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EFFECTS OF NATURAL OXYCAROTENOIDS ON THE IMMUNE FUNCTION OF JAPANESE QUAILS

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ABSTRACT

Adult *Japanese quail* layers were immunized by a combination of goat red blood cells and bovine serum albumin (100 µg/animal/ i.m.). The control group (C) was fed with commercial layer food, the other group (X) with the same food but supplemented with a mixture of natural xanthophylls (1000 ppm Capsantal EBS 40 NT: extraction of *Tagetes erecta*; active substances: 40 g/kg yellow xanthophyll: 0.8 % ß-carotene, 1.5 % cryptoxanthin, 82.0 % trans-lutein, 4.0 % trans-zeaxanthin, 11.7 % other carotenoids). Blood and egg samples were collected in two weeks intervals for six weeks. The samples were analyzed for retinoid and carotenoid spectrum by HPLC and for the avian immunoglobulin-Y (IgY) titres by ELISA and haemagglutination inhibition test (HAI). The colours of egg yolk and skin were characterized by CIELAB values (Micromatchs™ Sheen Ltd). The concentrations of oxycarotenoids were increased continuously in the blood of group X. There were no differences between the retinoid concentrations of blood. The yolk colour intensity increased in the 2nd w. in xanthophyll fed birds and the average colouration was higher ($p<0.05$) throughout the experiment. The skin CIELAB values were as follows: control group L*: 65.8; a*: 4.1; b*: 26; and in the xanthophyll treated group L*: 59.3; a*:7.8; b*: 41.7 respectively. The differences were significant ($p<0.05$) in the case of b* values. The blood IgY and HAI titres were raised in both the groups and the values were higher for xanthophyll supplemented birds. Recent studies on the role of natural oxycarotenoids have demonstrated that beside the colouration of egg yolk and skin they can enhance the immune function too in *Japanese quail*.

Key words: xanthophyll; lutein; IgY; CIELAB; *Japanese quail*

INTRODUCTION

The carotenoids are a group of over 600 naturally occurring coloured pigments in plants. More than 20 of them are common in human and animal foods. There is increasing evidence that dietary components that possess antioxidant properties can help protect the immune system from oxidative damage and thereby enhance cell-mediated immune responses (Hughes, 1999) thereby increasing resistance to infection (Chandra, 1992).

Immune responses are initiated by the stimulation of appropriate T lymphocytes by antigen presenting cells (Unanue and Cerottini, 1998). One of the antigen presenting cell function is the expression of major histocompatibility complex (MHC) molecules which are present on the cell membrane. It is possible that a mechanism by which carotenoids act as local cytoprotective agents can also enhance cell mediated immune responses which affects the cell surface expression of these molecules (Hughes et al., 2000). The humoral immune response depends on the transition of mature activated B cells to antibody-secreting plasma cells. The role of carotenoid in B cell mediated processes remains unexecuted.

Poultry industry uses carotenoids not only for colouration of different body parts but these substances are also involved in metabolism and fertility. Some carotenoids serve as precursors for the synthesis of vitamin A (Sklan et al., 1989), and some other act as physiological antioxidants (Miller et al., 1996), and thus enhance the immune response (Sklan et al., 1989). Poultry cannot synthesize these compounds and thus must obtain carotenoids from their diets (Perez-Vendrell et al., 2001).

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In the present experiment the effect of marigold (*Tagetes erecta*) originated xanthophylls as diet supplements was tested on colouration and immune modulation in *Japanese quail*.

MATERIAL AND METHODS

Experimental animals and set up

Two groups of adult *Japanese quail* layers (n=10) were kept in pens. The control animals (group C) were fed with commercial layer diet. The another group of quails (group X) were fed with the same basal food but it was supplemented with mixture of natural xanthophylls (1000 ppm Capsantal EBS 40 NT: extraction of *Tagetes erecta*; active substances: 40 g/kg yellow xanthophyll: 0.8 % ß-carotene, 1.5 % cryptoxanthin, 82.0 % trans-lutein, 4.0 % trans-zeaxanthin, 11.7 % other carotenoids). The feed and water access were *ad libitum* for the animals throughout the six weeks of experiment.

Antigen

Goat red blood cells (gRBC) were washed three times with isotonic phosphate buffered saline (PBS). Tannic acid in 1:125 000 dilution was added to the 5% cell suspension for the treatment of outer cell membrane. Bovine serum albumin (BSA) was added for this suspension. The final concentration was adjusted to 100 µg/animal for immunization.

Immunization

Both groups were immunized with a combination of goat red blood cells and bovine serum albumin (gRBC-BSA). All immunizations were given intra muscularly (i.m.) into the breast muscle without Freund's adjuvant for primary 1st d. and booster injections at 4th wk., respectively.

Analytical methods

Blood retinoid and carotenoid concentrations were measured by isocratic reverse phase HPLC (Jasco Ltd., Japan; Kerti and Bárdos, 2006). The major class of circulating avian immunoglobulin (IgY) was measured by a modified ELISA technique described previously (Losonczy et al., 1999). Haemagglutination inhibition (HAI) test was carried out as a micro method (Allan and Gough, 1974) in 10 % (w/v) PBS which caused a more complete precipitation and a maximum antibody content could not be measured at salt concentrations of less than about 8% successfully (Goodman et al., 1951). The colour of egg yolk was compared with the Yolk Colour Fan (DSM, Switzerland) values. Surface colour of skin was evaluated according to the CIELAB scale by the measurement by handy spectrometer (Micromatch™ Plus, Sheen Ltd., United Kingdom).

RESULTS AND DISCUSSION

The xanthophyll colorants are dyes of animal tissues, because there are characteristic differences of pigment versus dyes. Pigments are organic or inorganic colour materials that are practically insoluble in the medium in which they are dispersed. They are distinct particles, which gives the medium their colour. On the contrary, dyes are soluble in the medium in which they are dispersed. This leaves the medium with no visible particles.

Concentrations of lutein as the main components of xanthophylls (oxycarotenoids) in the sera of Japanese quails increased continuously in group X (Fig. 1.).

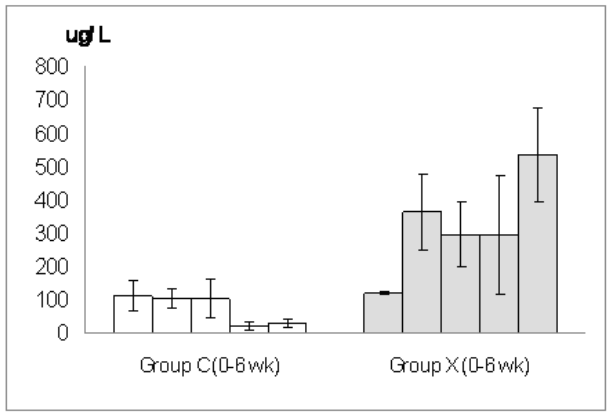

Fig 1: Lutein concentration in the blood of Japanese quails

In the retinoid concentrations of blood there were no differences between the groups (Fig. 2). This indicates that the xanthophylls have no provitamin A activity because their terminal rings have oxo-groups (substituents), bat not the ß-jonon structure, which is the essential part of retinoids.

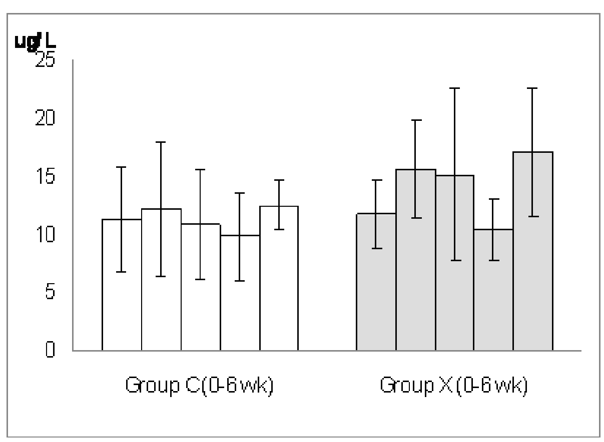

Fig 2: Retinol concentration in the blood of Japanese quails

At the end of the experiment all the birds were exterminated *lege artis*. The skin was flenched from the breast. Colour intensity of the plain inner surface of the skin was measured (Table 1.).

Table 1: CIELAB values of inner skin surface (x±s)

	L	a*	b*
Group C	65.80±0.90	4.0±3.50	25.90±8.70
Group X	59.30±3.10	7.80±1.90	41.70±6.70
P<	0.01	0.08	0.01

According to Perez-Vendrell and co-workers (2001), the CIELAB coordinate b* measured in breast skin is a good indicator of xanthophyll content in feed, whereas a* coordinate measured on the shank showed a linear relationship with the dietary cantaxanthin level. In our experiment significant differences were found between the b* values. Cantaxanthin was not represented among marigold xanthophylls.

The yolk colour intensity measured by YCF increased in the 2nd w. in xanthophyll fed birds (Fig. 3) and the average colouration was higher (P<0.05) throughout the experiment (Fig.4). These results indicate that the applied naturally occurring xanthophylls are absorbed, metabolised and deposited in the organs (yolk, subcutaneous fat) in *Japanese quails*.

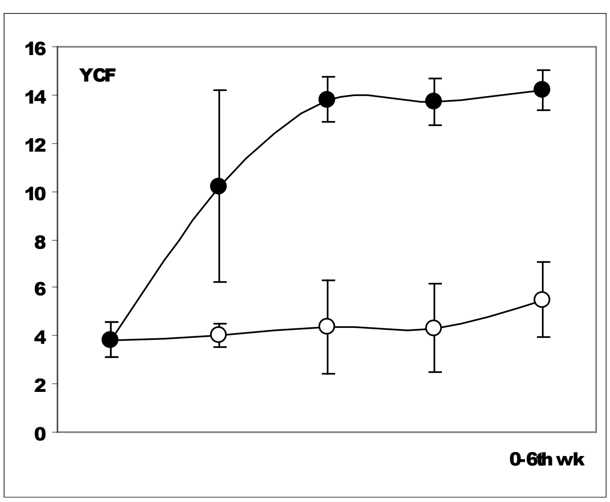

Fig. 3: Comparison of yolk colour between group C (o) and group X (●)

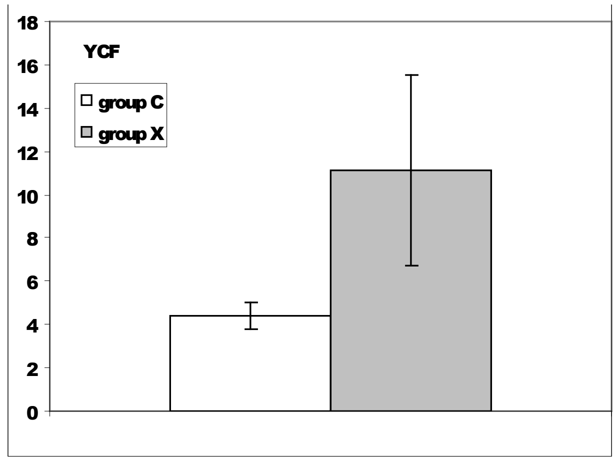

Fig. 4: Differences of yolk colour throughout the experiment

Toyoda et al. (2002) demonstrated similar results in serum, liver, fat and retina of this bird as well. These dyes act as intensive colorants. Carotenoids and mostly the xanthophylls are the natural colorants of birds. Their colours result in change in colour of egg yolk, skin, feather, beak and legs depending on the actual carotenoid content in the birds' diet and the resultant colours range from light yellow to reddish orange. Hen eggs normally contain 0.3 to 0.5 mg of total xanthophylls, with just over half present as lutein. As with most fats and fat-soluble compounds, the composition of the egg is responsive to manipulation of such nutrients in the layer diet (Leeson and Caston, 2004).

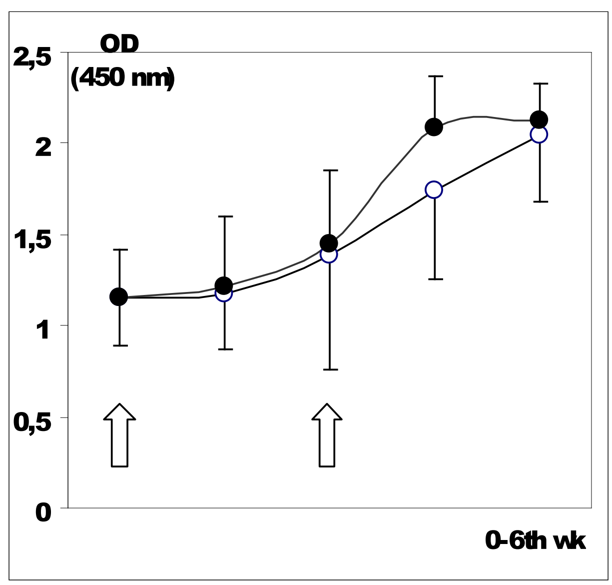

Fig. 5: Serum titres of IgY in control (group C, o) and xantophyll supplemented (group X, ●) *Japanese quails*

The blood IgY and HAI titres were raised in both groups. Mean titres of IgY in blood was higher in group X than in group C of *Japanese quails*. The highest difference was measured after the second (booster) antigen administration (Fig. 5). Similar tendency was established in the case of HAI-test (not shown).

These colorants mainly have physiological functions, protecting cells and tissues from oxidative damage (Miller et al., 1996; Ribaya-Mercado et al. 2004) as well as stimulating the immune system. Xanthophylls modulate immunity and cancer cell proliferation in humans (Ribaya-Mercado et al., 2004). Characteristic elevation of serum IgG titres were demonstrated proceeding to dietary supplementation of lutein in cats (Hong et al., 2000a) and in dogs (Hong et al., 2000b) too.

Kevin et al. (2004) found that lutein-supplemented and zeaxanthin-supplemented male zebra finches mount similar cell-mediated immune responses (to phytohaemagglutinin, or PHA). In our experiment we also found a moderate enhancement of humoral immunity as well as higher IgY titres against *i.m*. BSA administration and higher HAI in the blood of xanthophyll supplemented Japanese quails.

CONCLUSION

Recent studies on the role of marigold (*Tagetes erecta*) originated oxycarotenoids have demonstrated beside the colouration of egg yolk and skin that they may enhance immune function in Japanese quail.

REFERENCES

ALLAN, W. H. – GOUGH, R. E. 1974. A standard haemagglutination inhibition test for Newcastle disease. A comparison of macro and micro methods. *Vet Rec*., vol. 95, 1974, p.120-123.

CHANDRA, R. K. 1992. Effect of vitamin and trace element supplementation on immune responses and infection in elderly subjects. *Lancet* vol. 340, 1992, p.1124-1127.

GOODMAN, M. – WOLFE, H. R. – NORTON, D. 1951. Precipitin Production in Chickens. VI. The effect of varying concentrations of NaCl on precipitate formation. *J. Immunol*., vol. 66, 1951, p. 225-236.

HONG, W. K. – CHEW, B. P. – WONG, T. S. – PARK, J. S. – WENG, B. B. C. – BYRNE K. M. – HAYEK, M. G. – REINHART, G. A. 2000a**.** Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. *Vet. Immunol. Immunopath*., vol. 73, 2000, p. 331-341.

Hong, W. K. – Chew, B. P. – Wong, T. S. – Park, J. S. – Weng, B. B. C. – Byrne K. M. – Hayek, M. G. – Reinhart, G. A. 2000b**.** Dietary lutein stimulates immune response in the canine. In: *Vet. Immunol. Immunopath*., vol. 74, 2000, p. 315-327.

Hughes, D. A. 1999. Effects of dietary antioxidants on the immune function of middle-aged adults. In: *Proc. Nutr. Soc.***,** vol. 58, 1999. p.79-84.

HUGHES, D. A. – WRIGHT, A. J. A. – FINGLAS, P. M. – POLLEY, A. C. J. – BAILEY, A. L. – ASTLEY, S. B. – SOUTHON, S. 2000. Effects of lycopene and lutein supplementation on the expression of functionally associated surface molecules on blood monocytes from healthy male nonsmokers. *J. Infect. Diseases,* vol. 182, 2000, p. S11-S15.

KERTI, A. – BÁRDOS, L. 2006. Simultaneous determination of retinoids (retinol, retinyl-palmitate) carotenoids (lutein, zeaxanthin, ß-crypoxanthin, lycopene, ß-carotene) and vitamin E by RP- HPLC. *Clin. Exper.Lab.Med.,* 2006, vol. 32, Abstract. p.106.

KEVIN, J.– MCGRAW, K. J. – ARDIA, D. R. 2004. Immunoregulatory activity of different dietary carotenoids in male zebra finches. *Chemoecology*, vol. 14, 2004, p. 25-29.

LEESON, S. – CASTON, L. 2004. Enrichment of eggs with lutein. *Poultry Sci.,* vol. 83, 2004, p.1709–1712.

LOSONCZY, S. – SZABÓ, CS. – KISS, ZS.– BÁRDOS, L. 1999. Application of an anti-HQIgY antibody for the measurement of IgY concentratuon of hen's and quail's serum and yolk. *Acta Physiol. Hung.,* vol. 86, 1999, p. 253-258.

MILLER, N. J. – SAMPSON, J. – CANDEIAS, L. P. – BRAMLEY, P. M. –RICE-EVANS. C. A. 1996. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett*., vol. 384. 1996, p. 240-242

PARK, J. S. – CHEW, B.P. – WONG, T. S. 1998. Dietary lutein absorption from Marigold extract is rapid in BALB/c mice. *J. Nutrition* vol. 128, 1998, p. 1802-1806.

PEREZ-VENDRELL, A. M. – HERNANDEZ, M. – LLAURADO, L. – SCHIERLE, J. – BRUFAU, J. 2001. Influence of source and ratio of xanthophyll pigments on broiler chicken pigmentation and performance. *Poultry Sci.,* vol 80, 2001, p. 320-326.

RIBAYA-MERCADO, J. D. – BLUMBERG, J. B. 2004. Lutein and zeaxanthin and their potential roles in disease prevention. *J. Am. Col.l Nutr.,* vol. 23, 2004, p. 567-587.

SKLAN, D. – YOSEFOV, T. – FRIEDMAN, A. 1989. The effects of vitamin A, β-carotene and canthaxanthin on vitamin A metabolism and immune responses in the chick. *Internat. J. Vit. Nutr. Res.*, vol. 59, 1989, p. 245–249.

TOYODA, Y. – THOMSON, L. R. – LANGNER, A. – CRAFT, N. E. – GARNETT, K. M. – NICHOLS, C. R. – KIMBERLY, M. – CHENG, K. M. – DOREY, C. K. 2002. Effect of dietary zeaxanthin on tissue distribution of zeaxanthin and lutein in quail. *Invest. Ophthalm. Vis. Sci.,* vol. 43, 2002, p.1210-1221.

UNANUE, E. R. – CEROTTINI, J. C. 1989. Antigen presentation. *FASEB J.*, vol. 3, 1989, p. 2496-2502.