



Effects of Octacosanol Extracted from Rice Bran on the Laying Performance, Egg Quality and Blood Metabolites of Laying Hens

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ABSTRACT: A 42-d study with 384 Hy-line brown laying hens was conducted to assess the effects of dietary octacosanol supplementation on laying performance, egg quality and blood metabolites of laying hens. Hens were randomly allocated into 4 dietary groups of 8 cages each, which were fed basal diet supplemented with 0 (Control), 9 (OCT9), 18 (OCT18), and 27 (OCT27) mg/kg diet of octacosanol isolated from rice bran, respectively. The experiment was conducted in an environmental controlled house and hens were fed twice daily for *ad libitum* intake. Laying performance was determined over the 42-d period, and egg quality as well as blood metabolites were estimated on d 21 and d 42. Diets in OCT18 and OCT27 increased ($p < 0.05$) laying rate, egg weight, egg mass, egg albumen height, Haugh unit and eggshell strength on d 42, but decreased ($p < 0.05$) feed conversion rate and levels of total cholesterol, triglyceride and low density lipoprotein cholesterol in the serum as compared to those of Control. Feed intake, yolk color, yolk diameter, eggshell thickness and high density lipoprotein cholesterol were similar ($p > 0.05$) among treatments. Results demonstrate that supplementing 18 to 27 mg/kg diet of rice bran octacosanol can improve laying rate and egg quality and reduce blood lipid of laying hens. (**Key Words:** Octacosanol, Laying Performance, Egg Quality, Blood Metabolites, Laying Hens)

INTRODUCTION

Octacosanols are high molecular weight aliphatic alcohols that are main constituents of natural wax products such as beeswax, sugarcane wax and rice bran (Taylor et al., 2003). It has been reported that octacosanols affected lipid metabolism and enhanced stamina and energy (Kabir and Kimura, 1994; Taylor et al., 2003; Chen et al., 2007). In particular, their cholesterol-lowering effect, cytoprotective use, and ergogenic properties have been extensively investigated (Saint and McNaughton, 1986; Arruzazabala et al., 1994; Carbajal et al., 1995). Recently, an octacosanol isolated from rice bran has been reported to improve growth performance, enhance immunity and anti-oxidant status, and significantly reduce diarrhea of weanling piglets (Long

et al., 2015a) as well as promote the secretion of growth hormone and up-regulate the gene expressions of glucose transporter protein (Long et al., 2015b). Moreover, octacosanol has been shown to be very safe to animal with the median lethal dose (LD_{50}) to rats being 18,000 mg/kg body weight (BW) which is greater than that 3,000 mg/kg BW of sodium chloride (Pons et al., 1993). Octacosanols are currently used in health food, cosmetic and pharmaceutical industries. However, their potentials as natural feed additive have not been fully explored. Particularly, there is little information on effects of octacosanols on poultry productive performance. The objective of this study was to evaluate the effects of rice bran octacosanol on the laying performance, egg quality and blood metabolites of laying hens.

MATERIALS AND METHODS

Preparation of octacosanol by high vacuum distillation method

Octacosanol used in this study was obtained from a

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crude octacosanol extract of rice bran wax (Huzhou Shengtao Biological Co., Ltd., Zhejiang, China). The crude extracts contained approximately 13.6% (dry matter basis) octacosanol, and was processed further by high vacuum distillation (HVD) method (Long, 2014) to 53.7% of octacosanol in the product.

Animals, dietary treatments, and experimental design

Three hundreds and eighty four healthy laying hens (Hy-Line Brown, 25-week) with average initial BW of 1.03 ± 0.10 kg and similar laying rates were used. The laying hens were randomly divided into 32 cages (12 hens per cage). The cages (0.9 m×0.6 m×0.4 m) equipped with nipple drinkers and trough feeders were located in an environmentally controlled room (24°C and 16 h of light at 20 lx/d) and raised to 30 cm above the ground. The 32 cages of hens were randomly assigned to 4 dietary treatments (8 cages per treatment) which were basal diet supplemented with 0 (Control), 9 (OCT9), 18 (OCT18), and 27 (OCT27) mg/kg diet of octacosanol. The octacosanol product (purify 53.7%) used in this study was firstly mixed with lime powder at ratio of 1:6 and then incorporated into the basal diet upon feeding at levels of 0, 100, 200, and 300 mg/kg diet to achieve corresponding levels of 0, 9, 18, and 27 mg/kg diet of octacosanol. The lime powder was used as a carrier to uniformly distribute octacosanol into diet. The treatments were arranged as completely randomised design with 8 replications (cages) per treatment. The basal diet (Table 1) was formulated to meet or exceed the nutrient recommendations of NRC (1994), and its nutrient composition was analyzed by the method of AOAC (2001). The experiment consisted of a week of adaptation followed by 6-week data collection periods. Diets and feeding management were the same for both periods. Hens were fed twice daily at 08:00 and 16:00 h for *ad libitum* intake and had free access to water throughout the entire feeding period. The animal protocol for this study was approved by the Animal Welfare Committee of China Agricultural University and was conducted in accordance with the guidelines for experimental animals. The health of hens was closely monitored by technicians and feeding staff.

Determination of laying performance and egg quality

Feed intake was measured daily by the difference between diet offered and residue collected prior to morning feeding. Eggs from individual cages were collected, counted and weighed daily. Laying rate was calculated as number of eggs per hen per day in each cage, and egg mass was calculated as the daily total egg weight dividing by number of hens in each cage. Feed conversion rate (FCR) for the 6-week experimental period was determined weekly as feed intake/egg mass. On d 21 and d 42 of the

Table 1. Composition and nutrient content of basal diet (DM basis)

Items	
Ingredient	
Corn	62.50
Soybean meal	19.33
Cottonseed meal	3.00
Rapeseed meal	3.00
Fish meal	1.50
DL-Methionine	0.12
Limestone	8.30
CaHPO ₄	1.00
NaCl	0.25
Premix ¹	1.00
Total	100.00
Nutrient concentration	
Metabolisable energy (MJ/kg)	11.09
Crude protein (%)	16.55
Calcium (%)	3.30
Total phosphorus (%)	0.56
Available phosphorus (%)	0.37
Lysine (%)	0.75
Methionine (%)	0.39
Methionine+cysteine (%)	0.68
Threonine (%)	0.65
Tryptophan (%)	0.21
Arginine (%)	1.05

¹ Premix provides the following per kilogram of the basal diet which contained: vitamin A, 3,600 µg; vitamin D₃, 50 mg; vitamin K₃, 31 mg; vitamin E, 4.6 mg; vitamin B₁₂, 6 mg; d-pantothenic acid, 5 mg; folic acid, 0.1 mg; niacin, 7 mg; Cu (as CuSO₄•5H₂O), 8 mg; Zn (as ZnO), 40 mg; Fe (as FeSO₄•H₂O), 70 mg; Mn (as MnSO₄), 30 mg; I (as KI), 0.175 mg; Se (as Na₂SeO₃•5H₂O), 0.075 mg.

experiment, five eggs were randomly picked out from each cage and individually measured for egg weight, yolk diameter and albumen height with an egg analyzer (EA-01, ORKA, Ramat HaSharon, Israel), for eggshell thickness at three different points in the middle part of the egg using a dial gauge micrometer (FHK-P1, FHK, Tokyo, Japan), eggshell strength using an Eggshell Strength Tester (EFR-01, ORKA, Israel), and yolk color using method of the National Egg Products Association (NEPA) values according to Kahlenberg (1949). The Haugh unit (HU) score for each individual egg was calculated using following equation (Silversides and Scott, 2001):

$$HU = 100 \times \text{Log}_{10} (h - 1.7w^{0.37} + 7.6)$$

Where h is the albumen height (mm) and w is the weight of egg (g).

Determination of serum metabolites

Blood samples were collected from wing vein of

Table 2. Effects of dietary supplementation of octacosanol isolated from rice bran on laying performance of laying hens

Items	Treatments ¹			
	Control	OCT9	OCT18	OCT27
Feed intake (g/hen/d)	113.04±2.56	112.57±2.89	109.90±1.21	111.06±1.75
Laying rate (%)	82.31±0.72 ^b	83.90±0.91 ^b	88.14±0.98 ^a	87.63±0.83 ^a
Egg weight (g/egg)	60.45±0.55 ^b	61.07±0.48 ^b	61.56±0.89 ^a	61.62±0.53 ^a
Egg mass (g/hen/d)	49.76±0.46 ^b	51.24±0.52 ^b	54.26±0.55 ^a	54.00±0.64 ^a
Feed conversion rate (g/g)	2.27±0.13 ^b	2.20±0.09 ^b	2.02±0.17 ^a	2.06±0.04 ^a

¹ Control, basal diet; OCT9, basal diet supplemented with 9 mg/kg diet of octacosanol; OCT18, basal diet supplemented with 18 mg/kg diet of octacosanol; OCT27, basal diet supplemented with 27 mg/kg diet of octacosanol.

^{a, b} Mean values within the same row not sharing a common superscript are statistically different at $p < 0.05$.

randomly selected eight hens (one per cage) in each treatment using Vacutainer tubes (BD Franklin Lakes, NJ, USA) before morning feeding on d 21 and d 42 of the experiment. The blood samples were kept at room temperature for 20 min, followed by centrifugation at $8,000 \times g$ for 10 min and the resultant serum was stored at -80°C until analysis. The serum samples were analyzed for total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLC) and high density lipoprotein cholesterol (HDLC) using corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to manufacturer recommended procedures.

Statistical analysis

All data were analyzed statistically by one-way analysis of variance using SPSS Statistics Base 17.0 (SPSS Inc., 2007) with treatment as main effect and cage as statistical unit and were expressed as mean±standard error of the mean. Data obtained at d 21 and d 42 were analyzed separately. Differences between treatments were tested

using Duncan's multiple range test system and the significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of octacosanol on laying performance and egg quality

Hens had similar feed intake over the entire experimental period regardless of the treatments (Table 2). However, hens fed OCT18 and OCT27 diet had greater ($p < 0.05$) laying rate, individual egg weight and egg mass but lower ($p < 0.05$) FCR than those fed OCT9 and Control diet. In addition, there was no difference ($p > 0.05$) in the laying rate and egg weight between OCT18 and OCT27 groups. Eggs obtained on d 21 of the experiment had similar ($p > 0.05$) albumen height, HU, yolk color, yolk diameter, and eggshell thickness, but eggs in OCT18 and OCT27 treatment had greater ($p < 0.05$) eggshell strength compared to that of Control and OCT8 treatment (Table 3). At d 42, eggs from all octacosanol treatments had higher

Table 3. Effects of dietary supplementation of octacosanol isolated from rice bran on egg quality of laying hens

Items	Control	OCT9	OCT18	OCT27
Albumen height (mm)				
Day 21	6.61±0.14	6.68±0.35	6.70±0.22	6.69±0.30
Day 42	6.59±0.52 ^b	6.74±0.63 ^a	6.79±0.31 ^a	6.76±0.46 ^a
Haugh unit				
Day 21	80.92±3.17	81.09±4.25	81.02±2.16	80.96±1.47
Day 42	80.69±3.21 ^b	81.49±2.19 ^a	81.63±1.98 ^a	81.42±2.03 ^a
Yolk color				
Day 21	8.25±0.39	8.35±0.44	8.40±0.17	8.50±0.32
Day 42	8.40±0.42	8.40±0.31	8.50±0.29	8.60±0.28
Yolk diameter (mm)				
Day 21	8.89±0.21	9.09±0.08	9.20±0.08	9.01±0.09
Day 42	8.90±0.24	9.07±0.35	9.24±0.12	9.11±0.20
Eggshell strength (kg/cm ²)				
Day 21	3.62±0.09 ^b	3.72±0.13 ^{ab}	3.84±0.29 ^a	3.88±0.13 ^a
Day 42	3.87±0.29 ^b	3.98±0.62 ^b	4.25±0.36 ^a	4.22±0.25 ^a
Eggshell thickness (mm)				
Day 21	0.41±0.31	0.42±0.09	0.43±0.14	0.41±0.23
Day 42	0.42±0.05	0.41±0.08	0.42±0.16	0.43±0.47

^{a, b} Mean values within the same row not sharing a common superscript are statistically different at $p < 0.05$.

($p < 0.05$) albumen height and HU as compared with that of Control, and eggshell strength of eggs from OCT18 and OCT27 were greater ($p < 0.05$) than that from Control and OCT9 treatments. Similar to that found on d 21, there was no difference among treatments in yolk color, yolk diameter or eggshell thickness on d 42.

The similar feed intake of laying hens across all treatments indicated that dietary supplementation of octacosanol up to 27 mg/kg diet did not affect feed intake. On the other hand, the increased laying rate and egg weight for hens fed OCT18 and OCT27 indicated that octacosanol supplemented at the levels of 18 and 27 mg/kg diet increased egg production. This, comparing with the observation that hens fed OCT9 (9 mg/kg diet of octacosanol) had similar laying rate and egg weight to that of Control, suggests that laying performance responded to the octacosanol in a dose response manner. Information about effect of rice bran octacosanol on laying performance of laying hens is lacking, but dietary concentration of rice bran up to 10% has been shown no adverse effect on laying performance (Samli et al., 2006). Long et al. (2015a) reported that supplementing 8 mg/kg diet of the same octacosanol increased growth rate and improved feed efficiency of piglet. The same growth-promoting effect of octacosanol was also reported by Xiang et al. (2012) in rate. All these results suggested that octacosanol has the potential as natural feed additive to laying hens and piglets although the mechanisms by which octacosanols improve animal performance are unknown. Limited studies showed that octacosanol could stimulate fat metabolism, improve energy efficiency and increase protein synthesis (Kato et al., 1995; Castano et al., 2000; Singh et al., 2006). Long et al. (2015b) demonstrated that 8 mg/kg diet of octacosanol greatly promoted secretion of growth hormone of weaning piglets. Furthermore, Yang (2012) found that octacosanol increased the gene expressions of glucose transporter-4, glutamine synthetase and adenosine monophosphate and activated protein kinase thereby improved energy metabolism and increased protein synthesis in rats. All of these could have positive effect on animal productive performance and therefore further research in this area is needed.

HU is a measurement of the internal quality of an egg, which is positively correlated to its weight and albumin height (Loh et al., 2014). Higher albumin height and HU values indicate better quality and longer shelf life of eggs (Dai et al., 2012). To authors' knowledge, there is no information about the effects of octacosanol on egg quality of laying hens. This study demonstrated that octacosanol supplemented at the dietary concentrations of 9 to 27 mg/kg diet significantly increased albumen height and HU of eggs at d 42, but not at 21 d of the experiment. This suggests that octacosanol supplementation improved the egg internal

quality via increasing albumin height. The fact that this egg quality-promoting effect octacosanol was not observed for eggs collected at 21 d of the experiment may indicate that there is lag time between initiation of octacosanol supplementation and animal productive response to octacosanol. Kabir and Kimura (1993) reported that absorption of octacosanol is very low and mainly excreted through feces. Moreover, the same authors suggested that octacosanol may be partly oxidized and degraded to fatty acids through beta-oxidation. This suggests that it may need time for octacosanol to be accumulated to sufficient concentration in the body to exert its effects on HU. This study showed that supplementing 18 to 27 mg octacosanol/kg diet did not affect eggshell thickness but increased eggshell strength, which suggests that external quality of egg was increased. Eggshell quality is closely related to calcium metabolism in laying hens (Gordon and Roland, 1998). It is not known whether octacosanol has the direct effect on calcium absorption/metabolism or through other mechanism. However, it has been reported that dietary octacosanol supplementation at the level of 8 mg/kg diet significantly increased growth hormone concentration in the blood of piglets (Long et al., 2015b). Growth hormone has been reported to have positive effect on calcium absorption and retention in animal (Braithwaite, 1975).

Effect of octacosanol on blood fat metabolism related metabolites

Concentration of TC in the serum was lower ($p < 0.05$) for hens fed OCT18 and OCT27 diets than for hens fed Control and OCT9 diets and TC concentration of OCT9 was also lower ($p < 0.05$) than that of Control on d 21 and d 42 of the experiment (Table 4). Similarly, OCT18 and OCT27 hens had lower ($p < 0.05$) serum LDLC than Control

Table 4. Effects of dietary supplementation of octacosanol isolated from rice bran on serum metabolites of laying hens

Items (mmol/L)	Control	OCT9	OCT18	OCT27
TC				
Day 21	5.39±1.52 ^a	4.28±1.50 ^b	3.24±0.95 ^c	3.18±0.76 ^c
Day 42	4.82±1.78 ^a	3.60±1.59 ^b	2.89±1.37 ^c	2.76±0.98 ^c
TG				
Day 21	4.25±0.92	3.58±0.70	3.56±0.83	3.47±0.65
Day 42	4.75±0.96 ^a	3.41±0.60 ^b	3.26±0.58 ^b	3.21±0.73 ^b
HDLC				
Day 21	0.97±0.20	1.02±0.15	1.04±0.28	0.99±0.31
Day 42	1.03±0.18	1.06±0.14	1.10±0.05	1.05±0.21
LDLC				
Day 21	1.52±0.37 ^a	1.37±0.25 ^a	0.74±0.16 ^b	0.68±0.20 ^b
Day 42	2.14±0.43 ^a	2.09±0.23 ^a	1.07±0.30 ^b	1.12±0.25 ^b

TC, total cholesterol; TG, triglyceride; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol.

^{a,b,c} Mean values within the same row not sharing a common superscript are statistically different at $p < 0.05$.

and OCT9 hens, but TC concentration of OCT9 was similar ($p>0.05$) to that of Control on both dates. In contrast, all hens irrespective of the treatments had similar ($p>0.05$) HDLC concentration in the serum on both dates. All octacosanol diets fed hens had lower ($p<0.05$) TG than that control hens on d 42, but this difference was not observed on d 21 of the experiment.

Previous researches have shown that dietary supplementation of octacosanol reduced blood cholesterol and TG in various test models (Hernandez et al., 1992; Kato et al., 1995; Castano et al., 2000; 2002; Taylor et al., 2003). In this study, the similar blood cholesterol and TG lowering effect of dietary octacosanol was also observed in laying hens. The exact mechanism by which supplementary octacosanol reduced cholesterol and TG in laying hens is not known but may partly attributable to its ability to regulate some enzymatic activities in lipid metabolism (Kato et al., 1995; Mas et al., 2004). However, whether cholesterol content in egg yolk is affected by the dietary octacosanol is not known and is of interest to be further studied in terms of its high relevance to human health.

CONCLUSION

Supplementation of octacosanol up to 27 mg/kg diet increased laying rate, egg weight and improved egg quality, but reduced serum TC and total fat concentrations. The responses of these variables are dose-dependent and optimum supplementation level is about 18 mg/kg diet under the conditions of this study. The information from this preliminary study suggest that rice bran octacosanol could be a potential natural feed additive to laying hens but further study is needed to elucidate the mechanisms by which octacosanol improve laying performance of laying hens.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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