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Pigmentation Efficiency of Croatian Corn Hybrids in Egg Production

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Summary

To achieve desirable egg yolk colour producers enhance laying hen's diet with pigments, usually from synthetic origin. More recently, consumers have become more concerned about the use of synthetic additives in foods and feeds and interest in natural alternatives has increased. In Croatia, corn grain is the base of laying hen's diet, but contribution of corn carotenoids to egg yolk colour was neglected. Therefore, aim of this research was to explore the pigmentation efficiency of corn grain hybrids in egg production. Three cages, each with three laying hens were allotted to one of the eight dietary treatments differentiated only in corn hybrid (56 % of diets) and fed for 63 days. There were no added synthetic pigments in the treatments. Total carotenoid content of the corn grain and egg yolk was quantified spectrophotometrically as β-carotene equivalents, while colour measurements of egg yolk were recorded instrumentally using a Minolta Chromameter (CR-410) in the CIE L a* b* space, and subjectively by Roche yolk colour fan (RYCF). The level of total carotenoids in corn grain ranged from 17.32 to 31.52 mg/kg. Total carotenoids in egg yolks significantly differ (P<0.001) between dietary treatments. The RYCF, as conventional measure of yolk colour, ranged from 8 to 10 and correlated (r=0.81, P<0.001) with CIE a* value (redness). Both, the RYCF and a* value in egg yolk increased linearly with an increase in the level of total carotenoids in corn grain (b=0.0746, P<0.001; b=0.1856, P<0.001). This research showed that corn grain, without added pigments, in laying hen diet could provide adequate carotenoids supply for acceptable egg yolk colour. Better results (RYCF>10) could be achieved with higher portion of corn grain in the diet. In addition, the CIE a* value correlated well with RYCF scale and therefore could be used for more objective colour measurements of egg yolk.*

Keywords: egg yolk; carotenoids; colour; corn grain; poultry

Introduction

Colour of egg yolk is an important factor in consumer's acceptance of a product. Desirable egg yolk colour varies between markets, but yellow to golden colours are usually considered as an indication of better egg quality. Natural colour of egg yolks is a result of carotenoid accumulation. Hens are not able to synthesise colour pigments, but have the ability to transport pigments to the yolk from the ingested feed and, therefore, carotenoid profile of yolk reflects carotenoid profile of diets (Karadas et al., 2006). To achieve the desirable intense colour of egg yolk, laying hen's diet is enriched with carotenoid pigments which are, due to the low cost and good pigmentation efficiency, usually of synthetic origin. However, more recently there has been a concern about the use of synthetic supplementation in foods and feeds. Moreover, the use of synthetic supplementation has been banned in some countries (Lokaewmanee et al., 2010). Consequently, alternative sources of natural pigments to enhance egg yolk colour are widely investigated (Karadas et al., 2006; Lokaewmanee et al., 2010; Baiao et al., 1999; González et al., 1999).

Yellow corn grain is usual dietary base for laying hens diet, e.g., its content in hen's diet in Croatia reach up to 70 %. Carotenoids responsible for the colour of corn grain are also the pigments for egg yolks colour. Yellow corn grain is source of lutein, zeaxanthin, β-cryptoxanthin and β-carotene (Kurilich and Juvik, 1999). Their contents in total carotenoids content of corn grain depend on corn hybrid, but the major substances are lutein and zeaxanthin (Kurilich and Juvik, 1999; Egesel et al., 2003). Although monohydroxy- and monoketocarotenoids (β-cryptoxanthin and β-carotene) could be converted to vitamin A (Hencken, 1989), lutein and zeaxanthin are effectively transferred to egg yolk (Karadas et al., 2006).

Because of diverse content of total carotenoids among the corn hybrids used in laying hen's diet, the accepted idea is that the intensity of yolk colour from hen fed corn-soy diet is low (usually ranked in number 7 from Roche Yolk Colour Fan; Kijparkorn et al., 2010). Contribution of corn carotenoids to yolk colour is neglected, although there are some hybrids with high carotenoid content. Therefore, the aim of this research was to explore the pigmentation efficiency of Croatian corn grain hybrids with diverse content of total carotenoids in egg production.

Materials and Methods

Plant material

Eight yellow (Bc 282, Bc 354. Bc 394, Bc 462, Bc 572, Bc 678, Bc 778 and Pajdaš) and one white corn grain hybrid (Bc 38 w) were provided from Bc Institute, Zagreb, Croatia. Corn grains were produced under the same agro-climate and production conditions. Each hybrid was planted on one test lot. Corn grain were manually harvested from the central area of lot and stored in paper bags at 4 ˚C until animal experiment. From each bag (n=3), representative sample for further analysis was taken. For laying hen's diet corn grains were grounded through 4-mm screen. Sample corn grains were grounded in laboratory mill (Cyclotec 1093, Foss Tecator, Hoganas, Sweden) provided with 1-mm screen. Once in use, all samples were analysed for dry matter (DM) content (HRN ISO 6496:2001 en).

Hens and diets

All procedures related to the holding and conducting an experiment with live animals were conducted under the approval of the Ethics Committee of the Faculty of Agriculture, Zagreb.

A total of 72 Hissex-brown laying hens (24 weeks old) were randomly assigned to eight dietary treatments (n=9 per

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treatment). Three hens were allotted in one cage (665 cm^2 per hen), and there were three replicates per treatment (three cages with three hens per treatment).

Diets were prepared to meet or exceed the minimum requirements of laying hens according to the NRC (1994). Composition and calculative nutritional value of diets are presented in Table 1. Dietary treatments were differentiated only in a corn hybrid; its share and share and origin of all other components in diets was the same. There were no added synthetic dyes – corn grain was the only source of pigments, and only minimally required 4000 IU of vitamin A was added in the diets. The trace elements were added in the form of Bioplex (Alltech ®, Nicholasville, USA).

Diets and water was given to hens *ad libitum*. First two weeks after allocation to the cages all hens were fed with dietary treatment containing white corn grain with intent to clean hens digestive tract and liver from pigments given before coming to the test object. After the cleaning period, cages were randomly assigned to the dietary treatments and hens were fed with diets differentiated only in corn grain hybrid for next 5 weeks. Total carotenoid content in egg yolk was determined weekly (one egg per cage – 3 eggs per treatment). After stabilization of carotenoid content, 2 eggs from each replicate in two consecutive days (12 eggs per dietary treatment) were collected for total carotenoid determination. In the same week, 3 eggs from each replicate in two consecutive days (18 eggs per dietary treatment) were collected for colour analysis

Table 1. *Composition and calculated composition of dietary treatments.*

Ingredient	Content (%)	Calculated composition	
Corn grain	56.00	w (metabolic energy) / MJ/kg	12.20
Soybean meal	25.00	w (crude protein) / %	18.00
Vitex yeast	4.85	w (crude fat) / %	2.70
Linseed oil	2.00	w (crude fiber) / %	3.00
Calcium carbonate	9.80	w (crude ash) / %	13.85
Monocalcium phosphate	1.30	w (methionine) / %	0.45
Sodium chloride	0.40	w (lysine) / %	1.00
Methionine	0.15	w (calcium) / %	4.15
Premix[1]	0.50	w (phosphorus) / %	0.65

[1]Supplied per kg of diet: 4000 IU Vitamin A, 750 IU vitamin D, 10 mg vitamin E, 0.75 mg Vitamin K, 1.2 mg thiamine, 3.75 mg riboflavin, 3.75 mg pantothenic acid, 15 mg niacin, 0.006 mg vitamin B12, 400 mg choline chloride, 0.15 mg biotin, 0.375 mg folacin, 4.5 mg pyridoxine, 500 mg magnesium, 30 mg manganese, 50 mg zinc, 50 mg iron, 6 mg copper, 0.3 mg iodine, 0.1 mg selenium.

Carotenoid analysis

Total carotenoid content of corn grain was extracted using hexane. Sample (0.6 g) was hydrated with 2 mL of water for 1 hour. Acetone (4 mL) was added and mixture was ultrasonificated (Sonorex TK52, Bandelin, Berlin, Germany) for 5 min. Hexane (2 mL) was added and mixture was vortexed (37600 Mixer, Barnstead Thermolyne, Dubuque, USA) for 30 sec. Hexane layer was discarded after centrifugation (Centric 322A, Tehtnica, Železniki Slovenia; 4000 rpm, 5 min). The procedure without sonification was repeated until colourless hexane layer. Hexane extracts were combined and total volume was made up to 10 mL.

Egg yolk carotenoids were extracted according to the procedure described elsewhere (Surai et al., 2001). In this procedure, total carotenoids were extracted with hexane after homogenisation of egg yolk with equivolume mixture of ethanol and 5 % NaCl solution. Total volume of hexane extract was made up to 10 mL.

A UV-Vis spectrum of hexane extracts was recorded in range 380-520 nm versus hexane (Helios γ, Thermo Electron Corporation, Waltham, USA). Absorbance in maximum was used and total carotenoid (TC) content was calculated as β-carotene equivalents (mg/kg of sample on a dry weight basis, final concentrations of β-carotene for calibration curve 0.2-2.5 mg/L).

Colour determination

Colour of grounded corn grain and egg yolk was determined instrumentally by Minolta Chroma-meter (CR-410, Konica Minolta, Osaka, Japan) in the CIE L* a* b* space. The L* value indicates the lightness, representing dark to light (0–100). The a* (redness) value gives the degree of the red–green colour, with a higher positive a* value indicating more red colour. The b* (yellowness) value indicates the degree of the yellow–blue colour, with a higher positive b* value indicating more yellow colour. White calibration with the specifications of Y=94.5, x=0.3158 and y=0.3323 was used to standardise the chroma meter. Samples were in a 7-cm diameter glass cell with an optically clear bottom. Corn samples replicates were recorded in triplicate and average value was taken. Three egg yolk samples from each cage were combined and colour was recorded in triplicate.

Subjective colour was determined on the same combined egg yolk samples as the instrumental measure using the Roche Yolk Colour Fan (RYCF; DSM Nutritional Products, Kaiseraugst, Switzerland) by one person.

Statistical analysis

Statistical analysis was performed by Statistical Analysis System 9.1. (2003) using the PROC GLM procedure for the comparison of corn hybrids and egg yolks of different dietary treatments, and PROC CORR procedure for the analyses of Pearson correlation coefficients between the variables. Effect of total carotenoid content of tested hybrids on the distribution of carotenoids in the egg yolks and their colour parameters were examined using the linear regression in PROC GLM. Differences were considered significant if P<0.05.

Results and Discussion

Corn grain

Total carotenoid content and colour parameters in CIE Lab space were determined for eight yellow corn hybrids (Table 2). Total carotenoid content ranged from 17.32 (Bc 394) to 31.52 (Bc 572) mg β-carotene (βC) equivalents/kg of sample and varied significantly among tested hybrids. Quantification of total carotenoid content estimated as total absorption of hexane extract was reported in research performed by Hulshof et al. (2007). Reported total carotenoid content ranged in similar values (9.90–39.96 mg/kg) as obtained in this research. Moreover, Kurilich and Juvik (1999) reported on 0.15 to 33.11 mg/kg total carotenoids in 44 corn lines.

The lightness value was highest for hybrid Bc 778 (88.18) and lowest for hybrid Bc 282 (79.77). The redness values ranged from -1.44 to 2.55, the lowest being for hybrid Bc 778 and the highest being for hybrid Bc 282. Hybrids Bc 394, Bc 462, Bc 678 and Bc 778 had a negative a* value indicating the presence of green colour. The yellowness ranged from 28.86 to 37.59, with the lowest and the highest values observed for Bc 354 and Bc 572 hybrid, respectively. Analysis of variance revealed the significant differences (P<0.05) among tested hybrids for all three colour parameters. Sandhu et al. (*2007*) reported similar L* values (from 81.94 to 86.96) for nine corn hybrids, but lower a* (from -2.28 to -2.81) and b* (from 7.83 to 24.12) values than observed in this study.

Table 2. *Total carotenoid content and colour parameters of eight yellow corn grain hybrids. Results are expressed as mean±standard deviation (n=3).*

Hybrid[b]	Total carotenoids (mg βC/kg d.w.)	Colour[1]		
		L*	a*	b*
Bc 282	25.31[d]	79.77[d]	2.55[a]	33.02[b]
Bc 354	21.30[f]	82.02[c]	2.49[a]	28.86[c]
Bc 394	17.32[g]	85.75[b]	-0.79[cd]	19.16[c]
Bc 462	28.39[c]	84.83[b]	-0.94[cd]	36.44[a]
Bc 572	31.52[a]	85.63[b]	1.00[b]	37.59[a]
Bc 678	29.84[bc]	85.07[b]	-0.49[c]	33.09[b]
Bc 778	23.15[e]	88.18[a]	-1.44[d]	29.03[c]
Pajdaš	30.85[ab]	82.40[c]	1.96[a]	32.99[b]

[1]L* = black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+).
[abcdefg]Means within a column lacking a common superscript differ (P<0.05).

Lightness and redness did not correlate with the total carotenoid content, but they showed a high negative mutual correlation (r = -0.80; P<0.05) indicating that hybrid with the highest redness value will have the lowest lightness value and *vice versa* as it was observed for hybrids Bc 282 and Bc 778, respectively. Yellowness did not correlate with the other colour parameters. However, there was a high positive correlation between b* and total carotenoid content (r = 0.82; P<0.05), which indicates the possibility of using the yellowness as a rapid and low cost measure of total carotenoid content in yellow corn hybrids.

Egg yolks

Total carotenoid content in egg yolks was analysed every seven days starting with the day when the dietary treatments began with intent to determine carotenoid content and egg yolk colour stabilisation. After two weeks content was stabilised (data not shown). Egg yolks were collected in fourth week for further carotenoid analysis, and results of total carotenoid content from eight dietary treatments are shown in Table 3. The total carotenoid content in egg yolks ranged from 15.34 to 29.82 mg β-carotene (βC) equivalents/kg of sample, with the lowest and the highest values in dietary treatment with Bc 394 and Bc 572 hybrid, respectively. Dietary treatment had a significant effect on egg yolk carotenoid content showing a linear relationship between the carotenoid content in corn grain and egg yolk (Figure 1). It has already been shown (Karadas et al., 2006; Na et al., 2004; Surai et al., 1998) that the concentration of carotenoids in egg yolk reflects the concentration of carotenoids in the diet and our results are consistent with previous findings.

The lightness of egg yolks colour ranged from 73.11 to 75.54 with the lowest and the highest values for dietary treatment with Bc 572 and Bc 394 corn hybrids, respectively

Table 3. *Total carotenoid content and colour parameters of egg yolks from eight dietary treatments differentiated in corn grain hybrid. Results are expressed as mean±standard deviation (n=12).*

Hybrid	Total carotenoids (mg βC/kg)	Colour			
		CIE Lab[1]			RYCF[2]
		L*	a*	b*	
Bc 282	24.62[bc]	73.85[bc]	7.12[ab]	77.18	8.58[bc]
Bc 354	20.91[c]	73.99[ab]	7.70[ab]	77.14	8.83[bc]
Bc 394	15.34[d]	75.54[a]	5.27[b]	76.45	8.08[c]
Bc 462	22.97[c]	74.84[abc]	5.75[b]	76.99	8.50[bc]
Bc 572	29.82[a]	73.11[bc]	9.32[a]	76.58	9.77[a]
Bc 678	24.68[bc]	73.65[b]	6.86[ab]	76.78	8.92[ab]
Bc 778	20.98[c]	74.33[abc]	5.36[b]	76.46	8.92[ab]
Pajdaš	28.47[ab]	73.34[b]	7.72[ab]	76.55	9.08[ab]

[1]L = black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+).
[2]Egg yolk colour based on Roche Yolk Colour Fan: 15, dark orange; 1, light pale yellow.
[abcd]Means within a column lacking a common superscript differ (P<0.05).

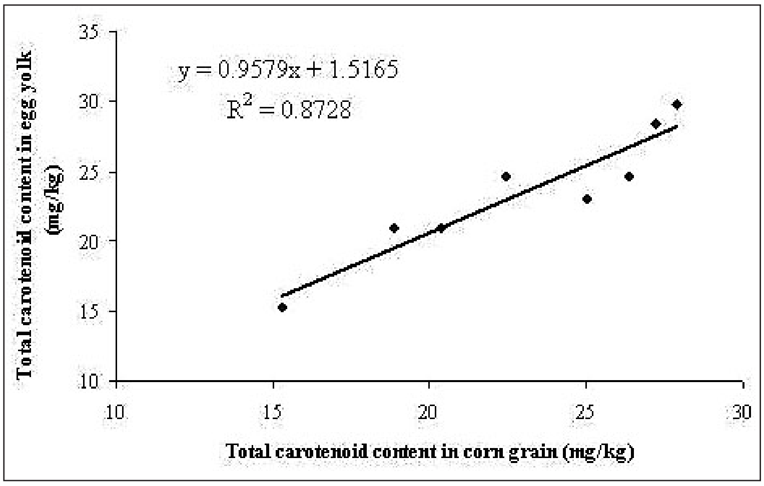

Figure 1. *Relationship between total carotenoid content in corn grain and egg yolks from different dietary treatments.*

(Table 3). Although all L* values were numerically similar, the difference between some dietary treatments was significant (Bc 394 *vs*. Bc 572 and Pajdaš; P<0.05). The redness value ranged from 5.27 (Bc 394 dietary treatment) to 9.32 (Bc 572 dietary treatment), with the significant difference between dietary treatment Bc 572 and dietary treatments Bc 394, Bc 462 and Bc 778 (P<0.05). The values for yellowness were similar (P>0.05) between dietary treatments ranging from 76.45 for Bc 394 to 77.18 for Bc 282.

Regression analysis revealed that carotenoids from dietary treatments affected only the redness of egg yolks (Figure 2.), making it the parameter to determine pigmentation efficiency of corn carotenoids. Also, this observation suggests that some hybrids had higher content of red coloured carotenoids (zeaxanthin; Baiao et al., 1999). Karadas et al. (2006) proposed that the carotenoid profile is much more important for egg yolk pigmentation than quantity of total carotenoids in egg yolk. Although the further characterisation and quantification of carotenoids was not performed in this study, based on mentioned conclusion, we had observed that some corn hybrids have adequate carotenoid profile for desirable egg yolk pigmentation.

Roche Yolk Colour Fan (RYCF) is a common tool used to determine yolk colour. Croatian consumers prefer egg yolks with intensive golden, almost orange colour with 10 to 12 score on RYCF scale (Senčić et al., 2006). In this research, the average RYCF values ranged from 8.08 for dietary treatment with hybrid Bc 354 to 9.77 for hybrid Bc 572. Furthermore, it was observed linear relationship between RYCF value and total carotenoid content in corn grain (Figure 3). Results obtained in this research are much higher than those reported by Kijparkorn et al. (2010) who used 54.5 % of corn grain as the only source of pigments in hen's diet. With 55.67 % of corn grain in hen's diet Lokaewmanee et al. (2010) obtained RYCF average of 8.64, which is similar to the most of the results of corn hybrids tested in this study, except for Bc 572 and Pajdaš which resulted in considerably higher RYCF score. In present study, only the egg yolks from dietary treatment with Bc 572 corn hybrid achieved the RYCF score considered as desirable for Croatian market. However, as the carotenoid intake increases with the dietary level of corn, the better RYCF results could be achieved with higher portion of corn grain in laying hen's diet.

The RYCF colour determinations are highly subjective which makes them difficult to compare with determinations made in different conditions. Correlation analysis between the RYCF and instrumentally measured a* values indicated a highly positive relationship (r=0.6274; P<0.05). This result agrees with findings of Baiao et al. (1999) who concluded that both methods are of equal practical value for evaluating yolks colour. In our opinion, better and more objective insight in egg yolk colour could be achieved by measuring the CIE a* colour parameter.

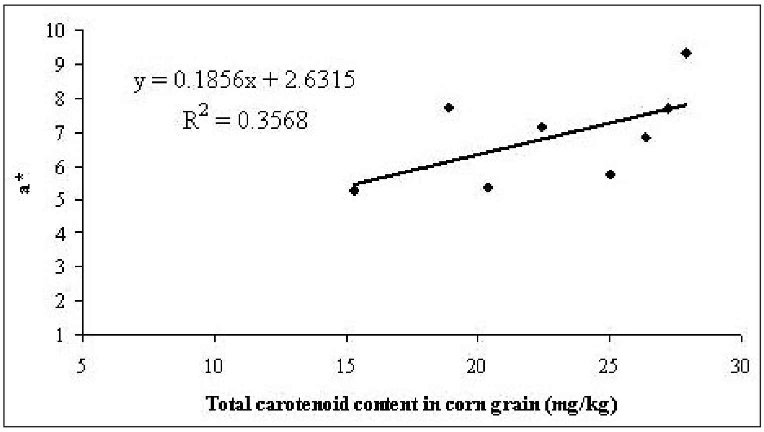

Figure 2. *Relationship between total carotenoid content in corn grain and CIE a* value of egg yolks from different dietary treatments.*

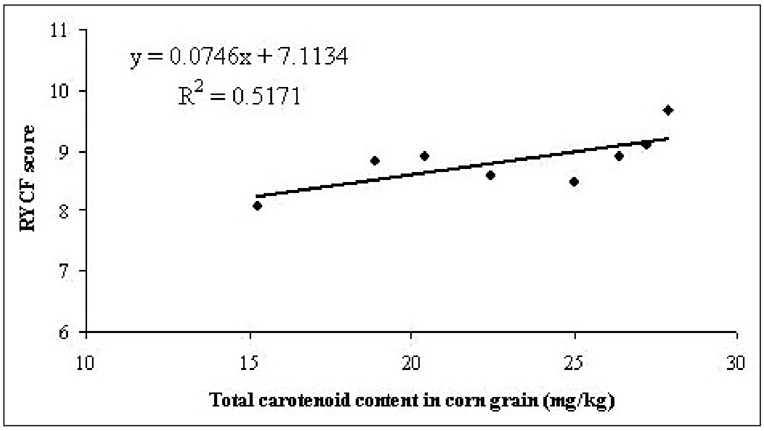

Figure 3. *Relationship between total carotenoid content in corn grain and RYCF value of egg yolks from different dietary treatments.*

Conclusion

This research showed that corn grain in laying hen's diet without added pigments, could be used for adequate carotenoids supply for acceptable egg yolk colour. Higher colour intensity (RYCF>10) could be achieved with higher portion (>56 %) of corn grain in laying hen's diet. The total carotenoid content in corn grain related positively with both the subjectively (RYCF score) and instrumentally (CIE redness value) measured egg yolk colour. In addition, the CIE redness showed a high and positive correlation with RYCF score, and therefore could be used for more objective colour measurements of egg yolk.

References

Baiao N.V, Mendez J., Mateos J., Garcia M., Mateos G.G. (1999) Pigmenting efficacy of several oxycarotenoids on egg yolk. *Journal of Applied Poultry Research* 8: 472-479.

CIE: Supplement No.2 to CIE Publication No. 15 (E-1.3.1) (1978) 1971/ (TC-1- 3), Recommendations on uniform color spaces-color difference equations, Psychrometric Color Terms, Commission Internationale de I ´Eclairage, Paris, France.

Egesel C. O., Wong J. C., Lambert R. J., Rocheford T. R.(2003) Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Science* 43: 818–823.

González M., Castaño E., Avila E., González de Mejía E. (1999) Effect of capsaicin from red pepper (*Capsicum sp*) on the deposition of carotenoids in egg yolk. *Journal of the Science of Food and Agriculture* 79: 1904-1908.

Hencken H. (1989) Chemical and physiological behaviour of feed carotenoids and their effects on pigmentation. *Poultry Sci*ence 71: 711-717.

HRN ISO 6496:2001 en. (2008) Animal feeding stuffs – Determination of moisture and other volatile matter content. Croatian Standards Institute, Zagreb, Croatia.

Hulshof P.J.M., Kosmeijer-Schuila T., West C.E., Hollman P.C.H. (2007) Quick screening of maize kernels for provitamin A content. *Journal of Food Composition and Analysis* 20: 655-661.

Karadas F., Grammendis E., Surai P.F. (2006) Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *British Poultry Science* 47: 561-566.

Kijparkorn S., Plaimast H., Wangsoonoen S. (2010) Sano (*Sesbania javanica* Miq.) flower as a pigment source in egg yolk of laying hens. *Thai Journal of Veterinary Medicine* 40: 281-287.

Kurilich A.C., Juvik J.A. (1999) Quantification of carotenoid and tocopherols antioxidants in *Zea mays*. *Journal of Agricultural and Food Chemistry* 47: 1948-1955.

Lokaewmanee K., Yamauchi K., Komori T., Saito K. (2010) Effects on egg yolk colour of paprika or paprika combined with marigold flower extracts. *Italian Journal of Animal Science* 9: 356-359.

Na J.-C., Song J.-Y., Lee B.-D., Lee S.-J., Lee C.-Y., An G.-H. (2004) Effect of polarity on absorption and accumulation of carotenoids by laying hens. *Animal Feed Science and Technology* 117: 305–315.

National Research Council (NRC; 1994), Nutrient requirements of poultry, 9th revised edition, National Academy Press, Washington, DC, USA.

Sandhu K.S., Singh N., Malhi N.S. (2007) Some properties of corn grains and their flours I: Physicochemical, functional and chapati-making properties of flours. *Food Chemistry* 101: 938–946.

Senčić Đ., Butko D. (2006) Productivity of layers and egg yolk quality in free range and cage system of housing. *Poljoprivreda* 12: 48-51 (in Croatian).

Statistical Analysis System (SAS; 2003), OnlineDoc® Software Release 9.1., SAS Institute Inc., Cary, NC, USA.

Surai P.F., Speake B.K. (1998) Distribution of carotenoids from the yolk to the tissues of the chick embryo. *Journal of Nutritional Biochemistry* 9: 645– 651.

Surai P.F., Speake B.K, Wood N.A.R., Blount J.D., Bortolotti G.R., Sparks N.H.C. (2001) Carotenoid discrimination by the avian embryo: a lesson from wild bird. *Comparative Biochemistry and Physiology B* 128: 743-750.