

## Effect of different levels of marigold and paprika on egg production and yolk colour

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### SUMMARY

Yolk colour is a very important sensory characteristic of table eggs. It is considered as the most important egg characteristic when it comes to selection and assessment by consumers. In the last two decades, public opinion more clearly expresses aversion to synthetic additives to improve colour of egg yolks, and some countries introduced statutory prohibition on the use of artificial pigments in the diet of laying hens. Eggs enriched with natural pigments are desirable in human food chain due to numerous health benefits. In this study, we investigated the effect of inclusion of natural sources of pigments into laying hens' diet on egg production and yolk colour. Different levels of marigold and red sweet pepper were used as natural sources of pigments. The experiment which included 150 Lohmann Brown layers (38th week of production) lasted 4 weeks. Laying hens were divided into five groups (1 control and 4 experiments) with six replicates of five birds each, which makes total of thirty hens per group. Hens were housed in individual wire cages with feed and water available *ad libitum*. Egg yolk colour was measured by Minolta, spectrophotometer and Roche yolk colour fan. The changes in egg, yolk, eggshell and albumen weights were not observed. On the other hand, significant differences between groups for  $L^*$ ,  $a^*$ ,  $b^*$ ,  $\beta$ -carotene and Roche yolk colour were found. The largest differences were found between control and the group with 1.5% red sweet pepper for  $a^*$  from -0.79 to 17.66, respectively and for RYCF from 7.67 to 14.71, respectively.

Keywords: egg, yolk colour, marigold, paprika,  $\beta$ -carotene

### INTRODUCTION

Colour of the egg yolk is the most important characteristics which determines egg selection by consumers. Even though followed by no shift in nutritional quality, extreme changes in the colour of egg yolk can make it

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unacceptable for consumers, because many of them consider that eggs with pale yolk colour is neither healthy nor tasty (Chowdhury et al., 2008). Depending on the geographical location, culture and tradition, consumers prefer a certain colour of egg yolk. For instance, Germany, Netherlands, Spain, and Belgium consumers prefer yolks with the values of 13–14 per RYCF scale (Roche Yolk Colour Fan), while egg yolks with the values of 11–12 per RYCF scale are more favoured in France, south England, and Finland (Bovšková et al., 2014). On the other hand, US consumers prefer egg yolk colour from 7 to 10 per RYCF scale (Galobart et al., 2004), while consumers in Ireland, north England, and Sweden prefer yolks with the values of 8–9 per RYCF scale (Bovšková et al., 2014).

Colour of egg yolks depends on the presence of carotenoids in feed for laying hens, that are capable of transferring about 20-60% of colour from feeds in egg yolk, but not able to synthesize pigments by their own biochemical processes (Karadas et al., 2006; Li et al., 2012). In order to achieve desirable coloration of egg yolks synthetic or natural pigments can be added to a diet (Galobart et al., 2004). Colour of egg yolk commonly derives from synthetic pigments. However, due to the increasing public concern about the use of synthetic pigments, alternative natural sources of pigments have been introduced (Spasevski et al., 2016). Possible natural sources of carotenoids in poultry feed are yellow maize, marigold, alfalfa, carrot, pumpkin, paprika etc. (Lokaewmanee et al., 2010).

The main goal of the present study was to investigate the possibility of replacing synthetic pigments with marigold flower and paprika in laying hens diets, and to determine their effects on egg production and yolk colour.

## MATERIAL AND METHODS

### *Dietary treatment*

The experiment included 150 Lohmann Brown layers (in the 38th week of production) which were divided into five treatments (1 control and 4 experiments) with six replicates of five birds each, which makes total of thirty hens per treatment. The basal diet were formulated for all treatments of laying hens and composed of conventional raw materials with the addition of paprika and marigold flower in the experimental treatments according Table 1. Hens were housed in individual wire cages with feed and water available *ad libitum*. The chemical composition of control and experimental diets were shown in Table 1.

### *Sample collection and analysis*

Laying hens were fed for four weeks and after this period 10 eggs from each treatment were randomly chosen and analysed in order to identify

any differences in the physical characteristics of eggs. Weight of whole egg, yolk, shell and albumen were weighed separately and shown in Table 2.

Content of  $\beta$ -carotene was determined according to the method described by Islam (Islam, 2015). The results were expressed as  $\mu\text{g } \beta\text{C/g}$  of sample.

Table 1. Composition of basal and experimental diets g/kg

Ingredients (%)	C	E1	E2	E3	E4
Maize	58.7	57.8	57.7	57.7	57.6
Soybean cake	21.1	20.6	20.7	20.7	20.8
Sunflower meal 33%	9.8	9.8	9.8	9.8	9.8
Limestone	6.28	6.28	6.28	6.28	6.28
Premix	4	4	4	4	4
Methionine-DL (99%)	0.018	0.018	0.018	0.018	0.018
Marigold flower	-	1.5	1	0.5	-
Paprika	-	-	0.5	1	1.5
Chemical composition (%)					
Dry matter	89.64	90.01	89.76	89.84	89.79
Moisture	10.36	9.99	10.24	10.16	10.21
Crude protein	16.59	16.61	16.80	16.67	16.73
Crude fat	3.92	3.99	4.14	3.89	4.24
Crude ash	11.10	12.19	11.15	11.41	11.64
Crude fibre	3.63	3.56	3.82	3.71	4.06
Calcium	3.26	3.24	3.20	3.26	3.36
Available phosphorus	0.41	0.55	0.52	0.55	0.55
Methionine	0.35	0.34	0.33	0.34	0.35
Lysine	0.75	0.74	0.76	0.75	0.76

Colour of the egg yolks was determined visually with RYCF (Hoffmann-La Roche Ltd, Basel, Switzerland), which is a scale of colours ranging from 15, dark orange to 1, light pale. The yolk colour was also measured with a Minolta Chroma Meter (Model CR-400, Minolta Co., Osaka, Japan), using the application contact surface diameter of 8 mm. The results are presented according to the CIELab color system, where the colour values were expressed as  $L^*$  (lightness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness). The colour measurement was conducted in three replications for each of the sample.

#### *Statistical analysis*

The influence of the hen nutrition on egg production and yolk colour was determined by one-way analysis of variance (ANOVA). The comparison among means was performed by Tukey's HSD test, and the significant differences were calculated at  $p < 0.05$ . Statistical methods were performed using the Statistica Software version 13 (Statsoft Inc. 2013, USA).

## RESULTS AND DISCUSSION

The physical characteristics of the eggs were shown in Table 2. According to presented results it can be concluded that the egg weights of experimental groups were not significantly different from the egg weight of control group. Moreover, the addition of paprika and marigold lower to the laying hens' diet did not affect the weight of egg shell, yolk, and albumen. These results are in agreement with Lokaewmanee et al. (2010) and Rowghani et al. (2006) who reported no effect of marigold and paprika addition on egg quality properties. Chowdhury (2008) and Gurbuz (2003) found that supplementation of neither 40 g/kg of marigold nor 40 g/kg of paprika significantly affected egg weight. The obtained results indicated that the supplementation of natural pigments to laying hens' diet did not deteriorate egg quality.

Table 2. Physical characteristics of the eggs

	Egg weight, g	Yolk weight, g	Egg shell weight, g	Albumen weight, g
C	68.39 ± 6.7	17.60 ± 2.2	6.8 ± 2.2	43.99 ± 4.2
E1	72.36 ± 6.8	19.14 ± 3.0	7.86 ± 0.5	45.36 ± 3.9
E2	69.56 ± 4.2	17.51 ± 0.6	6.89 ± 1.0	45.16 ± 3.0
E3	65.72 ± 6.7	16.56 ± 0.6	7.25 ± 0.8	41.90 ± 5.3
E4	68.98 ± 7.4	16.06 ± 1.1	7.17 ± 0.4	49.77 ± 6.4

Results are expressed as mean ± SD (n=10); All variables showed no significant differences among dietary treatments

The obtained data regarding the effect of marigold and paprika on egg yolk colour were shown in Table 3. The content of  $\beta$ -carotene in control treatment was the lowest (23.17  $\mu\text{g/g}$ ), as it was expected. This result is in agreement with literature data (Barbosa et al., 2011; Kljak et al., 2012). The addition of natural pigments to the hens' diet increased the content of  $\beta$ -carotene. The addition of paprika (E4) affected the greatest increase in  $\beta$ -carotene content than marigold supplementation (E2). Concerning the combination of the pigments, higher content of  $\beta$ -carotene was observed in group E3 which contained 1% of paprika and 0.5% of marigold. Therefore, paprika showed to be the main source of  $\beta$ -carotene in egg yolks.

In addition, yolk lightness ( $L^*$ ) significantly ( $p < 0.05$ ) decreased when paprika was added alone (E4) or in combination with 0.5% of marigold (E3). The supplementation of natural pigments to hens' diet significantly ( $p < 0.05$ ) affected yolk redness. Paprika supplementation provided higher value of yolk redness than the addition of marigold alone, being in accordance with the results of  $\beta$ -carotene content and RYCF. In combination, higher redness value was reported in group in which paprika was more prevalent (E3). The value of yolk yellowness significantly ( $p < 0.05$ ) decreased when paprika was added alone or in combination with

0.5% of marigold (E3) to the diet. On the other hand, the supplementation of marigold (E1) significantly ( $p < 0.05$ ) increased yolk yellowness value. No significant ( $p > 0.05$ ) changes in yolk yellowness was obtained in group E2.

Table 3. The content of  $\beta$ -carotene and parameters of egg yolks colour from control and four dietary treatments

	Content of $\beta$ -carotene ( $\mu\text{g/g}$ )	Colour			RYCF
		CIELab			
		$L^*$	$a^*$	$b^*$	
C	23.17 $\pm$ 0.9 <sup>a</sup>	51.68 $\pm$ 1.7 <sup>b</sup>	-0.79 $\pm$ 0.7 <sup>a</sup>	36.19 $\pm$ 3.2 <sup>b</sup>	7.67 $\pm$ 0.5 <sup>a</sup>
E1	31.67 $\pm$ 0.4 <sup>b</sup>	51.23 $\pm$ 1.8 <sup>b</sup>	1.25 $\pm$ 0.9 <sup>b</sup>	40.36 $\pm$ 2.6 <sup>c</sup>	8.60 $\pm$ 0.5 <sup>a</sup>
E2	31.93 $\pm$ 0.1 <sup>b</sup>	50.08 $\pm$ 1.2 <sup>b</sup>	6.21 $\pm$ 1.2 <sup>c</sup>	36.61 $\pm$ 1.5 <sup>bc</sup>	12.56 $\pm$ 1.0 <sup>b</sup>
E3	39.60 $\pm$ 0.3 <sup>c</sup>	47.27 $\pm$ 2.5 <sup>a</sup>	12.57 $\pm$ 1.0 <sup>d</sup>	31.85 $\pm$ 4.3 <sup>a</sup>	14.57 $\pm$ 0.5 <sup>c</sup>
E4	47.97 $\pm$ 0.3 <sup>d</sup>	45.55 $\pm$ 2.1 <sup>a</sup>	17.66 $\pm$ 1.0 <sup>e</sup>	32.01 $\pm$ 4.2 <sup>a</sup>	14.71 $\pm$ 0.5 <sup>c</sup>

Results are expressed as mean  $\pm$  SD (n=10); Values with different letter in same row are statistically different ( $p < 0.05$ ).

The addition of marigold in hens' diet (E1) did not affect yolk colour determined by RYCF. Values 7.67 (C) and 8.60 (E1) per RYCF don't satisfy consumers in most countries in Europe and Asia, which means that marigold alone cannot provide the yolk colour more than 10 per RYCF scale, which these consumers demand (Galobart et al., 2004). Even though the highest yolk colour value (over 14 per RYCF) was achieved in E3 and E4 treatments, these yolk colours are not acceptable to consumers (Sandeski et al., 2014) but are preferred in cake industry (Shahsavari, 2014). Marigold is a source of yellow pigments while paprika is rich in red pigments which amplify egg yolk colour and best effect can be achieved with a combination of these two natural sources of pigments. The optimal yolk colour of 12.56 per RYCF was obtained for samples in E2 treatment which contained 1% marigold and 0.5% paprika. This value satisfies consumers in most European countries and wider which prefer yellow - orange colour of yolk.

## CONCLUSIONS

Supplementation of paprika and marigold into laying hens' diet did not negatively influence physical characteristics of eggs. Therefore, their addition to hens' diet was justified. Marigold alone cannot give the colour of the yolk which consumers demand and must be combined with paprika. The addition of paprika alone or in combination with marigold increased the content of  $\beta$ -carotene, redness value and RYCF. The optimal yolk colour was achieved in group E2 which was supplemented with 1% of marigold and 0.5 % of paprika. According to presented results it might be concluded

that marigold and paprika can be successfully added to hens' diet as alternative sources of pigments to intensify egg yolk coloration.

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