PHYSIOLOGY AND REPRODUCTION

Effects of *Deinococcus* spp. supplement on egg quality traits in laying hens

I-Chen Li,[*] Szu-Yin Wu,[*] Jenn-fa Liou,[†] Hsiao-Hui Liu,[*] Jiau-hua Chen,[‡] and Chin-Chu Chen[*,§,#,|,‖,&,1]

[*]*Grape King Bio Ltd, Zhong-Li Dist., Taoyuan City, Taiwan;* [†]*Physiology Division, Livestock Research Institute, Council of Agriculture, Hsinhua, Tainan City, Taiwan;* [‡]*Department of Food Science and Technology, Chia Nan University, Tainan City, Taiwan;* [§]*Institute of Food Science and Technology, National Taiwan University, Taipei City, Taiwan;* [#]*Department of Food Science, Nutrition, and Nutraceutical Biotechnology, Shin Chien University, Taipei City, Taiwan;* [|]*Department of Applied Science, National Hsin-Chu University of Education, Hsinchu City, Taiwan;* [‖]*Institute of Biotechnology, National Changhua University of Education, Changhua City, Taiwan; and* [&]*Department of Bioscience Technology, Chung Yuan Christian University, Taoyuan City, Taiwan*

ABSTRACT To counter the ill effects of synthetic dyes, bacterial pigment production as an alternative is now one of the promising and emerging fields of research. This study was conducted to evaluate the applicability of *Deinococcus* genus on the egg quality traits in laying hens. In study I, 24 single comb White Leghorn layers were fed with various 1 wt % *Deinococcus* bacterial strains for 10 d. In study II, 84 brown Hendrix layers were fed with one of 4 diets containing 0, 0.2, 1, or 5 wt % *Deinococcus* sp. GKB-Aid 1995 powder for 12 wk. In study III, 60 White Leghorn laying hens were fed either with or without 1 wt % *Deinococcus* sp. GKB-Aid 1995 powder, 1 wt % *Deinococcus* sp. GKB-Aid 1995 granules, or 1 wt % *Deinococcus* sp. GKB-Aid 1995 oily granules for 10 successive d. In all of the experiments, feeding *Deinococcus* powder did not affect egg quality traits except for the yolk color. In particular, supplementation with all *Deinococcus* powder treatments changed the yolk color ($P < 0.05$) in study I, with the best pigmentation score obtained by *D. grandis* and *Deinococcus* sp. GKB-Aid 1995. Moreover, longer supplementation of *Deinococcus* sp. GKB-Aid 1995 in study II had a significant effect on feed conversion ratio. With these findings under consideration, the present study suggests that the *Deinococcus* species, especially *Deinococcus* sp. GKB-Aid 1995, can be an excellent candidate for improving egg yolk color in laying hens.

Key words: *Deinococcus*, feed additive, fermentation, reproduction, yolk color

2018 Poultry Science 97:319–327
http://dx.doi.org/10.3382/ps/pex281

INTRODUCTION

Having the right color plays an extremely important role in food acceptance (Fletcher, 1999). In particular, the color of egg yolk is a major criterion not only to consumers but also to the industry in different parts of the world. Factors that may affect the degree of egg yolk coloration include the type of pigmenting substances, namely β-carotene and xanthophylls, their concentrations present in the feed, and the health of the hens (Galobart et al., 2004). Even though β-carotene is the representative of carotenes, it is found only as a minor component in egg yolks as hens can efficiently convert β-carotene to vitamin A (Surai et al., 1996). The most important xanthophylls that have the greatest influence on yolk color are lutein, capsanthin, zeaxanthin, β-apo-8′-carotenal, canthaxanthin, β-apo-8′carotenoic acid ethyl ester, β-cryptoxanthin, and citranaxanthin (Schlatterer and Breithaupt, 2006). Lutein, capsanthin, zeaxanthin, and β-cryptoxanthin are natural xanthophylls derived from various plants while others are produced by chemical synthesis. As hens cannot synthesize color pigments and can transfer only 20 to 60% of ingested pigments to the yolk (Lokaewmanee et al., 2010), xanthophylls with different carbon lengths and functional groups can be incorporated into poultry feed to change the yolk pigmentation.

Although sources of color-promoting agents can either be synthetic or natural, most of the ingredients sold on the market are usually synthetic due to their low cost and good pigmentation efficiency. Artificial pigments can be more stable than naturally derived forms, but safety concerns have arisen about the content of these synthetic ingredients, which have led to a significant decline in their use in some countries (Delgado-Vargas

Received May 8, 2017.
Accepted September 5, 2017.
[1]Corresponding author: gkbioeng@grapeking.com.tw

et al., 1998). For this reason, the reduction in the use of synthetic pigments as feed additives has led to new alternatives such as utilizing bacteria, fungi, microalgae, and yeast for their coloring effectiveness and their benefits to maximize the health and well-being of farm animals (Youssef et al., 2013).

Deinococcus spp. can be excellent sources of natural pigments. They are cells that exhibit remarkable resistance to radiation, oxidation, desiccation and high temperature, and they are found to form pink- or reddish-colored colonies because of their ability to synthesize carotenoids (Zhang et al., 2007b). Studies have shown that these natural carotenoids extracted from different species of *Deinococcus* will not only improve the yolks in terms of color, but also nutritionally as they possess strong protective abilities against oxidative damage (Fei et al., 2012b). Recently, a new member of the *Deinococcus* lineage, GKB-Aid 1995, was found to be non-toxic in rats via oral administration for 90 d (Jhou et al., 2016) and was susceptible to ampicillin, vancomycin, chloramphenicol, streptomycin, tetracycline, and kanamycin (Wu et al., 2016). Moreover, when prepared with fermented soymilk, GKB-Aid 1995 was known to possess enhanced antioxidant and potent anticarcinogenic effects by mediating the downregulation of autophagy and upregulation of apoptosis (Yao et al., 2016). Thus, there is potential in developing *Deinococcus* bacteria, not only as an additive used for animal feeding to meet consumer expectations but also as an antioxidant and anticancer agent to improve animal health.

As numerous studies have focused on understanding the mechanisms of resistance to environmental hazards in *Deinococcus* bacteria, this study aimed to assess the applicability of the *Deinococcus* genus for industrial production by conducting 3 different trials to evaluate the additive (study I), dosage (study II), and dosage form (study III) effects of *Deinococcus* strains in the diet of laying hens.

MATERIALS AND METHODS

Preparation of Deinococcus spp. Powder

Five different *Deinococcus* spp. bacterial strains were used to determine their potentially beneficial effects when supplemented into animal feeds. The species tested included *Deinococcus grandis* BCRC 17,376, *D. radiodurans* BCRC 12,827, *D. proteolyticus* BCRC 17,377, *D. geothermalis* BCRC 17,378, and *Deinococcus* sp. GKB-Aid 1995 (Wu, Li, Lin and Chen, 2016). The selected strains were grown and then inoculated into a 200-L fermenter with a medium composed of 2% sucrose, 0.5% peptone, and 1% yeast extract. Fermentations were agitated at 80 rpm with an aeration rate of 0.5 vvm at 32°C (*D. geothermalis* at 42°C) for 2 to 5 d. At the end of the cultivation, the bacterial cells were removed from the fermentation broth, lyophilized, and stored at room temperature.

Preparation of Deinococcus spp. Granules

The selection of the right dosage forms and use of excipients in the formulation designs are extremely important as they can influence bioavailability. Hence, 2 different formulations of *Deinococcus* spp. in granule forms with excipients were prepared using the wet granulation method. In brief, 80% w/w of *Deinococcus* spp. powder was wet massed with predetermined quantities of either 15% w/w hydrogenated vegetable oil and 20% w/w potato starch or 20% w/w potato starch alone. The wet mass was then granulated by passing through an oscillating granulator using a 1-mm sieve, and dried in a hot air oven at 40°C for 1 h to obtain the final product.

Study Design I: Impact of various Deinococcus sp. on Egg Quality and Egg Yolk Color

Twenty-four single comb White Leghorn layers aged 42 wk were assigned randomly to 1 control and 5 experimental units of 4 hens each, where they were housed in pairs with continuous feed and fresh water supply provided. The dimensions of the cages were 40 cm in width × 45 cm in depth with 45 cm front height × 38 cm back height, and the housing was designed on the basis of the opened system. The hens were fed daily with either commercial corn-soy-type diets or 5 different diets of commercial corn-soy-type diets supplemented with various 1 wt % *Deinococcus* sp. powdered bacterial strains (*Deinococcus grandis*, *D. radiodurans*, *D. proteolyticus*, *D. geothermalis*, and *Deinococcus* sp. GKB-Aid 1995) for 10 d. All ingredients were thoroughly mixed in a mixer and fed in a dry form to the hens. Eggs produced by each group were collected during the trial, and 4 eggs from each group were selected randomly for egg quality assessment. Physical characteristics of eggs, such as weight and Haugh Unit score, were measured electronically by an automatic egg quality measurement system (QCM+ System, Technical Services and Supplies, England). The yolk height was measured using the caliper while the egg yolk color was evaluated visually with a Roche Color Fan (Roche Ltd., Basel, Switzerland). This study was conducted under our supervision in a commercial egg production facility in Nantou, Taiwan.

Study Design II: Impact of Different Concentrations of Deinococcus sp. GKB-Aid 1995 on Production Performance and Egg Quality

A total of 84 brown 22-week-old Hendrix layers were arranged randomly to 1 control and 3 treatment groups with powdered *Deinococcus* sp. GKB-Aid 1995 at 0, 0.2, 1 and 5 wt % for 12 wk, with twenty-one replicates per treatment. Hens were housed 3 to a cage with continuous feed and fresh water supply provided. The ingredients were thoroughly mixed in a mixer and fed in a dry

form to the layers. The dimensions of the cages were 120 cm in length x 23 cm in depth x 36 cm in height, and the housing was designed on the basis of the opened system. Starting from wk 3, twenty-one eggs harvested randomly from each group were collected, weighed, broken up, and evaluated for the following traits: Egg weight, shell weight, shell thickness, shell strength, albumen weight, yolk weight, yolk length, yolk height, yolk color, and Haugh unit score. Egg weight, albumen weight, yolk weight, and Haugh unit score were measured electronically by an automatic egg quality measurement system (QCM+ System, Technical Services and Supplies, York, England) while the yolk length and width were measured using a vernier caliper. The shell weight was measured via a digital scale; the shell thickness was analyzed with an egg shell thickness gauge (model 0.0001 mm, Mitutoyo, Japan). Furthermore, the shell strength was determined by an egg shell force gauge (model-II, Robotmation Co., LTD, Japan). The egg yolk color was evaluated visually with a Roche Color Fan (Roche Ltd., Basel, Switzerland). This experiment was conducted at a commercial egg production facility in Taichung, Taiwan under our supervision.

Study Design III: Impact of different Formulations of Deinococcus sp. GKB-Aid 1995 on Egg Quality and Egg Yolk Color

This experiment was conducted at a commercial egg production facility in Nantou, Taiwan for 20 d under our supervision. Sixty White Leghorn laying hens at 55 wk of age were divided randomly into 1 control and 4 treatment groups comprised of twelve layers, each designated as replicates. These hens were kept in pairs and had free access to poultry feed and water. All ingredients were thoroughly mixed in a mixer and fed in a dry form to the hens. The dimensions of the cages were 40 cm in width x 45 cm in depth x 45 cm front height x 38 cm back height, and the housing was designed on the basis of the opened system. The dietary treatments included: 1) commercial corn-soy-type diet (control), 2) commercial diet plus 1 wt % *Deinococcus* sp. GKB-Aid 1995 powder, 3) commercial diet plus 1 wt % *Deinococcus* sp. GKB-Aid 1995 granules (with potato starch alone) and 4) commercial diet plus 1 wt % *Deinococcus* sp. GKB-Aid 1995 oily granules (hydrogenated vegetable oil and potato starch). Animals were treated with 100 g of the diet supplemented with and without different formulations of *Deinococcus* sp. GKB-Aid 1995 for 10 successive d. From d 1 of treatment to 10 d after treatment, eggs from each group were obtained and preserved at 4 ± 1°C until the analysis step. The egg yolk color was evaluated visually with a Roche Color Fan (Roche Ltd., Basel, Switzerland).

Deinococcus spp. Color Measurement

Color traits L*a*b* of 5 *Deinococcus* sp. strains in powder form were measured 3 times by a SP60 series sphere spectrophotometer (X-Rite, Inc., Grand Rapids, MI) and determined according to the manufacturer's instructions. These values were then used to calculate C (chroma) and h (hue angle) values, in which C denotes the color saturation and h expresses the color itself by an angular measurement: 0° (red), 90° (yellow), 180° (green) and 270° (blue) (Weatherall and Coombs, 1992). Calibration was performed with standard white and black plates at the start of each session.

Deinococcus spp. Pigments Analysis by HPLC-DAD

Pigment extractions were performed on 0.2 g *Deinococcus* spp. powder suspended in 10 mL methanol under sonication for 30 min and filtered through a 0.45 μm filter. High-performance liquid chromatography (**HPLC**)-diode array detector (**DAD**) analyses of the filtrates were then conducted in a Hitachi D-2000-series system equipped with a L-2455 diode array detector. Chromatography was performed on a Kinetex 5 u C18 100A column (150 × 4.6 mm; particle size 5 μm, Phenomenex) with water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B) for the mobile phase at 40°C. The solvent gradient was programmed from 50 to 100% B in A in 30 min with a flow rate of 1 mL/min monitored at 480 nm.

Statistical Analysis

Data in all studies were reported as mean ± SD and were analyzed using one-way analysis of variance (**ANOVA**) followed by Duncan's post hoc test (SPSS for Windows, version 20.0; IBM-SPSS, Chicago, IL). The results of all statistical analyses were considered significant at $P < 0.05$.

RESULTS

Color Measurements of 5 Strains of Deinococcus sp. Powder

The color of 5 freeze-dried strains of *Deinococcus* sp. powder were shown in Figure 1 and analyzed with a color meter (Table 1). The L* value of selected *Deinococcus* powder ranged between 52 to 62, whereas a* value (red/green) and b* values (yellow/blue) ranged between 15 to 25 and 9 to 21 respectively. The chroma value of *Deinococcus* spp. powder varied from 20 (gray) to 30 (bright). *D. geothermalis* showed the highest value of L*, suggesting its color was lighter than the rest of the *Deinococcus* strains. All *Deinococcus* powder types showed redness and yellowness, illustrated by the positive a* and b* values. The hue angle of the *Deinococcus* sp. GKB-Aid 1995 was 29.25, corresponding to the color red on the basis of a scale of 0 for redness, 90 for yellowness, 180 for greenness, and 270 for blueness. Values for the other strains were 30–55, indicating that they were more orangish red compared to *Deinococcus* sp. GKB-Aid 1995.

Figure 1. Powdered *Deinococcus* spp.

Table 1. Color characteristics of 5 freeze-dried strains of *Deinococcus* sp. powder.

Bacterial Strains	L*	a*	b*	Chroma[a]	Hue Angle[b]
D. radiodurans	52.75 ± 0.22	18.89 ± 1.00	13.43 ± 1.02	23.18	35.37
D. proteolyticus	61.07 ± 0.12	22.07 ± 0.07	20.61 ± 0.10	30.20	44.13
Deinococcus sp. GKB-Aid 1995	52.52 ± 0.09	17.84 ± 0.14	9.97 ± 0.10	20.44	29.25
D. geothermalis	61.40 ± 0.47	15.20 ± 0.23	21.06 ± 0.49	25.97	54.07
D. grandis	52.65 ± 0.08	25.80 ± 0.35	16.00 ± 0.29	30.36	30.96

[a]Chroma (C) = $[(a^*)^2+(b^*)^2]^{1/2}$.
[b]Hue angle (h) = $\tan^{-1}(b^*/a^*)$.

HPLC-DAD Analysis of Pigments in Deinococcus spp.

The various red pigments in *Deinococcus* spp. were further extracted with methanol and analyzed by HPLC-DAD, which produced the chromatogram shown in Figure 2. Chromatograms corresponding to *D. geothermalis* showed no peaks. HPLC of *D. radiodurans* showed one peak, which corresponded to the retention time of 6 min while *D. proteolyticus* showed 2 peaks at the retention times of 3 min and 5.2 min. *Deinococcus* sp. GKB-Aid 1995 showed identical absorption spectra and similar retention times to the red pigment that was also present in *D. grandis*, strongly suggesting that this component was the same and shared by the 2 strains.

Study I: Impact of Various Deinococcus spp. on Egg Quality and Egg Yolk Color

Egg quality traits and yolk characteristics are presented in Table 2. Groups receiving *D. radiodurans* and *D. geothermalis* had significantly higher average egg weights while hens consuming *D. proteolyticus* produced significantly lower average egg weights when compared to the control group ($P < 0.05$). Although egg weight decreased in the group receiving *Deinococcus* sp. GKB-Aid 1995 and increased in the group receiving *D. grandis*, these changes were not significant when compared to the control group. Other traits such as yolk height and Haugh unit were not affected by the supplementation of *Deinococcus* spp. within the 10-d period. However, the inclusion of *D. radiodurans, Deinococcus* sp. GKB-Aid 1995, and *D. grandis* in the diet significantly enhanced egg yolk pigmentation ($P < 0.05$) whereas adding *D. proteolyticus* and *D. geothermalis* in the diet showed lower yolk color values than the control.

Study II: Impact of Different Concentrations of Deinococcus sp. GKB-Aid 1995 on Production Performance and Egg Quality

The effects of diets supplemented with different levels of *Deinococcus* sp. GKB-Aid 1995 for a longer treatment period on production characteristics and egg quality traits are shown in Table 3. Regarding the production performance, 5.0 wt % *Deinococcus* sp. GKB-Aid 1995 supplementation significantly improved the feed conversion ratio ($P < 0.05$) compared to the

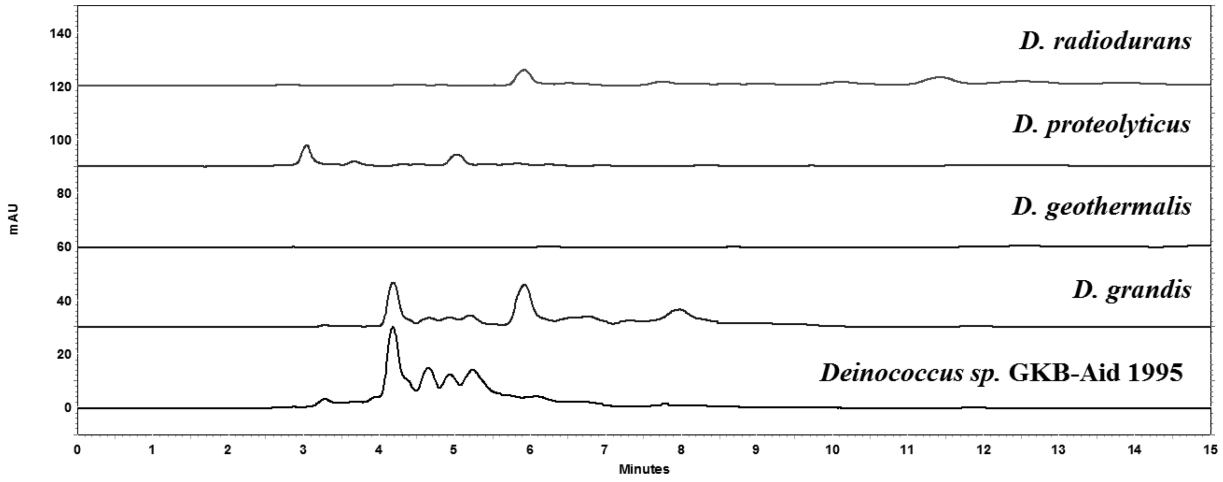

Figure 2. Chromatogram of pigments in selected *Deinococcus* spp. by HPLC-DAD.

Table 2. Effects of various *Deinococcus* sp. for 10 d on egg parameters.

Supplemented with 1 wt %	Egg Weight (g)	Yolk Height (mm)	Haugh Unit	Yolk Color
None	50.06 ± 3.32^{b,c}	2.92 ± 1.09^{a}	52.68 ± 13.77^{a}	7.53 ± 0.85^{b}
D. radiodurans	52.36 ± 3.02^{a}	3.17 ± 1.18^{a}	50.59 ± 16.74^{a}	8.69 ± 0.64^{c}
D. proteolyticus	45.84 ± 1.91^{d}	2.82 ± 0.92^{a}	51.46 ± 11.70^{a}	7.42 ± 1.16^{a}
Deinococcus sp. GKB-Aid 1995	48.63 ± 3.34^{c}	2.94 ± 1.02^{a}	51.92 ± 14.45^{a}	10.50 ± 1.59^{e}
D. geothermalis	52.71 ± 5.24^{a}	3.16 ± 1.00^{a}	51.23 ± 12.92^{a}	7.23 ± 1.19^{a}
D. grandis	51.10 ± 3.30^{a,b}	3.09 ± 0.98^{a}	52.26 ± 12.09^{a}	10.81 ± 0.98^{e}

Data were expressed as mean ± SD for 4 replicates with 4 hens each per treatment.
Means in the same row not sharing a common superscript are significantly different ($P < 0.05$).

Table 3. Effects of diets supplemented with different levels of *Deinococcus* sp. GKB-Aid 1995 for 12 wk on production characteristics and egg quality traits.

	Supplementation of *Deinococcus* sp. GKB-Aid 1995			
Variables	0 wt %	0.2 wt %	1.0 wt %	5.0 wt %
Feed consumption (g/hen/d)	99.25 ± 0.74^{a}	99.47 ± 0.57^{a}	99.17 ± 0.75^{a}	99.34 ± 0.87^{a}
Egg Production (%)	94.50 ± 3.46^{a}	90.60 ± 3.39^{b}	91.90 ± 1.65^{b}	95.50 ± 2.31^{a}
Feed Conversion Ratio (g/g)	1.72 ± 0.06^{a}	1.66 ± 0.07^{b}	1.67 ± 0.04^{a,b}	1.63 ± 0.04^{b}
Egg Weight (g)	59.10 ± 6.38^{a}	63.03 ± 4.18^{b}	64.64 ± 4.42^{b}	63.19 ± 3.73^{b}
Shell Weight (g)	7.44 ± 0.9^{a}	8.00 ± 0.69^{b}	7.97 ± 0.6^{b}	8.11 ± 1.02^{b}
Shell Hardness (kg cm$^{-1)}$	2.93 ± 0.95^{a}	3.68 ± 1.12^{c}	3.20 ± 1.12^{a,b}	3.61 ± 0.87^{b,c}
Shell Thickness (mm)	0.43 ± 0.05^{a}	0.45 ± 0.06^{a}	0.44 ± 0.05^{a}	0.44 ± 0.05^{a}
Albumin Weight (g)	33.94 ± 3.52^{a}	36.19 ± 4.81^{b}	38.03 ± 4.52^{c}	35.18 ± 3.56^{a,b}
Yolk Weight (g)	16.76 ± 1.76^{a}	17.84 ± 1.81^{b}	16.95 ± 1.26^{a}	18.19 ± 1.47^{b}
Yolk Diameter (mm)	4.90 ± 0.28^{a}	4.07 ± 0.21^{a}	4.05 ± 0.18^{a}	4.13 ± 0.23^{a}
Yolk Height (mm)	4.55 ± 1.22^{a}	4.94 ± 1.43^{a}	5.18 ± 1.38^{a}	4.93 ± 1.1^{a}
Yolk Color	7.78 ± 1.09^{a}	9.63 ± 0.87^{b}	10.92 ± 2.46^{c}	12.94 ± 1.79^{d}
Haugh Unit	62.94 ± 11.25^{a}	62.70 ± 13.62^{a}	66.88 ± 12.4^{a}	64.89 ± 9.26^{a}

Regarding feed consumption and egg production, the values are represented as mean ± SD with 21 replicate hens in each experimental group.
For egg quality, data were expressed as mean ± SD for 21 replicates of 7 adjacent cages with 21 hens each per treatment.
Means in the same row not sharing a common superscript were significantly different ($P < 0.05$).
Egg production (%) = Average daily egg production × 100.
Feed conversion ratio (g/g) = g feed/g egg mass.

control but had no effect on feed consumption and egg production. Additionally, inclusion of 5.0 wt % *Deinococcus* sp. GKB-Aid 1995 in the diet significantly enhanced egg weight, shell weight, shell hardness, albumin weight, yolk weight, and yolk color of the eggs (Figure 3) ($P < 0.05$). However, the addition of *Deinococcus* sp. GKB-Aid 1995 in the diet had no effect on other egg traits ($P > 0.05$).

Study III: Impact of Different Formulations of Deinococcus sp. GKB-Aid 1995 on Egg Quality and Egg Yolk Color

The effects of supplementing *Deinococcus* sp. GKB-Aid 1995 in various formulations and those without it for 10 d on egg quality traits are reported. Statistically significant differences between control and treated

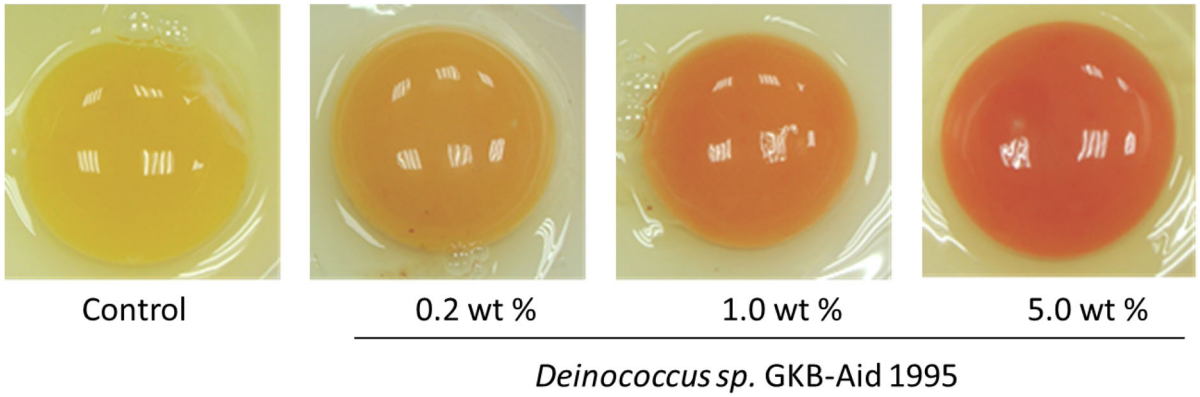

Figure 3. Enhancement of egg yolk color by different levels of *Deinococcus* sp. GKB-Aid 1995.

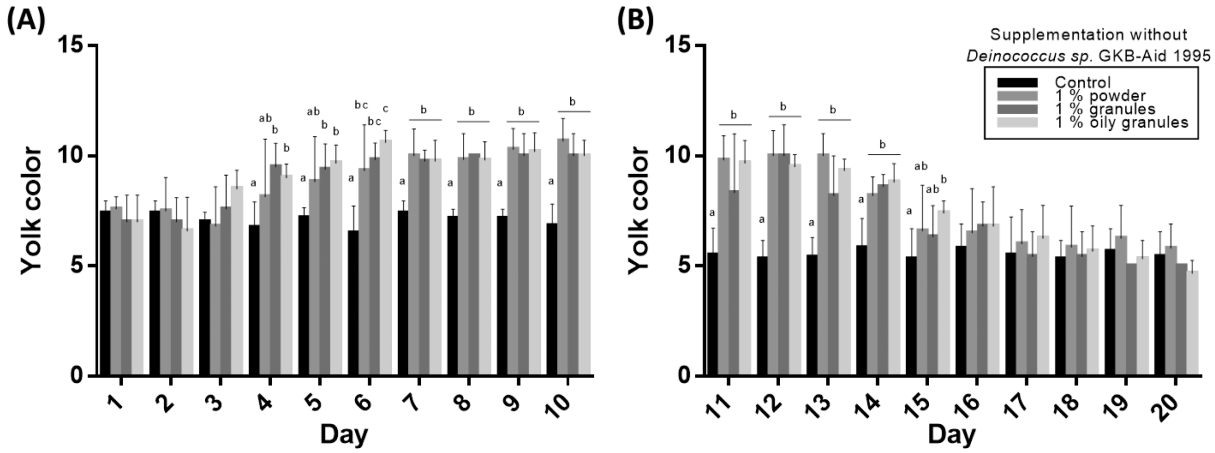

Figure 4. Changes in egg yolk color with (A) supplementation of *Deinococcus* sp. GKB-Aid 1995 in various formulations and (B) without it for 10 d. Data were expressed as mean ± SD for 12 replicates of 6 adjacent cages with 12 hens each per treatment. Means that do not share a common superscript were significantly different ($P < 0.05$).

groups were noted in egg weight, yolk height, shell thickness, and Haugh unit, but all differences were small in magnitude and were not consistent throughout the study (data not shown). Therefore, these differences were not considered to be treatment related. Regarding the yolk color (Figure 4), a significant improvement in yolk pigmentation was observed between 4 to 10 d with the addition of 1 wt % oily *Deinococcus* sp. GKB-Aid 1995 granules compared to the control, and this coloration continued up to 15 d even after the supplementation has stopped. The *Deinococcus* sp. GKB-Aid 1995 powder and granule groups showed a similar pattern but with shorter time intervals. The addition of *Deinococcus* sp. GKB-Aid 1995 granules at 1 wt % gradually increased yolk redness over time, reaching significant differences from d 4 to 14, whereas such redness in yolk colors from *Deinococcus* sp. GKB-Aid 1995 granules-treated White Leghorn laying hens was only detected from d 6 to 14. Moreover, the highest egg-yolk color value (10.60 ± 0.55) was observed in 1 wt % oily *Deinococcus* sp. GKB-Aid 1995 granules on d 6 during the 10-d feeding period.

DISCUSSION

Deinococcus spp. are a group of coccus- or rod-shaped, red or pink pigment-producing bacteria that have a remarkable DNA repair system and an efficient reactive oxygen species (**ROS**) scavenging capacity as they have the ability to adapt to harsh environments such as high temperature and UV rays (Zhang, Sun, Li, Yu, Zhou, Zhang, Xu and Jiang, 2007b). Knockout constructs of *crtB* and *crtI* genes in a previous study suggested that the carotenoids present in *D. radiodurans R1* act as free radical scavengers to protect this organism against the deleterious effects of oxidative DNA-damaging agents (Zhang et al., 2007a). This unique carotenoid was later isolated from the radioresistant bacterium *Deinococcus radiodurans* in 1997 and identified as deinoxanthin (Lemee et al., 1997).

Compared to other carotenoids such as lycopene, lutein, or zeaxanthin, deinoxanthin had a stronger scavenging ability on reactive oxygen species and free radicals (Tian et al., 2009; Ji, 2010). Currently, there are more unknown carotenoids being discovered from different *Deinococcus* species (Fei et al., 2012a). Although the nature of these compounds is largely unknown, these carotenoids found in this genus are worthy of special attention. As *Deinococcus* spp. are enriched with carotenoids, they offer a sustainable avenue as food colorants with potential health benefits. Hence, the application of red carotenoid-producing *Deinococcus* spp. as a feed supplement for hens and other avians was discussed.

Considering that even closely related strains of the same microbial species may possess different physiological effects (Gerritsen et al., 2011), 5 different strains of *Deinococcus* spp. were selected in this study. As anticipated, various red colors produced by different *Deinococcus* sp. under the same fermentation conditions were easily distinguished with the naked eye. A clear difference in the color of the samples (lyophilized *Deinococcus grandis*, *D. radiodurans*, *D. proteolyticus*, *D. geothermalis,* and *Deinococcus* sp. GKB-Aid 1995) was also observed based on the hue angle and chroma values. Moreover, different pigments showed significantly different retention times from each strain via HPLC analysis, which explained the reason behind the various shades of red exhibited by separate strains of the same species. As *Deinococcus* sp. GKB-Aid 1995 exhibited 97.9% and 98.4% similarity to *D. grandis* based on the results from the Vitek 2 GN system and 16S rRNA gene sequencing respectively (Wu, Li, Lin and Chen, 2016), it was not surprising that they shared similar absorption spectra at similar retention times.

However, little is known about the mechanisms of these compounds on coloration and pigmentation effects when administered *in vivo*. Compared to the nonsupplemented diet, only *D. proteolyticus* decreased egg weight slightly, whereas no differences between the remaining groups were observed for the other variables. On the other hand, the addition of *D. radiodurans*, *D. grandis*, and *Deinococcus* sp. GKB-Aid 1995 significantly improved the yolk color, with the best pigmentation score obtained by *D. grandis* and *Deinococcus* sp. GKB-Aid 1995. These findings suggested that the pigments of *Deinococcus* spp. can be absorbed from the intestinal tract and be deposited into the egg yolk without additional extraction steps.

Unfortunately, the egg yolk color was not significantly influenced in the groups fed with *D. proteolyticus* or *D. geothermalis*. Pigment composition analysis further showed that *D. grandis* and *Deinococcus* sp. GKB-Aid 1995 strains shared highly similar spectra with retention times of 4.2 min, but these pigments were not detected in *D. radiodurans*, *D. proteolyticus*, or *D. geothermalis* strains. These results suggested that the color of egg yolk is closely related to the type and ratio of carotenoids shared by *D. grandis* and *Deinococcus* sp. GKB-Aid 1995 strains, rather than being dependent on the color of the bacterial cell itself. Since the medium compositions and culture conditions have always been suggested to be the key strategy for pigment production (Chen et al., 2015), further studies on improving the appropriate colorant yields in bacterial cells may represent an alternative strategy to enhance the color of egg yolks.

Deinococcus sp. GKB-Aid 1995 and *D. grandis* were effective feed additives for improving egg yolk color in Study I. Seeing that *Deinococcus* sp. GKB-Aid 1995 produced higher biomass yield than *D. grandis* in our unpublished data, additional larger-scale, longer-term studies with different doses (Study II) and different dosage forms (Study III) of *Deinococcus* sp. GKB-Aid 1995 were conducted. When the amount of *Deinococcus* sp. GKB-Aid 1995 supplemented into the diet for laying hens was increased, no changes were observed for yolk diameter, yolk height, shell thickness, and Haugh unit. However, there were some significant differences between treatment and control regarding shell weight, shell hardness, albumin weight, and yolk weight, but these changes showed a lack of consistency or dose-relationship and were therefore considered to be chance occurrences. However, supplementation of *Deinococcus* sp. GKB-Aid 1995 had a significant effect on feed conversion ratio. Although study II results showed that 0.2%, 1%, and 5 wt % *Deinococcus* sp. GKB-Aid 1995 supplemented diets improved egg weight, a similar trend was not observed in Study III when supplementing different dosage forms of 1 wt % *Deinococcus* sp. GKB-Aid 1995 to the diet of laying hens. Such inconsistency may be explained by the differences in the environmental background and hen strains used among different studies.

Nevertheless, findings from the 3 studies were similar in that egg yolk color was enhanced by the addition of *Deinococcus* sp. GKB-Aid 1995 in a dose-dependent manner. Yolk redness was gradually increased by *Deinococcus* sp. GKB-Aid 1995 over the time, reaching significant differences starting from d 4, which lasted for 6 d even after the supplementation has ended. The addition of lipids has been said to enhance carotenoid absorption in animals as well (Nagao, 2014). Although *Deinococcus* sp. GKB-Aid 1995 granules and oily *Deinococcus* sp. GKB-Aid 1995 granules can advance or delay yolk coloring, these values were not significantly different from those of the *Deinococcus* sp. GKB-Aid 1995 powder group. Despite these 3 supplement formulations having no significant impact on yolk color, they can be useful as references for future studies.

Currently, the applications of *Deinococcus* strains in the feed industry have received considerable attention due to their potential to improve feed utilization and performance of livestock. For instance, in the present study, *Deinococcus* sp. GKB-Aid 1995 was beneficial

not only in increasing yolk color intensity but also in improving the feed conversion ratio. In another study, supplemental *D. radiodurans* powder increased the number of piglets born alive, stimulated milk production, improved litter weight at weaning, and provided sows a rapid return to estrus after weaning (Guohui et al., 2010). Moreover, when administering *Deinococcus radiopugnance* in moths, results showed better fecundity, silkworm development, and cocoon productivity (Zhenli, 2013). Based on the results of these studies, it can be concluded that the use of *Deinococcus* strains as feed additives is feasible, imparting benefits for the animals.

Nevertheless, feed safety is still of the utmost importance. Although there have been no safety studies on *Deinococcus* strains in animal feeds, the acute toxicity, genotoxicity (Wu, Li, Lin and Chen, 2016), 90-d subchronic oral toxicity (Jhou, Jiang, Lin, Tsai and Chen, 2016), and teratogenicity test (Li et al., 2017) of *Deinococcus* sp. GKB-Aid 1995, in particular, have all been conducted in rodents. In support of these findings, the present study has provided further evidence on the safety of *Deinococcus* sp. GKB-Aid 1995, where no signs of toxicity were shown based on a 12-wk feeding period for hens. In considering that antimicrobial resistance strains may pose a health risk to humans, the results of the antibiotic susceptibility test revealed that this strain exhibited no tolerance to the 6 antibiotics tested (Wu, Li, Lin and Chen, 2016). Since the consumption of *Deinococcus* sp. GKB-Aid 1995 demonstrated a yolk-coloring effect and did not result in any adverse effects in the experimental animals, it may be valuable to explore its possible application as a feed additive with various health benefits.

CONCLUSION

In this study, the egg yolks ranged in color from pale yellow to deep orange, where the degree of yolk pigmentation depended largely on the types and levels of carotenoids present in different *Deinococcus* species. Out of 5 *Deinococcus* species fed to hens, *Deinococcus* sp. GKB-Aid 1995 and *D. grandis* showed the best color values and had significant effects on the color of egg yolks. *Deinococcus* sp. GKB-Aid 1995 was beneficial not only in increasing yolk color intensity but also in improving the feed conversion ratio. Moreover, oily *Deinococcus* sp. GKB-Aid 1995 granules can delay yolk coloring when compared to other dosage forms. As no apparent effect on morbidity or mortality was observed in hens in 3 trials, *Deinococcus* sp. GKB-Aid 1995 have demonstrated their potential application in the livestock industry as feed additives in the present study.

ACKNOWLEDGMENTS

The authors thank Hsin-Yun Yang for editing the manuscript.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Chen, G., K. Shi, D. Song, L. Quan, and Z. Wu. 2015. The pigment characteristics and productivity shifting in high cell density culture of Monascus anka mycelia. BMC Biotechnol. 15:72.

Delgado-Vargas, F., O. Paredes-López, and E. Avila-González. 1998. Effects of sunlight illumination of marigold flower meals on egg yolk pigmentation. J. Agric. Food Chem. 46: 698–706.

Fei, W., F. Qiong, L. Chengzhi, L. Lin, and T. Bing. 2012a. Screening of antioxidant activities of carotenoid extracts from *Deinococcus*. J. Nucl. Agric. Sci. 26:900–905.

Fei, W., F. Qiong, L. Chengzhi, L. Lin, T. Bing, and H. Yuejin. 2012b. Screening of antioxidant activities of carotenoid extracts from *Deinococcus* Acta Agric. Nucl. Sin. :900–905.

Fletcher, D. L. 1999. Broiler breast meat color variation, pH, and texture. Poult. Sci. 78:1323–1327.

Galobart, J., R. Sala, X. Rincón-Carruyo, E. G. Manzanilla, B. Vilà, and J. Gasa. 2004. Egg yolk color as affected by saponification of different natural pigmenting sources. J. Appl. Poult. Res. 13:328–334.

Gerritsen, J., H. Smidt, G. T. Rijkers, and W. M. de Vos. 2011. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr. 6:209–240.

Guohui, M., H. Qihua, W. Liangyan, and C. Anguo. 2010. Use of radioresistant cocci in preparing feedstuff additive for improving fertility of sow. Patent CN101326960A, assignee. Pat. No. CN101326960A.

Jhou, B.-Y., Y.-M. Jiang, Y.-C. Lin, Y.-T. Tsai, and C.-C. Chen. 2016. A 90-day subchronic toxicological assessment of *Deinococcus grandis* fermented soymilk in Sprague-Dawley rats. Int. J. Pharm. Pharm. Sci.8:207–215.

Ji, H. F. 2010. Insight into the strong antioxidant activity of deinoxanthin, a unique carotenoid in Deinococcus radiodurans. Int. J. Mol. Sci. 11:4506–4510.

Lemee, L., E. Peuchant, M. Clerc, M. Brunner, and H. Pfander. 1997. Deinoxanthin: A new carotenoid isolated from Deinococcus radiodurans. Tetrahedron 53:919–926.

Li, I. C., W.-P. Chen, Y.-C. Lin, C.-C. Chyau, and C.-C. Chen. 2017. Teratogenicity and volatile composition evaluation of soymilk fermented with a Deinococcus member. J. Toxicol. Health 4:1.

Lokaewmanee, K., K.-e. Yamauchi, T. Komori, and K. Saito. 2010. Effects on egg yolk colour of paprika or paprika combined with marigold flower extracts. Ital. J. Anim. Sci. 9:e67.

Nagao, A. 2014. Bioavailability of dietary carotenoids: intestinal absorption and metabolism. Japan Agric. Res. Q. 48:385–391.

Schlatterer, J., and D. E. Breithaupt. 2006. Xanthophylls in commercial egg yolks: quantification and identification by HPLC and LC-(APCI)MS using a C30 phase. J. Agric. Food Chem. 54:2267–2273.

Surai, P. F., R. C. Noble, and B. K. Speake. 1996. Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. Biochim. Biophys. Acta 1304:1–10.

Tian, B., Z. Sun, S. Shen, H. Wang, J. Jiao, L. Wang, Y. Hu, and Y. Hua. 2009. Effects of carotenoids from Deinococcus radiodurans on protein oxidation. Lett. Appl. Microbiol. 49:689–694.

Weatherall, I. L., and B. D. Coombs. 1992. Skin color measurements in terms of CIELAB color space values. J. Invest. Dermatol. 99:468–473.

Wu, S. Y., I. C. Li, Y. C. Lin, and C. C. Chen. 2016. Characterization and safety evaluation of a *Deinococcus* member as feed additive for hens. Regul. Toxicol. Pharmacol. 76:121–127.

Yao, C.-A., C.-C. Chen, N.-P. Wang, and C.-T. Chien. 2016. Soy-based multiple amino acid oral supplementation increases the anti-sarcoma effect of cyclophosphamide. Nutrients 8:192.

Youssef, A. W., H. M. A. Hassan, H. M. Ali, and M. A. Mohamed. 2013. Effect of probiotics, prebiotics and organic acids on layer performance and egg quality. Asian J. Poult. Sci. 7:65–74.

Zhang, L., Q. Yang, X. Luo, C. Fang, Q. Zhang, and Y. Tang. 2007a. Knockout of crtB or crtI gene blocks the carotenoid biosynthetic pathway in Deinococcus radiodurans R1 and influences its resistance to oxidative DNA-damaging agents due to change of free radicals scavenging ability. Arch. Microbiol. 188:411–419.

Zhang, Y. Q., C. H. Sun, W. J. Li, L. Y. Yu, J. Q. Zhou, Y. Q. Zhang, L. H. Xu, and C. L. Jiang. 2007b. Deinococcus yunweiensis sp. nov., a gamma- and UV-radiation-resistant bacterium from China. Int. J. Syst. Evol. Microbiol. 57:370–375.

Zhenli, T. 2013. Feed additive for improving output and quality of silkworm cocoons and preparation method thereof. Patent CN 102626183 A, assignee.