# Carotenoid-enriched Transgenic Corn in Poultry Nutrition

*J. Díaz-Gómez, J.A. Moreno, E. Angulo, G. Sandmann, M. Portero, T. Capell, C. Zhu, P. Christou, C. Nogareda*

Vitamin A deficiency (VAD) is one of the most important micronutrient deficiencies globally. It is prevalent throughout the world, but it is particularly severe in Africa and South-East Asia. The main underlying cause of VAD is a chronic insufficient vitamin A intake in the diet, which can lead to disorders such as xerophthalmia, anaemia and weakened resistance to infection. It has been estimated that VAD affects up to one third of the world's pre-school-age children and up to 15% of pregnant women[1].

Vitamin fortification programs are unsustainable due to poor governance, inefficient food distribution networks, and the prevalence of subsistence agriculture in rural populations[2]. Biofortification of staple crops with organic nutrients is a cost-effective and sustainable approach as exemplified by Golden Rice[3] with high-levels of β-carotene, and Multivitamin Corn[4] accumulating high levels of β-carotene, zeaxanthin, lutein, lycopene, ascorbic acid, and folate.

Vitamin A and carotenoid metabolism in chickens is closely related to the equivalent processes in humans. Consequently chickens are also susceptible to vitamin A deficiency with similar symptoms[5].

## Importance of carotenoids in poultry production

Skin color plays a major role in consumer preference for poultry meat. It is the first quality attribute that the consumer can evaluate, so possible shortcomings related to color might have a negative impact in terms of consumer buying preferences. A golden skin color is desirable because it is associated with better health, albeit consumer preferences vary by region.

The commercial poultry diet based on corn/soybean does not supply enough carotenoids to produce the golden skin preferred by many consumers and does not confer additional health benefits such as enhanced protective immunity. The antioxidant activity of carotenoids can result in their depletion from the circulation during immune stress periods and lead to reduced pigmentation[6].

Like other animals, chickens cannot synthesize carotenoids *de novo* and must obtain them from their feed. Natural pigments such as marigold flower rich in lutein and zeaxanthin, paprika rich in capsanthin, and canthaxanthin, or synthetic pigments such as β-apo-8'-carotenal have to be added to poultry feed to meet consumer demands and health benefits, but this increases the production costs[7].

## Transgenic high-carotenoid corn delivers nutritionally important carotenoids to poultry

The presence of carotenoids in poultry breast and thigh meat is important from a production point of view due to their activity as antioxidants. Oxidation affects skin color as well as the shelf life of poultry meat.

We evaluated commercial broilers fed diets supplemented with different types of corn including: control white corn; high carotenoid corn which accumulates high levels of β-carotene, lycopene, lutein and zeaxanthin[8]; a standard commercial corn-based diet with the colour additives normally used in commercial poultry production; and a standard corn-based diet without additives. Birds reared on the high-carotenoid diet accumulated higher carotenoid levels in breast meat.

The skin and meat color was evaluated with the CIELAB trichromatic system based on three-dimensional color space produced by plotting in rectangular coordinates, L*, a*, and b*. L* is the value for lightness, a* for redness, and b* for yellowness. The birds fed the high-carotenoid diet exhibited much more intense color than birds fed on the control diet (**Fig. 1**). Importantly the yellow skin color did not lose its intensity in shelf life studies, demonstrating the strong antioxidant activity achieved by consuming the carotenoid-rich diet[9].

## Bioavailability of carotenoids and conversion to retinol

The bioavailability of nutrients in staple crops is a better indicator of nutritional quality than the nutrient content alone. The health-promoting effects of vitamins depend on overall intake and bioavailability, which is influenced by food processing, absorption efficiency, and the utilization or retention of the vitamin in the body[10].

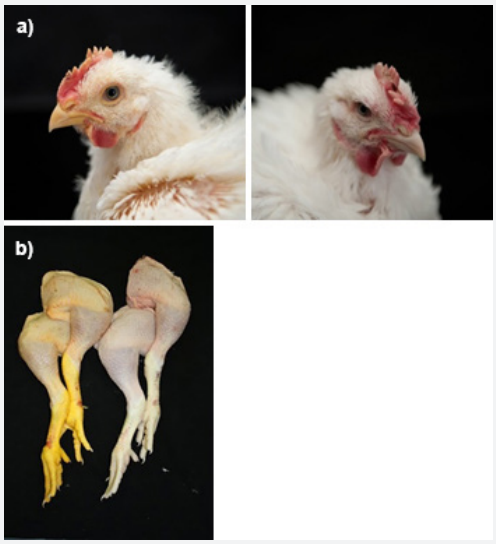


**Figure 1. Chickens fed the high-carotenoid corn (left) and the control (right) diets.** (a) Beak, crest, eyelids and facial feathers. (b) Dissected thighs.

The serum carotenoids represent the mobile pool of pigments, whereas the liver is the primary storage organ for carotenoids. Carotenoid and retinol levels tend to be maintained at constant levels in serum until storage pools are depleted; thus, liver carotenoid and retinol pools provide a more reliable indicator of nutritional status.

The livers of birds fed the high carotenoid diet contained much higher levels of carotenoids than the other diet groups, including the commercial diet supplemented with natural pigments. Birds reared on the high-carotenoid diet accumulated the highest levels of liver retinol (814 µg/g DW) compared to the control group (471 µg/g DW) and the commercial diets with and without color additives (573 and 531 µg/g DW, respectively). Most likely this reflects the greater supply of β-carotene and β-cryptoxanthin in the carotenoid-enriched diet.

## Protection of poultry against *Eimeria tenella*

Birds succumb to coccidiosis, caused by protozoan parasites of the genus *Eimeria*, when they ingest sporulated oocysts that are found in abundance on poultry farms. It is an economically important poultry disease which is currently controlled using drugs and vaccines. Plants and commercial products rich in carotenoids have been tested for their ability to prevent coccidiosis[11].

As control strategies depend on vaccination and the incorporation of anticoccidial drugs in the diets, development of drug resistant coccidian strains is becoming an increasing problem.

In our experiments, the high-carotenoid diet appears to delay the *E. tenella* reproductive cycle. Enhanced resistance against oocysts was observed in chickens fed the high carotenoid diet and the excretion of massive numbers of oocysts observed in all the other diets was reduced or delayed. Poultry reared on the high carotenoid diet exhibited a reduction in the severity of coccidiosis symptoms concomitant with a delay in the parasite life cycle, reducing the oocyst load in the feces.

The bursa of Fabricius is a lymphoid gland located on the posterodorsal wall of the cloaca that regresses with sexual maturity but plays an important role in disease resistance in poultry. This organ was heavier in the birds fed the high-carotenoid diet, providing a basis for their much improved immunomodulatory response to vaccination. The ability of the high-carotenoid diet to interact beneficially with vaccination schemes suggests that carotenoid-enhanced corn could be used as a complementary strategy to boost resistance to coccidiosis and increase the efficacy of co-presented vaccines against coccidiosis and other diseases.

Litter conditions influence poultry performance. Poor litters cause birds to develop foot pad dermatitis or pododermatitis characterized by inflammation and ulcers on the foot pad and toes. Feed composition helps to prevent pododermatitis lesions by maintaining general animal welfare, thus avoiding the economic losses of the disease. The high-carotenoid diet may have promoted faster follicular repopulation, thus reducing initial inflammation and enhancing the overall immune response. The incidences of footpad dermatitis and digital ulcers were significantly lower in animals fed the carotenoid-rich diet, suggesting that this diet protects against lesions in the presence but also in the absence of coccidiosis.

## Economic analysis

The poultry industry worldwide raises approximately 40 billion chickens annually. Avian coccidiosis results in annual economic losses of approximately $2.4 billion, including production losses, disease prevention and treatment costs[12].

Control strategies depend mainly on vaccination and the incorporation of anticoccidial drugs in the diet. The development of drug resistant coccidian strains is an increasing challenge for the poultry industry. It has been estimated that the research and development costs of an effective anticoccidial drug is about $500 million. Thus, a diet based on the high-carotenoid corn would be a safe and more economical option to combat avian coccidiosis.

Appearance often determines product preference or rejection by the consumer. In the case of poultry meat, a bright coloration is preferred, especially in North America and the Asia-Pacific markets. In the broiler industry, genetic selection has made the growth period shorter; thus, a high concentration of pigments is necessary to be added to the feed to achieve the desirable skin pigmentation. The pigmentation of poultry feed increases costs from $5 to $15/tonne[13].

## Summary

Poultry raised on a high-carotenoid corn diet were healthy and accumulated bioavailable carotenoids. Our results confirm that incorporation of this new strain of corn into commercial poultry diets could maintain poultry health and confer nutritional value to poultry products. In addition we noted a very substantial reduction in the severity of coccidiosis, prevention of pododermatitis lesions, and a general enhancement of the overall immune response.

## Acknowledgements

*We thank CESAC (Poultry Health Center of Catalonia and Aragon) for conducting the coccidia experiments; HIPRA Spain for providing E. tenella sporulated oocysts; IRTA-Mas de Bover for diet formulation and manufacture; and X. Subirana, S. Isanta andN. Duran for their involvement in the trials. This research was supported by grants BIO2011-22525 and PIM2010PKB-00746 CAROMAIZE, from Ministerio de Economia y Competitividad (MINECO), Spain; European Research Council IDEAS Advanced Grant Program (BIOFORCE to P.C.); European Research Council IDEAS Proof of Concept Program (Multinutrient Maize to P.C.) and RecerCaixa PC084082. J D-G acknowledges the Universitat de Lleida for a PhD fellowship.*

**References**

1. WHO (World Health Organization) (2009) Global Prevalence of Vitamin A Deficiency in Populations at Risk 1995-2005. Geneva.
2. Zhu C, Sanahuja G, Yuan D, Farré G, Arjó G, Berman J, Zorrilla-López U, Banakar R, Bai C, Pérez-Massot E, Bassie L, Capell T and Christou P (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11: 129–141.
3. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL and Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23: 482–487.
4. Naqvi S, Zhu C, Farré G, Ramessar K, Bassie L, Breitenbach J, Perez Conesa D, Ros G, Sandmann G, Capell T and Christou P (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci USA 106: 7762–7767.
5. Pretorius B and Schönfeldt HC (2013) Effect of different maize meal diets on growth and vitamin A : Case-study on chickens. Food Chem 140: 520–525.
6. Hamelin C and Altemueller U (2012) The effect of carotenoids on yolk and skin pigmentation. World Poult 8: WP010752W.
7. Castañeda MP, Hirschler EM and Sams AR (2005) Skin pigmentation evaluation in broilers fed natural and synthetic pigments. Poult Sci 84: 143-147.
8. Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P and Capell T (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. Proc Natl Acad Sci USA 105: 18232–18237.
9. Nogareda C, Moreno JA, Angulo E, Sandmann G, Portero M, Capell T, Zhu C and Christou P (2015) Carotenoid-enriched transgenic corn delivers bioavailable carotenoids to poultry and protects them against coccidiosis. Plant Biotech J DOI: 10.1111/pbi.12369.
10. Sanahuja G, Farré G, Berman J, Zorrilla-López U, Twyman RM, Capell T, Christou P and Zhu C (2013) A question of balance – achieving appropriate nutrient levels in biofortified staple crops. Nutr Res Rev 26: 235–245.
11. Arczewska-Wlosek A and Swiatkiewicz S (2012) The effect of a dietary herbal extract blend on the performance of broilers challenged with Eimeria oocysts. J Anim Feed Sci 21: 133-142.
12. Quiroz-Castañeda RE and Dantán-González E (2015) Control of avian coccidiosis: Future and present natural alternatives. BioMed Res Int 430610: 1-11.
13. Williams WD (1992) Origin and impact of color on consumer preference for food. Poult Sci 71: 744-746.

*J. Díaz-Gómez[1,6], J.A. Moreno[1], E. Angulo[1], G. Sandmann[2], M. Portero[3], T. Capell[4], C. Zhu[4], P. Christou[4,5], C. Nogareda[1]*

*[1]Department of Animal Production, ETSEA, University of Lleida-Agrotecnio Center, Lleida, Spain*
*[2]Biosynthesis Group, Department of Molecular Biosciences, J. W. Goethe Universitat, Frankfurt, Germany*
*[3]Department of Experimental Medicine, University of Lleida-Institut de Recerca Biomedica de Lleida (IRBLleida), Lleida, Spain*
*[4]Department of Plant Production and Forestry Science, ETSEA, University of Lleida-Agrotecnio Center, Lleida, Spain*
*[5]Institucio Catalana de Recerca i Estudis Avanc_ats, Barcelona, Spain*
*[6]Department of Food Technology, ETSEA, University of Lleida-Agrotecnio Center, Lleida, Spain*