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Effect of dietary octacosanol concentration extracted from triticale sprout on laying performance, egg quality, and blood parameters of laying hens

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Abstract

This study was conducted to investigate the effect of dietary supplementation of octacosanol (OCT) extracted from triticale sprout on laying performance, egg quality, and blood parameters of laying hens. A total of 192, Hyline brown laying hens aged 43 weeks were divided into 4 dietary groups of 48 birds each and they were randomly subjected to one of the experimental diets containing OCT at the levels of none, 10, 20, and 30 mg/kg of diet. All birds were fed with isoenergetic and isonitrogenous mash diets for 6 weeks. The result showed that hens supplemented with 20 and 30 mg/kg OCT in diet significantly increased ($p < 0.05$) egg production than those fed with the basal diet. OCT concentration in the egg yolk of hens fed with 20 and 30 mg/kg OCT was significantly higher than in those fed the control diet. Hens fed 20 and 30 mg/kg OCT exhibited greater high-density lipoprotein (HDL) cholesterol and interleukin (IL) concentrations and reduced serum concentrations of cholesterol and triglyceride compared to those fed with 0 and 10 mg/kg OCT. This study indicates that supplementing the diet of laying hens with 20 and 30 mg/kg of OCT can improve the performance, egg quality, and health status of laying hens.

Keywords: Blood parameter, Laying hens, Octacosanol, Triticale sprout, Performance

INTRODUCTION

Eggs are one of the most important sources of nutrients to meet human physiological needs. Some effects discovered on human health, added to easy availability and affordability, increase the egg consumption and give it the attributes of essential aliment in the global food market [1]. As the global population is growing, egg production will need to be increased in order to meet the needs of the 9 billion people predicted to inhabit the Earth by the year 2050. However, reduced egg-laying rates, lower egg quality of older hens, and the emergence of diverse diseases affecting the laying hens industry, place this target out of reach. Feed additives such as octacosanol (OCT) may provide a solution to these problems. OCT [$HO\text{-}CH_2\text{-}(CH_2)_{26}CH_3$], a high molecular weight alcohol with evident physiological

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Lim CI, Ryu KS.
Data curation: Lim CI.
Formal analysisLim CI.
Software: Lim CI.
Validation: Lim CI.
Investigation: Ryu KS.
Writing - original draft: Lim CI, Ryu KS.
Writing - review & editing: Lim CI, Ryu KS.

Ethics approval and consent to participate
The protocol for these experiments was approved, and animals were cared for according to the guidelines of the Animal Care and Use Committee of Jeonbuk National University, Jeonju, Korea (JBNU 2021-0168).

activity and pharmacological effects, is the main constituent of a natural wax product, existing in wheat germ oil, rice bran oil, fruits, and leaves [2,3]. OCT products are typically a blend of long-chain aliphatic components, specifically fatty acids, aldehydes, ketones, primary and secondary alcohols, and alkanes of chain lengths C20–C36, as well as C38–C70 alkyl esters [4]. OCT has diverse biological functions including anti-fatigue properties [5], antioxidant effects [6], cholesterol-lowering effects [7], and cytoprotective features [8]. OCT isolated from rice bran has been reported to enhance growth performance, improve immunity and antioxidant levels, significantly decrease diarrhea in weanling piglets [9], induce the production of growth hormones, and up-regulate glucose transporter protein gene expressions [10]. OCT is a safe feed additive [11], and Long et al. [10] and Peng et al. [12] reported that the addition of OCT to the diet of laying hens, at various levels, significantly improved their performance, egg quality, and concentrations of certain blood parameters.

Most investigations concerning OCT's effects on laying hens have examined OCT in humans and mice [2,3,13,14]. Moreover, the OCT used in previous experiments was typically extracted from rice bran [9,10,12,15]. To our knowledge, no research has been performed concerning OCT contained in triticale sprouts, nor on the transfer of OCT from laying hens into their eggs, which affects the nutritional value of the eggs. Therefore, our study aims to investigate effect of dietary supplementation of OCT from triticale sprout on performance, egg quality, and blood parameters of laying hens.

MATERIALS AND METHODS

Experimental birds, housing, and diets

The study was conducted at the Poultry Experimental Station of the Department of Animal Sciences at Jeonbuk National University in the Korea. The protocols for the experiment were approved by the Jeonbuk National University Institutional Animal Care and Use Committee (JBNU 2021-0168). The triticale sprout powder (cultivar *Joseong*) used for OCT extraction was obtained from the National Institute of Crop Science located in Suwon (Korea). The triticale sprout contained in approximately 2.33 mg/g of OCT, was extracted by Singh et al. [16].

A total of 192, Hy-line brown laying hen aged 43 weeks old, were individually weighed and randomly divided into 32 cages (6 hens per cage). The cages (1,205 × 50 cm floor space; 67 cm height) were equipped with metal feeders in front and two nipple drinkers inside an environmentally controlled hen house (25±5℃ temperature and 16/8 h light/dark cycle). The hens in the 32 cages were divided into four dietary groups (48 birds per group) which were fed diets supplemented with 0 (control), 10, 20, and 30 mg/kg of OCT, respectively. The groups were arranged in 4 replications (2 cages per replication). Feed and water were available *ad libitum* throughout the experimental period. The diets were based on corn and soybean meal, formulated to meet the nutrient requirements of laying hens as recommended in the RDA, NIAS [17]. The basal diet composition and nutrient contents are presented in Table 1. All birds were fed with isoenergetic (2,800 kcal/kg) and isonitrogenous (17.0%) mash diets from 43 to 48 weeks of age.

Laying hens performance

We recorded the number of eggs produced daily while egg weight was measured weekly. The remaining feed was documented every three weeks during the experiment. These data were used to determine egg production, feed intake, and feed efficiency. The feed conversion ratio (FCR) was calculated as the amount of feed consumed per unit of egg weight for six weeks.

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Table 1. Composition and nutrient content of the basal diet

Ingredients	Contents (g/kg)
Corn	60.6
Soybean meal	23.7
Corn gluten meal	2.69
Beef tallow	1.20
Limestone	9.33
MDCP	1.88
Choline chloride	0.08
Salt	0.29
Mineral premix[1)]	0.22
Vitamin premix[2)]	0.05
Total	100
Calculated chemical composition	
Metabolic energy (kcal/kg)	2,800
Crude protein (%)	17.0
Total lysine (%)	0.820
Total methionine (%)	0.360
Calcium (%)	4.00
Available phosphorus (%)	0.360

[1)]Minerals supplemented per kilogram of diet: Fe, 60 mg; Cu, 10 mg; Zn, 80 mg; Mn, 110 mg; Iodine, 0.48 mg; Se, 0.40 mg.
[2)]Vitamins supplement per kilogram of diet: vitamin A, 10,000 IU; vitamin D, 2,000 IU; vitamin E, 30 IU; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 0.15 mg; vitamin B_{12}, 0.025 mg; vitamin K_3, 3 mg; niacin, 50 mg; vitamin C, 200 mg; pantothenic acid, 12 mg; choline, 500 mg; biotin, 0.15 mg; folic acid, 1.5 mg.

Egg quality

Twenty eggs from each treatment randomly collected and were used to evaluate egg quality at the end of the experiment. Albumen height was measured using the TSS Egg multi-tester (Technical Services and Supplies, York, UK). Haugh unit was calculated by the following formula: 100 logs (albumen height − 1.7 egg weight$^{0.37}$+7.57). Eggshell thickness without the shell membrane was measured using a micrometer (Digimatic micrometer, Series 293 330, Mitutoyo, Tokyo, Japan). OCT level was determined using gas chromatography. First, egg samples from each of the four diets were prepared as follows: after placing 5 g of the egg sample into a saponification flask, 50 mL of ethanol potassium hydroxide solution was added, and the flask was heated for 1 hour to boil and saponify. The saponification solution was then transferred to a separator filter, 50 mL of water was added, and the mix was cooled. After that, 100 mL of ether was put in the mix; the gas was discharged and left until the two layers were separated. The separated lower layer was transferred to a second separator filter, 50 mL of ether was added, and it was then transferred to a third separator filter. After the third separation, the lower layer was discarded. The first, second, and third ether layers were collected and dehydrated using anhydrous sodium sulfate, then most of the ether was distilled to make a final sample. Ultra-performance liquid chromatography (UPLC) analysis was performed using an Agilent technology 6890N Network GC system (6870N-5973, Agilent, Santa Clara, CA, USA).

Blood chemical analysis

Blood samples were taken by puncturing each hen's wing vein. Samples were collected in sterile syringes, centrifuged, and then subjected to hematological and biochemical examination. The separated serum was stored at −20℃. The serum concentrations of cholesterol, high-density

lipoprotein (HDL) cholesterol, and triglyceride were measured via the colorimetric method using a biochemical analyzer (Automatic Biochemical Analyser, Konelab 20, Thermo Scientific, Vantaa, Finland). Interleukin-2 (IL-2) and IL-6 concentrations were analyzed using ELISA kits (Elabscience, E-EL-Ch0120; E-EL-Ch0228) following the manufacturer's instructions.

Statistical analysis

Data analyzed with one-way ANOVA of the SAS (Statistical Analysis System). Duncan's multiple range test was used to determine significant differences between treatments. Statistical significances were preset at $p < 0.05$.

RESULTS AND DISCUSSION

Laying performance

The egg laying performance results of hens fed a diet supplemented with OCT are presented in Table 2. Our results indicate significantly ($p < 0.05$) higher egg production in hens fed with 30 mg/kg OCT than those fed with 0 and 10 mg/kg OCT. These results are supported by Peng et al. [12], who showed that treatments containing 18 and 27 mg/kg of OCT significantly increased egg production. Similar outcomes were highlight by El-Wardany et al. [18] and Long et al. [10] following OCT treatment. OCT has been reported to elevate follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and prostaglandin (P4) [10] involved in egg maturation and production. Accordingly, our findings could be the result of the beneficial effects of OCT supplementation on egg production, possibly related to reproductive hormones. Furthermore, the increased egg production of hens fed diets supplemented with 20 and 30 mg/kg of OCT, in addition to similar effects between hens receiving the control and 10 mg/kg of OCT diets, suggests that laying performances are OCT dose dependent [12]. The higher egg production by hens fed with 30 mg/kg OCT relative to those provided with 10 mg/kg of OCT confirms this notion. These results suggest that 20 or 30 mg/kg of OCT supplementation in laying hens' diets can increase egg production.

Egg quality

The effect of dietary supplementation OCT on the egg quality of laying hens is presented in Table 3. Hens fed with a supplemental 30 mg/kg OCT showed increased ($p < 0.05$) egg albumen height and Haugh unit compared to those fed with the control diet. No significant differences in eggshell thickness were observed between the OCT-added and control diet groups. Long et al. [10] and Peng et al. [12] obtained similar results by supplementing the diet of hens with 9 to 27 mg/kg OCT. With the growing concern of human food safety, egg quality is becoming the most important

Table 2. Effect of dietary supplementation of octacosanol (OCT) extracted from triticale sprouts on egg laying performance of laying hens

OCT (mg/kg)	Egg production (%)	Feed intake (g)	Egg weight (g)	Feed conversion ratio
0	87.5^{c}	111	62.7	2.03
10	88.6^{bc}	111	63.0	1.98
20	90.2^{ab}	113	63.5	1.97
30	91.7^{a}	112	61.7	1.98
SEM	0.51	0.27	0.27	0.01
p-value	0.01	0.06	0.11	0.26

$^{a-c}$Means in each column with no common superscript differ significantly at $p < 0.05$.

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Table 3. Effect of dietary supplementation of octacosanol (OCT) extracted from triticale sprouts on egg quality of laying hens

OCT (mg/kg)	Albumen height (mm)	Haugh unit	Eggshell thickness (mm)	OCT in yolk (μg/g)
0	6.33[b]	79.7[b]	0.370	83.9[b]
10	6.69[ab]	81.1[ab]	0.389	145.0[ab]
20	6.71[ab]	81.6[ab]	0.396	166.2[a]
30	7.07[a]	83.3[a]	0.389	174.1[a]
SEM	0.19	0.53	0.004	8.95
p-value	0.04	0.02	0.18	0.01

[a,b]Means in each column with no common superscript differ significantly at $p < 0.05$.

aspect of the laying hen industry. Our results suggest that OCT extracted from triticale sprouts improved the internal quality of eggs by increasing albumen height.

OCT concentration in the egg yolk of hens fed with 20 and 30 mg/kg OCT in their diets was higher ($p < 0.05$) than in the yolk of hens fed with the control diet. Although OCT is hydrophobic, lipophilic, and has low bioavailability [19], oral administration of 100 mg/kg of policosanol (62% OCT) showed 510 ng/ml OCT in the plasma concentration in rats [20]. As fat-soluble substances, lipids including fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and carotenoids, are transferred from hens in the yolk of their eggs [21]. To our knowledge, there are no studies on the transfer of dietary fatty alcohol including OCT from chickens to eggs. However, our results might support earlier studies, whose findings showed that hens fed a diet with supplementary lipid components (α-tocopherol, isoflavone, and carotenoid) transferred the lipids into their eggs' yolk [22–24]. Furthermore, our results may be related to the transfer of dietary omega fatty acids into the egg yolk of laying hens [25]. OCT is known as a beneficial component of functional food due to its ability to lower cholesterol, as well as its anti-aggregative, cytoprotective, and ergogenic effects in the human body [2]. Accordingly, our results show that eggs from laying hens fed a diet supplemented with OCT are nutritionally superior to non-OCT fed hen eggs.

Serum biochemical analysis

Table 4 shows the effect of dietary supplementation of OCT on the blood chemical parameters of laying hens. Serum concentrations of triglyceride and cholesterol of hens fed with 20 and 30 mg/kg of supplementary OCT were significantly ($p < 0.05$) lower compared to those fed the control diet. Our results are consistent with previous reports that OCT supplementation lowers cholesterol and triglyceride concentrations in the blood serum of laying hens [12,18]. The reason for the reduction of cholesterol and triglycerides via supplementary OCT in laying hens is not well-understood, however it may be linked to OCT's capacity to regulate enzymatic activity in the lipid metabolism [26].

HDL cholesterol levels were higher ($p < 0.05$) in the group fed with 30 mg/kg of OCT compared to the control group, in contrast to other studies [12] in which OCT extracted from rice bran did not affect HDL cholesterol levels. It is reasonable to suspect that the higher level of OCT (30 mg/kg) supplementation compared to previous experiments, that used 27 mg/kg of diet-supplemented OCT, may be the reason for this discrepancy. In addition, the conflicting HDL cholesterol results may be explained by the different plant cultivars (triticale sprout or rice bran) used to extract the OCT.

The serum concentration of IL-2 in the supplemental 30 mg/kg OCT diet-fed group was significantly ($p < 0.05$) higher compared to the 0 and 10 mg/kg OCT diet-fed groups. However,

Table 4. Effect of dietary supplementation of octacosanol (OCT) extracted from triticale sprouts on blood chemical parameters of laying hens

OCT (mg/kg)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL cholesterol (mg/dL)	IL-2 (pg/mL)	IL-6 (pg/mL)
0	2,065[a]	163[a]	11.3[b]	43.3[b]	22.8
10	1,982[a]	155[ab]	12.8[ab]	62.8[b]	23.4
20	1,609[b]	134[bc]	12.5[ab]	78.7[ab]	27.7
30	1,600[b]	125[c]	13.1[a]	104.5[a]	26.9
SEM	52.14	5.33	0.24	7.14	1.33
p-value	0.01	0.03	0.03	0.01	0.49

[a–c]Means in each column with no common superscript differ significantly at $p < 0.05$.

HDL, high-density lipoprotein; IL, interleukin.

there was no effect of dietary supplementation of OCT on IL-6 concentration in the blood serum of laying hens. OCT's influence on IL-2 in our experiment is supported by de Oliveira et al. [3] who showed that OCT from the leaves of S. grisea has anti-nociceptive and anti-inflammatory properties. Guo et al. [15] demonstrated that OCT could significantly enhance the health condition of mice, and Long et al. [9] observed that dietary OCT reduced the occurrence of diarrhea in weaning piglets. Moreover, Kabir [8] reported that a natural mixture of high molecular weight alcohols, similar to OCT, showed increased anti-inflammatory activity effectively reduced chronic and acute inflammation. ILs, proteins belonging to the cytokines family, regulate inflammatory and immune responses, and increase in response to pathogens and other antigens [27]. IL-2 stimulates the activation of macrophages and cytotoxic lymphocytes [28,29]. Therefore, the higher IL-2 level induced by the supplemental OCT diet revealed that OCT improves anti-inflammatory and immune responses in laying hens.

CONCLUSION

The diet supplemented with OCT extracted from triticale sprouts increased egg production, and improved albumen height, Haugh unit, and OCT concentration in the eggs of laying hens. Hens fed with dietary OCT had higher serum concentrations of HDL cholesterol and IL-2, but reduced cholesterol and triglycerides. These positive effects were dose-dependent and the optimum level of OCT supplementation was approximately 20 to 30 mg/kg in the basal diet.

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