causing starvation and dehydration, and thus constitutes a welfare problem. Skeletal problems also increase the time that the bird spend sitting on the litter, which may cause breast skin lesions and hock-burns, further reducing bird well-being, as well as carcass quality. Housing conditions can also affect bird welfare (Dawkins et al., 2004) since high ambient temperatures and RH increase panting and prostration (Yahav et al., 2004), and reduce feed intake (Lu and Zhang, 2007). Due to this, the pressure for higher farm animal welfare standards have resulted in severe regulations in Europe (Veissier et al., 2008) and the United States (Centner, 2010), and elsewhere, and in new certification schemes that attempt to ensure animal welfare (Main et al., 2014). In Europe, this high level of legislation has positively changed the public perception of animal farming (Pouta et al., 2010). To increase profit and improve the public perception, producers can develop differentiated products with quality labels thorough alternatives in nutrition and production methods that are valued by consumers (Pouta et al., 2010). In Canada consumers have begun to be more willing to pay premium prices for products from animals that that have been grown under specific conditions that consumers associate with well-being (Spooner et al. 2014). Therefore, dietary MC and housing renovations may not only increase profitability by increasing the number of saleable birds at processing, but also may improve bird welfare. However, it is important to note that current methods of production in the poultry industry are intended to provide suitable conditions for production and welfare, but may fall short.

6.4 RECOMMENDATION FOR FUTURE RESEARCH

In the animal industry, environment, nutrition, genetic and health conditions influence sustainability and profitability. This research has provided direction for future studies related to

the effects of 25-OH-D_3 at different dietary levels and its limits of inclusion relative to levels commonly used by the industry. In addition, it will be important to clarify how genetics and other nutrients such as carotenoids interact with 25-OH-D_3 and affect broiler performance, immune response, bone quality, economics and welfare under controlled adverse conditions. The combination of early dietary MC and subsequent dietary MG has the potential to increase weight gain; however, feed efficiency and livability can be negatively affected; therefore, it would be important to determine if these adverse effects are due to the presence of two or more carotenoids in the diets or due to their levels of inclusion. As storage time is important in poultry and enriched food products are becoming more common, it will be useful to investigate the effect of MC and its components on the oxidative status of poultry products from birds fed diets with high concentration of polyunsaturated fatty acids and different storage conditions. Misting systems in tunnel-ventilated houses and in open-sided houses with forced ventilation positively influence broiler productivity and welfare. Therefore, it would be interesting to assess the effect of misting on those traits of birds placed in two-storey open-side houses. This could help broiler producers to develop guidelines for new house types.

6.5 STUDY LIMITATIONS

In the field trial, the logistics of the company and variability between housing conditions were some of the limitations that we faced. However, those limitations were overcome having a large sample size, a balanced distribution of treatments and standardized procedures. Additionally, to gather and analyze information of 4,922,130 broilers placed in 372 houses a very well-coordinated and planned work was mandatory. A permanent presence of a research team member during the field trial was not possible, this situation could have hampered the verification of

research conditions; however, frequent visits to the research site, regular contact and having a group of persons with very well identified roles facilitated the complete control of the experiment.

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