Journal of Poultry Research, 16(2): 80-85, 2019
DOI: https://doi.org/10.34233/jpr.600605

PoultRES Journal of Poultry Research

Available online, ISSN: 2147-9003 | www.turkishpoultryscience.com | Republic of Turkey Ministry of Agriculture and Forestry

# Effects of Supplementing Layer Hen Diet with Red Pepper (*Capsicum annuum L.*) Powder as Natural Yolk Colourant on Laying Performance, Pigmentation of Yolk, Egg Quality and Serum Immunoglobulin Levels

**Arda Sözcü**[1,a,*]

*Ödemiş Vocational High School, Ege University, Ödemiş/İzmir, Turkey*
[*]*Corresponding author*

A R T I C L E I N F O

*Research Article*

Received : 02/08/2019
Accepted : 11/11/2019

*Keywords:*
Carotenoid
Egg Yield
Immunoglobulin
Pigmentation
Red Pepper

A B S T R A C T

This study was carried out to investigate the effects of supplementing layer hen diet with red pepper powder (*Capsicum annuum L.*) as natural yolk colourant on laying performance, pigmentation of yolk, egg quality and serum immunoglobulin levels. One hundred sixty Super Nick Chick white laying hens at 78 week of age were randomly divided into four dietary groups, each containing supplements of control (commercial diet), red pepper supplementation groups as dose 1, dose 2 and dose 3 at 0.5%, 1.0% and 1.5% level, respectively. A higher hen-day egg production and egg mass, and an efficient feed conversion rate were observed in dose 2 and dose 3 groups. A lower yolk ratio and a higher albumen ratio were linearly observed in dose 2 and dose 3 groups. The darkest yolk colour was observed in dose 3 group with a value of 13.2 of Roche colour fan and 18.8 of redness value. A higher level of IgG was observed for the hens from dose 1 and dose 3 groups. As a conclusion, due to favourable effects for laying performance, quality parameters, yolk pigmentation and immunity, red pepper could be recommended as a natural source for yolk pigmentation.

Tavukçuluk Araştırma Dergisi, 16(2): 80-85, 2019

# Yumurtacı Tavuk Rasyonuna Doğal Sarı Renklendiricisi Olarak Kırmızıbiber (*Capsicum annuum L.*) Tozu İlavesinin Yumurtlama Performansı, Sarı Pigmentasyonu, Yumurta Kalitesi ve Serum İmmünoglobulin Düzeyileri Üzerine Etkileri

M A K A L E B İ L G İ S İ

*Araştırma Makalesi*

Geliş : 02/08/2019
Kabul : 11/11/2019

*Anahtar Kelimeler:*
Karotenoid
Yumurta verimi
İmmünoglobulin
Pigmentasyon
Kırmızıbiber

Ö Z

Bu çalışma yumurtacı tavukların rasyonuna doğal sarı renklendiricisi olarak kırmızıbiber (*Capsicum annuum L.*) tozu ilavesinin yumurtlama performansı, sarı pigmentasyonu, yumurta kalitesi ve serum immünoglobulin düzeyleri üzerine etkilerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada 78 haftalık yaşta toplam 160 adet Super Nick Chick beyaz yumurtacı tavuk kullanılarak dört deneme grubu oluşturulmuştur: kontrol (ticari rasyon), sırasıyla %0,5, %1,0 ve %1,5 düzeyinde kırmızıbiber tozu ilave edilen 1.doz, 2. doz ve 3. doz grupları. Çalışmada, 2. ve 3. doz gruplarında yumurta verimi ve yumurta kütlesinin daha yüksek ve yemden yararlanma oranınınsa daha iyi olduğu gözlenmiştir. 2. ve 3. doz gruplarında linear olarak sarı oranı daha düşük, albümin oranı ise daha yüksektir. Yumurta sarı renginin 3. doz grubunda 13,2 Roche sarı rengi ve 18.8 kırmızılık değeri ile en koyu tona sahip olduğu tespit edilmiştir. Serum IgG düzeyinin 1. ve 3. doz gruplarındaki tavuklarda daha yüksek olduğu saptanmıştır. Sonuç olarak, yumurtlama performansı, kalite parametreleri, sarı pigmentasyonu ve immünite üzerine olumlu etkilerinden dolayı, kırmız biber sarı pigmentasyonu için doğal bir kaynak olarak önerilebilir.

[a] arda.sozcu@ege.edu.tr https://orcid.org/0000-0002-0955-4371

## Introduction

In table egg production, the pigmentation of yolk has an importance for consumers' demand. Consumers usually have a tendency to choice the eggs with yolk colour from dark yellow to orange (Hasin et al., 2006). On the other hand, there is a growing interest and demand for animal rights and welfare all over the world. This trend has caused some alternative production systems against conventional systems in poultry production. Recently, free range and organic production systems have become widespread for egg production in many countries.

It is well known that the colour of egg yolk is affected by diet content with regard to the degree of pigmentation and especially xanthophyll content, type and the ratio of pigmentation compounds (Rossi et al., 2015). In commercial applications, these pigmentation compounds as feed additives have to be added to the diets to increase the pigmentation of yolk. Because, these compounds could not be synthesized in the body of bird (Breithaupt, 2007). In egg type chicken nutrition, these pigmentation compounds, named as carotenoids, are used with high levels of natural or synthetic pigments for colorization of egg yolk (Hammershøj et al., 2010; Shahsavari, 2014; Sirri et al., 2007). These carotenoids mainly include xanthophyll, capsanthin, and canthaxanthin (Perez-Vendrell et al., 2001).

Recently, instead of synthetic pigments, the use of natural pigment compounds has gradually increased in many countries, due to alternative production systems (McGraw, 2005). For that reason, plant origin carotenoids have become alternative sources as natural colorants for egg production (Wang et al., 2016), because of their advantages as stronger bioactivity and bioavailability, safety and cheaper (Rossi et al., 2015). The aim of the current study is to investigate the effects of supplementing layer hen diet with natural carotenoid source as red pepper powder on laying performance, pigmentation of egg yolk, egg quality and serum immunoglobulin levels.

## Material and Methods

All the procedures were in accordance with established standards for the care and use of animals for research purposes which was approved by Uludağ University Animal Use Local Ethical Committee (No: 2018-05/09).

The experiment was performed with a total of 160 hens of Super Nick Chick white laying strain at 78 weeks of age. The experiment was performed during 10 weeks (until 88 weeks of age). The laying hens were randomly assigned into four dietary groups which are all placed at eight battery cages (50 × 45 × 45 cm) with 5 birds in 8 replicates. The experimental groups were created by supplementation of red pepper (*Capsicum annuum L.*) powder: control (commercial diet), red pepper supplementation groups as dose 1, dose 2 and dose 3 at 0.5%, 1.0% and 1.5% level, respectively.

A standard commercial layer diet with a content of 16% CP and 2.700 ME kcal/kg was formulated according to the National Research Council (1994) to meet nutritional specifications. The chemical composition of the diet was given in Table 1. Pigment composition (total carotene, carotenoid and capsanthin content) of red pepper powder was measured according to the spectrophotometric method described by Jarén-Galán et al. (1999). According to the analyse results, total carotene, carotenoid and capsanthin content were found as 2637 mg/kg, 78 mg/kg and 5 mg/kg respectively.

During to the experimental period, feed and water were offered ad-libitum. All hens were kept under a constant lighting schedule (16 h light and 8 h dark periods daily). The laying hens were individually weighed with a digital scale with a precision of ±1 g at 78$^{th}$ and 88$^{th}$ weeks of age. Egg number from each cage were daily collected and hen-day egg production was calculated as daily. Feed intake and egg weight were recorded on weekly basis. Egg mass and feed conversion rate were calculated as ratio between egg production and egg weight, and ratio between feed intake and egg mass.

Egg interior and exterior quality parameters were measured at 88$^{th}$ weeks of age (n: 12 eggs/group). The egg shape index was calculated by a formula given by Reddy et al. (1979). Eggshell breaking strength (kg/cm$^2$) was measured by special equipment. The yolk weight was measured with a precision of 0.01 g. These yolk samples were also used for further yolk colour measurements.

The shells were carefully washed and dried 24 h in a drying oven at 105°C (Nuve FN-500, Ankara, Turkey) and then weighed with a precision of 0.01 g. Albumen weight was calculated by subtracting yolk and shell weights from the egg weight. Eggshell thickness was measured using a calliper with a precision of ± 0.01 mm. Egg yolk diameter, albumen length, albumen width (mm) were measured with digital calliper with a precision of ± 0.01 mm (Mitutoyo, 300 mm, Neuss, Germany). The albumen and yolk height (mm) were measured using a tripod micrometre. Egg yolk and albumen index values and Haugh Unit were calculated with the data.

The egg yolk colour (n = 12 eggs per experimental group) was measured visually assessing with Roche egg yolk colour fan (Roche Ltd., Switzerland). Pigmentation of yolk was determined by scoring ranging from the lightest pigmentation (score 1) to the darkest pigmentation (score 15). Besides, yolk colour characteristics as lightness (L*), redness (a*), yellowness (b*), chroma (C) and hue value (α) of yolk were measured using by a spectrophotometer (Konica- Minolta, Osaka, Japan).

At 88 week of age, blood samples were taken from ten hens from each group for serum immunoglobulin (IgA, IgG, and IgM) levels. Blood samples were centrifuged at 3000 rpm for 15 min. Serum immunoglobulin levels were measured by a commercial kit (Roche Cobas 6000 E601, Roche Diagnostics, North America) according to the analysis techniques described by Carew et al. (1997).

The parametric data were analysed by using GLM procedure of statistical analysis software of SAS (2013). Effects of the red pepper powder supplementation levels were determined by the contrast option of the GLM procedure. Orthogonal polynomial contrasts were also applied to determine the linear and quadratic responses to different levels of supplementation. Analysis for percentage data was conducted after an arcsine transformation of the data. Significant differences among means were compared with

Duncan's adjustment for multiple comparisons. Differences were considered significant at $P \leq 0.05$.

Table 1. Composition of the experimental diets containing red pepper extract

| Ingredients (%) | Control diet | Basal diet | Dose 1 | Dose 2 | Dose 3 |
|---|---|---|---|---|---|
| Corn | 64.35 | 64.35 | 64.35 | 64.35 | 64.35 |
| Soybean meal (46%) | 25.13 | 25.13 | 25.13 | 25.13 | 25.13 |
| Dicalcium phosphate | 1.08 | 1.38 | 1.25 | 1.23 | 1.22 |
| Calcium carbonate | 8.05 | 8.42 | 8.48 | 8.48 | 8.48 |
| DL-methionine | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| NaCL | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 |
| Vitamin premix[1] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[2] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Inert pigment | 0.70 | - | - | - | - |
| Pepper extract | - | - | 0.10 | 0.12 | 0.13 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Calculated composition | | | | | |
| Crude protein (%) | 16.05 | 16.05 | 16.05 | 16.05 | 16.05 |
| ME (kcal/kg) | 2755 | 2755 | 2755 | 2755 | 2755 |
| Ca (%) | 3.5 | 3.7 | 3.7 | 3.7 | 3.7 |
| Available phoshorus (%) | 0.32 | 0.35 | 0.35 | 0.35 | 0.35 |
| Sodium (%) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Total methionine (%) | 0.36 | 0.36 | 0.36 | 0.36 | 0.36 |
| Lysine (%) | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |

[1] each kg of premix: 20000 mg Fe, 120 mg I, 1600 mg Cu, 24000 mg Mn, 16000 mg Zn, 20 mg Se, 20 mg Co., [2] each kg of premix: 20000000 UI Vtamin A, 5000000 UI Vitamin $D_3$, 40500 UI Vitamin E, 4800 mg Vitamin $K_3$, 3600 mg Vitamin $B_1$, 12000 mg Vitamin $B_2$, 6000 mg Vitamin $B_6$, 28000 mcg Vitamin $B_{12}$, 87000 mg Niacin, 60 mg Biotin, 29000 mg Pantothenic acid, 1600 mg Folic acid.

**Results**

The effects of red pepper extract supplementation on performanca parameters were given on Table 2. Daily feed intake was significantly lower in the red pepper extract supplementation groups compared to the control group ($P<0.02$). It changed between 104.7-107.4 g in red pepper powder supplementation groups, whereas it was found as 118.3 g in the control group. Red pepper supplementation caused a linear and quadratic iincrement for hen-day egg production and egg mass. These parameters were found to be higher in dose 2 and dose 3 groups ($P<0.01$).

Control diet group (commercial diet), Dose 1 (0.5% red pepper supplementation); Dose 2 (1.0% red pepper supplementation); Dose 3 (1.5% red pepper supplementation)

As seen in Table 3, yolk weight and yolk ratio were found to be higher in control diet and dose 1 groups ($P<0.01$). Albumen weight was found to be the highest in eggs obtained from dose 2 group (46.4 g; $P<0.01$), whereas albumen ratio was higher the eggs obtained from dose 2 and dose 3 groups (66.6 and 65.4%, $P<0.01$). Eggs obtained from dose 1 and dose 2 groups had higher breaking strength. The darkest yolk color was observed in the eggs obtained from dose 3 group with 13.2 Roche yolk color and 18.8 redness value (Table 4, $P<0.01$). Yolk index value was linearly found to be the highest in dose 3 group, while the albumen index was found to be the highest in dose 2 group ($P<0.01$). The mean of α-value was the lowest in the control group compared to the red pepper supplementation groups ($P<0.01$). The highest value for Haugh unit was observed in dose 2 group, compared to the other groups ($P<0.01$).

Table 2. The effects of red pepper extract supplementation on performance parameters in layer chickens

| Experimental groups | Control diet | Dose 1 | Dose 2 | Dose 3 | Significance | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fixed | Linear | Quadratic |
| Body weight (g, 78th weeks) | 1730.0±88.2 | 1745.0±67.7 | 1740.0±87.6 | 1741.3±75.5 | 0.999 | 0.974 | 0.959 |
| Body weight (g, 88th weeks) | 1731.8±96.4 | 1756.7±87.2 | 1736.8±97.1 | 1746.8±78.2 | 0.953 | 0.652 | 0.879 |
| Daily feed intake (g) | 118.3±10.8[a] | 107.4±8.1[b] | 104.7±8.1[b] | 105.4±8.4[b] | 0.032 | 0.552 | 0.002 |
| Hen-day egg production (%) | 71.7±3.0[c] | 75.2±4.2[b] | 79.3±3.7[a] | 81.2±2.7[a] | <0.01 | <0.01 | 0.004 |
| Egg weight (g) | 66.5±5.5 | 68.8±4.9 | 71.0±5.4 | 69.5±4.6 | 0.099 | 0.062 | 0.153 |
| Egg mass (g/d/hen) | 47.7±2.0[b] | 51.7±2.6[ab] | 56.3±2.3[a] | 56.4±1.9[a] | <0.01 | 0.002 | 0.076 |
| Feed conversion rate | 2.48±0.23[a] | 2.08±0.19[b] | 1.86±0.15[c] | 1.87±0.16[c] | <0.01 | 0.030 | <0.01 |

[a,b] Differences in letters within colons indicates significant differences among the experimental groups, n = 40 layer hens / experimental groups

Table 3. The effects of red pepper extract supplementation on egg content and exterior egg quality parameters

| Experimental groups | Control diet | Dose 1 | Dose 2 | Dose 3 | Significance | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fixed | Linear | Quadratic |
| Yolk weight (g) | 18.5±0.6[a] | 19.0±0.3[a] | 17.7±0.4[b] | 17.7±0.5[b] | 0.029 | 0.164 | 0.189 |
| Yolk ratio (%) | 28.2±0.4[a] | 27.8±0.3[a] | 25.5±0.8[b] | 26.2±0.8[b] | <0.01 | 0.001 | 0.298 |
| Albumen weight (g) | 41.6±1.1[c] | 43.4±0.5[b] | 46.4±1.8[a] | 44.1±0.5[b] | <0.01 | 0.002 | 0.001 |
| Albumen ratio (%) | 63.5±0.8[b] | 63.5±0.5[b] | 66.6±0.9[a] | 65.4±0.7[a] | <0.01 | 0.003 | 0.281 |
| Eggshell weight (g) | 5.4±0.8 | 5.9±0.2 | 5.4±0.1 | 5.7±0.4 | 0.547 | 0.665 | 0.785 |
| Eggshell ratio (%) | 8.2±0.7 | 8.7±0.2 | 7.9±0.4 | 8.4±0.5 | 0.175 | 0.185 | 0.431 |
| Egg weight (g) | 65.5±3.4 | 68.4±4.0 | 69.6±3.5 | 67.5±4.2 | 0.330 | 0.242 | 0.056 |
| Shape index (%) | 74.4±1.9 | 75.2±1.4 | 75.4±1.7 | 73.6±2.1 | 0.158 | 0.723 | 0.223 |
| Eggshell breaking strength (g/cm$^2$) | 1.212±0.312[b] | 1.624±0.261[a] | 1.661±0.331[a] | 1.362±0.108[ab] | 0.014 | 0.123 | 0.004 |
| Eggshell thickness (mm) | 0.342±0.02 | 0.382±0.01 | 0.364±0.05 | 0.363±0.02 | 0.147 | 0.829 | 0.820 |

[a,b] Differences in letters within colons indicates significant differences among the experimental groups, n = 12 eggs / experimental groups, Control diet group (commercial diet), Dose 1 (0.5% red pepper supplementation); Dose 2 (1.0% red pepper supplementation); Dose 3 (1.5% red pepper supplementation).

Table 4. The effects of red pepper extract supplementation on egg yolk and albumen quality parameters

| Experimental groups | Control diet | Dose 1 | Dose 2 | Dose 3 | Significance | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fixed | Linear | Quadratic |
| Yolk color | 12.0±0.655[ab] | 11.8±0.447[b] | 12.4±0.894[ab] | 13.2±0.447[a] | <0.01 | <0.01 | <0.01 |
| Yolk height (mm) | 19.2±0.6[ab] | 19.3±0.7[ab] | 18.6±0.4[b] | 19.6±0.7[a] | 0.027 | 0.064 | 0.667 |
| Yolk diameter (mm) | 46.4±1.1[a] | 46.6±1.0[a] | 45.0±2.6[ab] | 43.1±1.0[b] | 0.030 | 0.005 | 0.227 |
| Yolk index (%) | 41.2±1.2[b] | 41.4±1.3[b] | 41.6±3.4[b] | 45.5±1.6[a] | 0.006 | 0.001 | 0.206 |
| L* | 53.6±3.5[b] | 56.4±1.5[a] | 55.5±1.7[ab] | 57.0±2.5[a] | 0.006 | 0.001 | 0.263 |
| a* | 14.4±2.3[b] | 12.3±1.0[b] | 13.3±1.6[b] | 18.8±2.8[a] | <0.01 | 0.03 | 0.285 |
| b* | 42.7±4.3 | 45.4±2.8 | 44.1±3.0 | 46.0±5.8 | 0.333 | 0.131 | 0.818 |
| C value | 46.8±3.9 | 46.2±2.8 | 46.1±3.2 | 47.9±6.1 | 0.402 | 0.067 | 0.915 |
| α-value | 66.1±3.9[b] | 74.5±0.7[a] | 73.3±1.4[a] | 73.8±1.7[a] | <0.01 | 0.009 | 0.120 |
| Albumen height (mm) | 6.8±0.8[c] | 7.1±0.5[bc] | 9.5±0.8[a] | 8.2±1.1[ab] | <0.01 | 0.004 | 0.067 |
| Albumen length (mm) | 100.2±6.5 | 98.9±6.2 | 96.1±7.0 | 97.6±3.4 | 0.254 | 0.090 | 0.260 |
| Albumen width (mm) | 78.8±4.5 | 79.2±5.4 | 75.3±5.4 | 77.5±6.1 | 0.674 | 0.843 | 0.993 |
| Albumen index (%) | 7.8±0.8[b] | 8.1±1.0[b] | 11.2±1.6[a] | 9.5±1.8[ab] | 0.002 | 0.011 | 0.110 |
| Haugh unit | 80.6±3.9[b] | 81.8±3.6[b] | 94.7±4.1[a] | 88.4±6.6[ab] | <0.01 | 0.005 | 0.115 |

[a-c] Differences in letters within colouns indicates significant differences among the experimental groups, n = 12 eggs / experimental groups, L*: lightness; a*: redness; b*: yellowness; C: chroma; α: hue value, Control diet group (commercial diet), Dose 1 (0.5% g red pepper supplementation); Dose 2 (1.0% red pepper supplementation); Dose 3 (1.5% red pepper supplementation).

The effects of red pepper extract supplementation on serum immunoglobulin levels were presented on Figure 1. A higher level of IgG was observed in the hens from dose 1 and dose 3 groups, with values of 30.1 and 31.9 mg/dl respectively ($P<0.05$).

**Discussion**

Results showed that red pepper extract supplementation as a natural carotenoid source had beneficial effect for hen-day egg production, egg mass and also feed conversion rate. This finding is consistent with another study performed by Abou-Elkhair et al. (2018) who reported a better performance for egg production, egg mass and feed conversion rate for laying hens fed with red pepper supplementation.

Observed decline in feed intake and improvements in performance parameters could be related with bioactive components of red pepper. There is a bioactive ingredient called as capsaicin in red pepper. It has a critical role for increasing of metabolism of nutrients and energy by stimulating enzyme activities, for example, glucose-6-phosphate dehydrogenase, lipoprotein lipase and pancreatic and intestinal enzymes (Platel and Srinivasan, 2004; Reddy and Lokesh, 1992).

Observed variations for yolk weight and yolk ratio could be related with changes of hen-day egg production among the experimental groups. Likewise, an increment for egg production by enhancement of ovulation in dose 2 and dose 3 groups could be resulted in smaller yolk formation. These findings are supported by Abou-Elkhair et al. (2018) who reported a decline in yolk weight with an increment egg production when laying hens fed with dietary supplementation of fennel seeds, black cumin seeds and hot red pepper, compared to the control diet.

Eggshell quality parameters as exterior egg quality has an economical importance for egg production chain. A stronger shell structure with a higher breaking strength and shell thickness is a one of the most important quality targets to reduce the breaking and cracks of eggshell during handling, storage and transportation (Ketta and Tůmová, 2016; Solomon, 2010). Current results showed that red pepper extract supplementation increased eggshell breaking strength compared to the control and basal diet groups. These findings could be related with a more efficient utilisation of some nutrients, as mentioned above, especially some minerals that has critial role for shell formation (Platel and Srinivasan, 2004; Reddy and Lokesh, 1992).

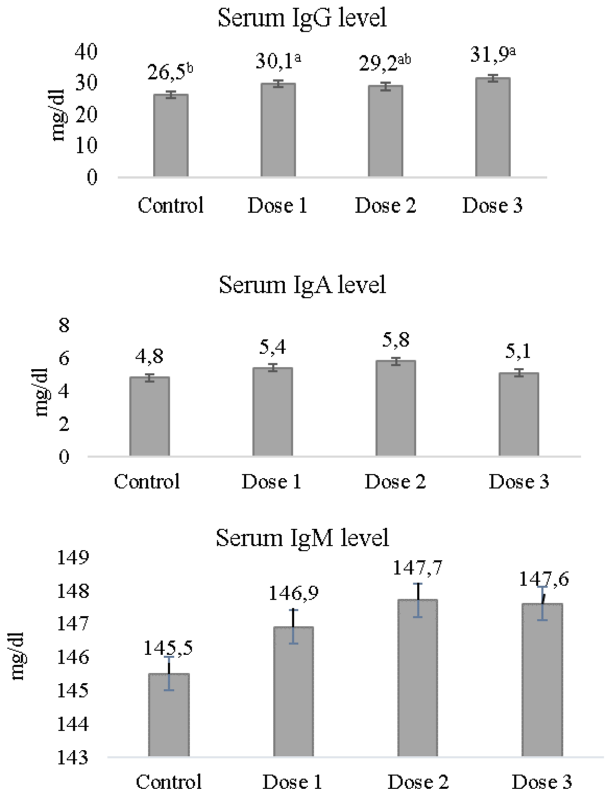


Figure 1. Serum immunoglobulin levels fed with red pepper powder supplementation

Each bar represents mean ± SEM; a,b indicaten significant differences at *P < 0.01*. Control diet (commercial diet), Dose 1 (0.5% red pepper supplementation); Dose 2 (1.0% red pepper supplementation); Dose 3 (1.5% red pepper supplementation).

Results showed that the efficiency of transition of yolk pigments in red pepper to yolk increased when the supplementation amount of red pepper extract increased. This finding is consistent with previous results reported by Niu et al. (2008), Lokaewmanee et al. (2010, 2013), and Rossi et al. (2015), Abou-Elkhair et al. (2018). This darkened color could be related with a higher content of capsanthin (Santos-Bocanegra et al., 2004). It is known that egg yolk colour is a combination of three colorimetric parameters read by a spectrophotometer, including hue value, L* value and chroma value (Lokaewmanee et al., 2010). Results showed that L* value tended to increase when red pepper extract supplementation increased. This finding was contrast to previous findings by Lokaewmanee et al. (2010) who concluded that a lower value of L* for paprika group.

A stronger immunocompetence and resistance to disease is essential to obtain optimum performance and profitability. Results showed that IgG level tended to increase in red pepper supplementation groups. This could be related with bioactive compounds of red pepper, for example chemo-preventive and chemotherapeutic effects (Puvaca et al., 2015). In the study, a higher laying performance in dose 3 group could be stimulated by a stronger immunocompetence and a higher protective effect against oxidative stress.

Results of current study showed that the red pepper extract supplementation could be recommended for improving laying performance and immunocompetence of hens, especially later term of laying period, and also exterior and interior egg quality parameters. Based on these findings, red pepper extract should have a potential feed ingredient instead of synthetic pigment ingredients, for yolk pigmentation, especially for alternative egg production systems.

## References

Abou-Elkhair, R., Selim, S. and Hussein, E., 2018. Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. Anim. Nutr. 4(4): 394-400.

Breithaupt, D.E., 2007. Modern application of xanthophylls in animal feeding. A review. Trends Food Sci. Technol. 18: 501-506.

Carew, L.B., Evarts, K.G. and Alster, F.A., 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult Sci, 76: 1398-1404.

Hammershøj, M., Kidmose, U. and Steenfeldt, S., 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (Daucus carota) varieties as forage material for egg-laying hens. J. Sci. Food Agr. 90: 1163-1171.

Hasin, B.M., Ferdaus, A.J.M., Islam, M.A., Uddin, M.J. and Islam, M.S., 2006. Marigold and orange skin as egg yolk color promoting agents. Int. J Poult. Sci. 5(10): 979-987.

Jarén-Galán, M., Nienaber, U. and Schwartz, S.J., 1999. Paprika (*Capsicum annuum*) oleoresin extraction with supercritical carbon dioxide. J. Agric. Food Chem. 47: 3558-3564.

Ketta, M. and Tůmová, E., 2016. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. Czech J. Anim. Sci., 61(7): 299-309.

Lokaewmanee, K., Yamauchi, K. and Okuda, N., 2013. Effects of dietary red pepper on egg yolk colour and histological intestinal morphology in laying hens. J. Anim. Physiol. Anim. Nutr. 97: 986-995.

Lokaewmanee, K., Yamauchi, K., Komori, T. and Saito, K., 2010. Effects on egg yolk colour of paprika or paprika combined with marigold flower extracts. Italian J. Anim. Sci. 9: 356-359.

McGraw, K.J., 2005. The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? Anim. Behav. 69: 757-764.

Niu, Z., Fu, J., Gao, Y. and Liu, F., 2008. Influence of paprika extract supplement on egg quality of laying hens fed wheat-based diet. Int. J. Poult. Sci. 7(9): 887-889.

NRC, 1994. Nutrient Requirements of poultry. Washington, USA, National Academy Press, 9th rev.ed.

Perez-Vendrell, A.M., Hernandez, J.M., Llaurado, L., Schierle, J. and Brufau, J., 2001. Influence of source and ratioof xanthophyll pigments on broiler chicken pigmentation and performance. Poult. Sci. 80: 320-326.

Platel, K., and Srinivasan, K., 2004. Digestive stimulant action of spices: a myth or reality? Indian J. Med. Res. 119: 167-179.

Puvaca, N., Kostadinovic, L., Ljubojevic, D., Lukac, D., Levic, J., Popovic, S., Novakov, N., Vidovic, B. and Duragic, O., 2015. Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. Europ. Poult. Sci. 79: https://doi.org/10.1399/eps.2015.73.

Reddy, A.C. and Lokesh, B.R., 1992. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol. Cell. Biochem. 111: 117-124.

Reddy, P.M., Reddy, V.R., Reddy, C.V. and Rap, P.S.P., 1979. Egg weight, shape index and hatchability in Khaki, Campbell duck egg. Indian J. Poult. Sci. 14: 26-31.

Rossi, P., Nunes, J.K., Rutz F., Anciuti M.A., Moraes P.V.D., Takahashi S.E., Bottega A.L.B. and Dornales, J.B., 2015. Effect of sweet green pepper on yolk color and performance of laying hens. J. Appl. Poult. Res. 24: 10-14.

Santos-Bocanegra E., Ospina-Osorio X., Oviedo-Rondon E.O., 2004. Evaluation of xanthophylls extracted from Tagetes erectus (Marigold Flower) and Capsicum Sp. (Red Pepper Paprika) as a pigment for egg-yolks compare with synthetic pigments. Int. J. Poult. Sci. 3: 685-689.

SAS, 2013. Statistical analysis system: user's guide: statistics. Version 9.3 User’s Guide. SAS Inst., Inc., Cary, NC.

Shahsavari, K., 2014. Influence of different sources of natural pigmenting on egg quality and performance of laying hens. Jordan J. Agr. Sci. 10(4): 786-796.

Sirri, F.N., Iaffaldano, M.G., Meluzzi, A., Rosato, M.P. and Franchini, A., 2007. Comparative pigmentation efficiency of high dietary levels of apo-ester and marigold extract on quality traits of whole liquid egg of two strains of laying hens. J. Appl. Poult. Res. 16: 29-43.

Solomon, S.E., 2010. The eggshell: strength, structure and function. Br. Poult. Sci. 51(1): 52-59.

Wang, D., Huifang, H., Zhou, L., Wei, L., Hanlin, Z., Guanyu, H., Jia, L. and Lin, H., 2016. Effects of dietary supplementation with turmeric rhizome extract on growth performance, carcass characteristics, antioxidant capability, and meat quality of Wenchang broiler chickens. Italian J. Anim. Sci. 14: 344-349.