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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Effects of Addition of the Red Pepper from 4th Harvest to Corn or Wheat Based Diets on Egg-yolk Colour and Egg Production in Laying Hens

Yavuz Gurbuz¹, Sulhaddin Yasar² and Mesut Karaman¹

¹Department of Animal Science, Faculty of Agriculture, The University of Sutcuimam, Kahramanmaraş, Turkey

²Department of Animal Science, Faculty of Agriculture, The University of Suleyman Demirel, Isparta, Turkey

Email: sulhattiny@yahoo.com

Abstract: In order to investigate the effects of locally grown red pepper on egg-yolk colour and egg production in laying hens, twelve diets based on white corn and wheat grain were supplemented by various amounts of red pepper and artificial colouring pigments, and offered to 96 layer hens for 13 weeks from the beginning of the laying circle. The characteristics of egg production and egg-yolk colour were examined under the influence of dietary treatments. The present results showed that egg weight, daily feed intake and egg yield did not significantly differ between the experimental diets, indicating that red chilli can be used as an alternative feed ingredient in layer diet with no significant alterations in the bird performance. Furthermore, the highest colour pigmentation were obtained from the diets (wheat + yellow corn) with 3.0 and 4.0% red chilli added whereas the lowest colour pigmentation was obtained from the diets (white corn + no wheat) with no chilli or artificial pigments. The most preferred colour pigmentation by the customer was obtained from the diets of 25% yellow corn and 32.4% wheat to which 0.5% red chilli added. Increasing amount of the red chilli in the yellow corn and wheat based diets resulted in an increasing reddish colour pigmentation of egg-yolk. On the other hand, we could not measure the colour of pigmentation by RCF scale of the diets containing only white corn (around 55%) in which the amount of the red chilli ranged from 2.0 to 4.0%. The addition of 0.30 and 0.25% artificial pigments to the diets of white corn and the diets of yellow corn + wheat resulted in optimum colour pigmentation. Although we did not test the interaction effects between the artificial pigments and red chilli on the egg-yolk colour pigmentation, the results suggested that an appropriate combination of artificial and natural colour pigments can be used to obtain optimum egg-yolk colour pigmentation. In short, the red chilli of 4th harvest, which is not suitable for human consumption, did not adversely alter the laying performance, and additionally the use of red chilli as a potential natural colour pigment caused to an optimum egg-yolk colour, especially at the rate of 0.5% in the layer diet.

Key words: Red pepper, layer hens, egg yolk colour

Introduction

Using the colour pigments in food industry has been received great impetus during the last few decades. However food used for human nutrition should be as natural as possible since artificial-free food is demanded by current market profile. Therefore, some of artificial colour pigmentation materials used in food industry have been banned in EU and USA recently (Erkek and Talug, 1990; Oktay and Olgun, 1972; Dogan, 1993).

Both artificial and natural colour additives are used in the hen's diet to improve egg-yolk colour. Both producer and consumers of the hen's egg do consider the basic characteristics such as size of the egg, colour of the shell and egg-yolk pigmentation (Erkek and Talug, 1990; Belyavin and Mrangos, 1988). However, the desires for egg-yolk colour are quite variable for different cultures. Hens can not synthesize the colour pigments, but they can transport 20-60 % of them to the egg yolk from the

feed they ingested (Bartov and Bornsteins, 1980; Streff, 1970). Colour additives given with diet of the hens to improve yolk pigmentation rely on so many factors (Belyavin, 1981; Hinton *et al.*, 1974). Some of the most important factors are crude material of diet and nature of colour additives used in the diet. In addition, the genus of hens, their age, health condition and the other environmental conditions may also affect the egg-yolk colour (El Baushly and Raterink, 1989; Fletcher and Halloran, 1983).

The plants containing colour pigments are widespread in the nature. For instance, corn is one of the essential feedstuff of the hen's diet and it contains lutein pigment which affects the egg-yolk colour. However when the corn is not available in the market, wheat subsidizes the corn as the main energy source in the diet whereas wheat contains insufficient colour pigments to enhance egg-yolk pigmentation. Therefore, when the wheat is practiced as main energy source, additional artificial or

natural colour additives should be added into the diet of laying hens.

The diets, containing insufficient amount of colour pigments, could be improved by adding natural or artificial colour additives to enhance yolk colour. Although corn is used at high levels (40%) in the diet of the laying hens, it does not enhance the red pigmentation of the yolk properly. Hence adding the artificial or natural colour additives to the diets is suggested (Kirkpinar and Erkek, 1996). As natural colour pigment sources ground-clover, marigold, and red chili are widely used in poultry diet. Furthermore, while some artificial colour additives result red or reddish yolk colour, some others give yellow colour to the yolk. For instance, β -apo-8-carotenoid acid ester gives yellow colour to the yolk, whilst canthaxanthin results in red colour pigmentation. Colour additives giving red pigmentation to the yolk, such as capsanthin and capsorubin dominate the ground chilli paper (Kirkpinar and Erkek, 1996; Kirkpinar and Erkek, 1997; Fletcher, 1980; Marusich and Bauernfeind, 1970).

As natural colour material, corn, marigold, ground-clover contain lutein, which leads to yellow colour whilst ground red pepper contains kapsantin and kapsorubin resulting red colour in the egg yolk (Fletcher and Halloran, 1981; Karunajeewa, 1980; National Research Council, 1993). Each colour material has different effects on egg yolk pigmentation. Bartov and Bornstain (1980) reported that laying hens can transform the 25.9-31.1% of the corn xanthophylls to the egg yolk. Marusich and Bauernfeind (1970) reported that when the hens ingest 0.3 mg d⁻¹ beta-apo-8-carotenoid acid ethyl ester, they transform the only 59 % of them to the egg yolk. However, transferring of the beta-apo-8-carotenoid acid ethyl ester is heavily affected by the age of hens and the amount and contents of the active substances within the diet (Papa *et al.*, 1985).

According to Hencken (1992) hens could transform the beta-apo-8-carotenoid acid ethyl ester more efficiently compared to corn. He determined that only 35-40% of canthaxanthin could be stored in egg yolk. Marusich and Bauernfeind (1970) reported that hens could transform the canthaxanthin at only 34-38%. Karunajeewa (1980) suggested that to obtain an orange colour egg yolk, the ratio between canthaxanthin (which gives red colour) and lutein (gives yellow colour) should be in 3:1 in the diet.

The present study was designed to investigate effects of 4th harvest of red pepper, not suitable for humans' consumption, as alternative feed and feed additive to improve egg-yolk pigmentation when added to the diet of laying hens at different levels.

Materials and Methods

Animals and feeds: The experiments were carried out using 22-week-old Hyline White hens (n = 96). The layer

diet was formulated to include in 17% crude protein (CP) and 2750 kcal/kg metabolizable energy (ME). The dietary ingredients were purchased from a private feed company and these were as follows: white corn, yellow corn, soybean meal, sunflower, vegetable oil, salt, limestone, vitamin mixture, mineral mixture di-calcium phosphate, DL-methionine, lysin.

The diet was prepared using mainly white corn and wheat, into which different levels of red pepper and artificial pigmentation materials (at standard level) were added. The added red pepper was purchased from a local red pepper factory and its nutritive information is given in Table 1. The ingredient composition of the experimental diets was presented in Table 2 and the nutrient analyses of the diets in Table 3.

Table 1: Chemical analysis data of 4th harvest red chilli used in the diet

Moisture	% 7.30
Crude Protein	% 12.80
Crude fibre	% 15.40
Crude lipid	% 8.50
Crude ash	% 28.00
Starch	% 0.40
Sugar	% 7.60
ME	1450 (kcal/kg)
Nitrogen- free- extract	% 28
Carotene	6.54 (mg/kg)
Xanthophylls	400 (mg/kg)
Phosphorus	0.20 (mg/kg)
Sodium	8.50 (mg/kg)
Magnesium	0.80 (mg/kg)
Manganese	0.10 (mg/kg)
Zinc	20 (mg/kg)
Copper	25 (mg/kg)
Cobalt	0.03 (mg/kg)
Potassium	3.5 (mg/kg)
Vitamin E	90 (mg/kg)
Vitamin C	97 (mg/kg)

Methodology: This study was carried out in Kahramanmaraş Sutcu Imam University research farm. Hens were kept in wire cage (four hens per cage). Pre-experiment (3 weeks) was conducted using a standard layer diet containing white corn and no additives. Twelve different diets were given to 96 hens for 13 weeks. Thus, each of the twelve diets was fed to 8 birds in two replicates, each replicate with 4 birds. Unlimited feed and water were made available and 16h d⁻¹ light was provided.

Chemical content of the diets was determined according to Association of Official Analytical Chemists (AOAC) (1980). Crude protein, dry matter content, crude oil content of the concentrates were analyzed as reported by Wende and crude fiber content was analyzed according to the method of Lepper Metabolic energy was

Table 2: The ingredient concentration (%) of the diets (groups A-N) given to hens

Feed Stuffs	A	B	C	D	E	F	G	H	K	L	M	N
White Corn	55.8	55.7	55.7	56.0	55.58	-	-	-	-	-	-	-
Yellow Corn	-	-	-	-	-	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Wheat	-	-	-	-	-	32.4	32.4	32.4	32.3	32.2	32.1	32.3
Soybean Meal	22.6	22.8	21.8	22.8	22.6	21.1	21.1	21.10	21.1	21.3	21.4	21.1
Sunflower Meal	4.5	4.40	4.40	5.0	4.50	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Soybean Lipid	3.0	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Wheat Bran	4.0	1.0	1.0	-	3.7	4.0	3.5	3.0	2.0	1.0	-	4.0
Limestone	8.0	8.0	8.0	8.0	8.0	8.5	8.2	8.2	8.5	8.5	8.0	8.0
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Mixed	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Mixed	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium Phosphate	1.3	1.3	1.3	1.3	1.3	1.20	1.20	1.2	1.20	1.2	1.3	1.3
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.15	0.15	0.15	0.15	0.15	0.15	0.13
Lysin	0.06	0.08	0.08	0.08	0.06	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium Bicarbonate	0.10	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.10	0.1	0.12
4th harvest paprika	-	2.0	3.0	4.0	-	-	0.50	1.0	2.0	3.0	4.0	-
Synthetic Pigment *	-	-	-	-	0.30	-	-	-	-	-	-	0.25
Total	100	100	100	100	100	100	100	100	100	100	100	100

*Synthetic Pigment Source (K-15 contained β -apo-8-carotenoid and canthaxanthin)

Vitamin Mixture: 2.5 kg da 12.000.000 IU A, 2.000.000 IU D₃, 35.000 IU E, 5000 mg K₃, 3000 mg B₁, 6000 mg B₂, 20.000 mg niacin 6000 mg calcium D-pentotenat, 5000 mg B₆, 15 mg B₁₂, 750 mg folic acid, 45 mg D-biotin, 125.000 mg Colin chloride, 50.000 mg C

Mineral Mixture: kg⁻¹; 80.000 mg zinc , 5000 mg copper, 500 mg cobalt, 2000 mg iodine, 235.680 mg calcium

calculated according to crude protein, crude oil, sugar, starch content of concentrates (Akyildiz, 1984). Determination of egg-yolk pigmentation is conducted by naked-eye scoring (Vuilleumier, 1969).

Laying performance of the hens was determined by daily controls, and egg weights were determined by taking 6 eggs group⁻¹ in 2 weeks intervals. Daily feed consumption of the hens was also determined. Feed conversion efficiency at each group was determined according to feed consumption for per kg egg production. Statistical analysis of data was carried out using SAS statistical package program (SAS, 1985).

Results

Egg-Yolk Colour: RCF is Roche Colour Fan for consideration of the degree of egg yolk pigmentation. RCF values with standard deviations were presented in Table 4. The RCF values of groups L and M were similar and were the highest. RCF of the group A which contained mainly white corn and no colour additives was 1.35 and showed the lowest RCF value. The groups of H (containing 1% red chilli), K (containing 2% red chilli) and N (containing artificial colour additive) showed similar RCF values (Table 4).

When 2, 3 and 4% (groups B, C, D respectively) red chilli were added to the diet containing mainly white corn, reddish yolk colour was obtained. Yolk colour of the B, C and D groups were out of the RCF scale, and hence they were not included in any analysis. When the synthetic colour additives were added to the diet containing mainly white corn (group E) RCF value was determined as 10.30. RCF value of the diet containing 25% yellow corn,

32.4% wheat and no colour additives was 4.25 (group F) which was found to be insufficient at the colour for consumers. However, when 0.5% red chilli was added to the diet of group G RCF value of the yolk colour increased to 9.55. When the red chilli concentration increased to 1% in the same diet (group H) RCF value of yolk colour increased to 11.45 resulting in an orange colour preferred by customers. When amount of red chilli increased to 2% at the diet RCF value at the yolk colour was determined as 12.55 (group K). Adding up to 2, 3 and 4% red chili to the diet resulted in reddish egg-yolk colour (groups B, C, D).

Characteristics of egg production: No significant differences were observed among groups for egg characteristics such as egg weight, daily feed intake, egg production performance.

Discussion

Red chilli level used in this study had no significant effect on the egg yield characteristics. Although nutritive value of red chilli was low, using as feed additive up to 4% at the diet had no adverse affect on egg production suggesting that red chilli could be used as feedstuff for laying hens. Therefore 4th harvest red chilli which is considered as worthless end-product in the industry could be used as feed additive of the laying hens. Hence, farmers will tend to import less artificial colour additives and this will result in an added value to the farm economy.

RCF value of the group A that contained neither artificial nor natural colour additive was 1.35. When 0.5% red chili

Table 3: Nutrient analyses (%) of the experimental layer diets

	A	B	C	D	E	F	G	H	K	L	M	N
Dry Matter	89.77	89.89	89.96	89.80	90.14	90.16	90.18	90.22	90.26	90.23	90.21	90.07
Crude Protein	16.98	17.08	16.90	17.05	16.90	17.03	17.02	17.00	16.98	16.90	17.02	17.01
Crude Lipid	5.73	5.89	5.94	5.74	5.90	5.96	5.98	6.04	6.09	6.15	6.23	5.95
Crude Fiber	4.05	4.19	4.23	4.06	3.79	3.82	3.85	3.90	3.95	4.0	4.13	3.85
Crude Ash	11.0	11.5	11.6	11.0	11.03	11.1	11.2	11.4	11.5	11.6	11.6	11.00
Calcium*	3.68	3.70	3.71	3.68	3.80	3.78	3.79	3.78	3.79	3.70	3.70	3.70
Phosphore*	0.58	0.58	0.59	0.58	0.58	0.58	0.57	0.56	0.55	0.63	0.60	0.61
Lysin*	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Methionine*	0.37	0.37	0.37	0.37	0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.37
ME	2753	2748	2745	2753	2758	2749	2751	2754	2749	2753	2750	2748

*They were estimated by calculation.

Table 4: Production performance and egg-yolk characteristics data of different groups

Groups	Colour Fan Score (RCF)	Egg Weight (g)	Feed Conversion (g feed/g egg)	Feed Intake (g/hen/day)	Egg Production (%)
A	1.35±0.50 ^e	56.34±1.12	1.801±0.05	87.08±0.77	85.35±2.86
B	-	56.16±1.51	1.796±0.01	89.21±1.14	88.94±1.71
C	-	55.33±1.23	1.777±0.03	86.58±0.42	87.98±1.47
D	-	55.47±1.57	1.857±0.11	87.89±2.89	86.25±3.04
E	10.30±0.30 ^c	57.19±1.35	1.907±0.05	91.05±4.32	88.82±1.46
F	4.25 ±0.35 ^d	57.93±1.71	1.84±0.01	91.00±2.81	84.99±1.38
G	9.55 ±0.40 ^c	56.34±1.01	1.827±0.02	92.22±0.68	89.76±1.66
H	11.45 ±0.45 ^b	57.56±1.54	1.858±0.06	90.85±2.07	85.36±1.35
K	12.55 ±0.40 ^b	56.46±1.10	1.863±0.02	90.09±3.78	88.39±1.88
L	14.30 ±0.45 ^a	57.06±1.15	1.822±0.02	89.40±1.38	85.40±2.31
M	14.45 ±0.45 ^a	56.25±1.42	1.809±0.04	87.95±1.63	86.78±1.60
N	12.45 ±0.40 ^b	56.35±1.67	1.852±0.01	92.60±0.20	88.37±1.56

^{a, b, c, d, e}: Means within a column with different superscript differed significantly $P < 0.05$.

was added to the layer diet of 25% yellow corn RCF value was close to 11 which is highly preferred by consumers. However, when the ration of added red chili was increased, yolk colour changed to reddish colour. These results were in agreement with the findings of Marusich and Bauernfeind (1970); Kirkpınar and Erkek (1996); Fletcher and Halloran (1981). RCF values at the groups of L and M were high due to the fact that red chilli levels of these groups were quite high (3 and 4% respectively).

To obtain the optimum yolk colour which is preferred by consumers, red chilli should be used with yellow pigments of such additives. Kaunajeewa (1980) reported that using red chilli together with yellow pigments at 3:1 ratio in layer diet containing no artificial colour is preferred by consumers. Therefore, colour pigment additives should be used in an appropriate ratio since both red and yellow colour additives have direct effects on egg-yolk colour. If the ratio is in favour of red colour additive, egg-yolk would result in reddish colour or vice versa. Both extreme colours are not preferred by consumers, thus 3:1 ratio should be considered for red:yellow colour additives in the diet of laying hens. When the red chilli was used as sole colour additive in the diet, reddish yolk colour was obtained. Therefore, yolk colour of the groups B, C and D could not be evaluated in RCF scale. This result was in agreement

with the findings of Marusich and Bauernfeind (1970). These results suggested that ratio of the colour additives (natural or artificial) should be considered for the diet of the laying hens since they have direct effect on egg-yolk pigmentation. Otherwise it would be impossible to obtain orange-yolk-colour preferred by consumers as previously reported by Erkek and Talug, 1990; Kirkpınar and Erkek, 1996; Fletcher and Halloran, 1981.

To avoid the adverse effect of the wheat and white corn used as main energy source of the laying hens either natural or artificial colour additives should be added into the diet. However, since some of the artificial colour additives have cancerous effects (Oktay and Olgun, 1972) the natural colour additives should have priority to improve yolk pigmentation. In particular, when the diet contained yellow corn as main nutritive component, red chilli should be added into the diet to obtain orange yolk colour preferred by the consumers. When the diet was composed of white corn or wheat as main energy source, colour additives resulting in red colour in yolk such as red chilli must added into the diets. Our results revealed that red chilli could be used as natural colour additive to enhance yolk pigmentation (in the favour of red colour). In addition, reddish or red yolk colours obtained by using colour additives are preferred in cake industry (Fletcher and Halloran, 1981; Papa *et al.*,

1985). This colour could be obtained by adding red chilli solely into the diet of laying hens.

Although the wheat is main crop produced in Turkey, it can not be used as main diet of the laying hens due to its adverse effect on yolk colour. RCF value of the diet (group F) containing 25% yellow corn and 35% wheat and no colour additive was found quite low (4.25). However, addition of 0.5% red chilli into that diet resulted in a higher RCF value (group G: 9.55), which is so close to the optimum RCF value (10.0) preferred by consumers. Thus, the present result can suggest that the wheat-based diets for layer hens can only be used with the 0.5% level of red chilli.

This study confirmed that 4th harvest red chilli enhances the yolk pigmentation and could be used up to 4% in the diet of laying hens, and these results were in agreement with the previous reports (Couch and Farr, 1971; Ozen, 1979; Splittgerber, 1972; Marusich and Bauernfeind, 1981). In addition, 4th harvest red chilli could be used as an alternative feed source for hens diet.

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