Sittiya J, et al., J Food Sci Nutr 2017, 3: 016
DOI: 10.24966/FSN-1076/100016

HERALD

HSOA Journal of Food Science and Nutrition

Research Article

Preliminary Study: Egg Production Performance, Egg Quality and Blood Plasma Cholesterol Concentration in Laying Hens Fed Dietary Dried Fermented Ginger and/or Fermented Corncob Powder

Sittiya Janjira[1]*, Khonyoung Duddoa[1], Kohsho Yamauchi K[2] and Koh-en Yamauchi[1]*****

[1]*Laboratory of Animal Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa-ken 761-0795, Japan*

[2]*Miyazaki Midori Pharms., Inc. 945 Hieda, Akae, Miyazaki-city 880-0912, Japan*

**Present address: Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, 76120, Thailand*

***Present address: Faculty of Animal Science and Technology, Maejo University, Phrae Campus 54140, Thailan*

Abstract

Dried Fermented Ginger (*Zingiber officinale* Rosc.) (DFG) was made by adding ground ginger to Japanese Mugwort (*Artemisia princeps* Pamp.) Silage juice (JMS) liquid. DFG is known to increase egg production performance of layer chickens and body weight gain in broilers. However, DFG has high production costs. Therefore, to develop a cheaper and more suitable agricultural by-product than the present expensive DFG to improve egg production performance, corncob powder was fermented with waste JMS. This Fermented Corncob Powder (FCP) and/or DFG was/were fed in small amounts to layer hens from 53-61 weeks of age, as well as from 62-71 weeks of age, and the hens' egg production performance and egg quality were compared. At the end of the second phase, Triglyceride (TG), High-Density Lipoprotein cholesterol (HDL), and Low-Density Lipoprotein cholesterol (LDL) concentrations were determined. Egg production performance did not differ among groups. The dietary 50ppm DFG + 250ppm FCP and 500ppm FCP elevated the shell weight, albumen weight and yolk color from 53-61 weeks of age, as well as the albumen weight from 62-71 weeks of age ($P<0.05$). The dietary 500ppm FCP increased blood HDL ($P<0.05$). When the egg mass of the control was expressed as an index of 100, most values for egg production, egg quality and blood TG, HDL and LDL were higher after feeding dietary DFG and FCP; the exception was the values for LDL, which were decreased in the 50ppm DFG and 50ppm DFG + 250ppm FCP groups. These results suggest that even a low concentration of DFG can be used as feed supplement for layer hens when FCP is added, and that even corncobs of low commercial value can also be used as a feed supplement with fermented JMS. Furthermore, DFG and FCP can be used as a functional feed to improve chicken health without inducing an environmental contamination. The current discovery benefits not only the poultry farming industry, due to lowered feed costs, but also human life due to the effective utilization of waste food.

Keywords: Blood chemical; Egg production; Egg quality; Fermented corncob; Fermented ginger

***Corresponding author:** Koh-en Yamauchi, Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, 76120, Thailand, Tel: +81 878913053; E-mail: yamauchi@ag.kagawa-u.ac.jp

Citation: Sittiya J, Khonyoung D, Yamauchi K, Yamauchi K (2017) Preliminary Study: Egg Production Performance, Egg Quality and Blood Plasma Cholesterol Concentration in Laying Hens Fed Dietary Dried Fermented Ginger and/or Fermented Corncob Powder. J Food Sci Nut 3: 016.

Received: February 17, 2017; **Accepted:** April 24, 2017; **Published:** May 08, 2017

Introduction

To protect human health and to prevent environmental contamination, organic food production and animal diets composed of natural feed ingredients have become popular in developed countries to counter the fact that continuous use of dietary chemicals has resulted in drug residues in the animal body [1] and the development of drug-resistant bacteria [2]. In our previous studies, dietary Dried Fermented Ginger (*Zingiber officinale* Rosc.) (DFG), fermented with Japanese Mugwort (*Artemisia princeps* Pamp.) Silage juice (JMS) liquid, increased the production performance of layer chickens [3] and body weight gain in broilers [4] due to the hypertrophy of intestinal villi and epithelial cells. However, this incurs high production costs due to the high purchase price of ginger. Therefore, to lower the production costs of natural supplementation, the use of agricultural products other than ginger is necessary. At the same time, egg quality is the most important factor in consumer acceptance. Many factors can affect egg shell quality and internal egg quality. Numerous nutritional factors have been reported to improve egg quality [5,6].

Corn is one of the most important foods for humans and feed ingredients for animals. Furthermore, corn has been employed in biogas production [7] after 1970 in the USA. Consequently, a by-product of the corn crop, namely the corncob, is produced in very large amounts from corn manufactory and represents an environmental problem. The majority of corncobs are either dumped or burned for fuel without being used effectively. One effective use of corncob agricultural waste has been removal of dyes from artificial textile dye effluent, such as dye adsorption [8] and adsorption of cadmium (II) [9]. As the corncob itself is well known to contain high fiber, it is very difficult to use it as a feed ingredient. In fact, daily body weight gain decreased in pigs with an increased level of corncob supplementation, because the high fiber in the corncob is resistant to the pig's digestive enzymes [10]. However, the fermentation of corncobs using rumen filtrate enhanced the crude protein value and reduced the crude fiber content [11]. Therefore, fermenting corncobs with the remaining JMS after formulating DFG [3] may enable the use of agricultural waste as a supplement in layer hen diets, specifically the effective use of waste corncob and reuse of JMS.

The purpose of this study was to develop a cheap and suitable agricultural by-product comprised as Fermented Corncob Powder (FCP) to improve egg production performance. Layer hens were fed dietary DFG and/or FCP diets from 53-61 weeks of age, as well as from 62-71 weeks of age, and their egg performance and egg quality were compared. At the end of the second phase, Triglyceride (TG), High-Density Lipoprotein cholesterol (HDL), and Low-Density Lipoprotein cholesterol (LDL) concentrations were determined.

Materials and Methods

Preparation of DFG, JMS and FCP

Preparation of the DFG and JMS were described in detail in our previous studies [3,4]. In short, Japanese mugwort plants were harvested and ensiled at room temperature to produce silage juice. The DFG was prepared as follows: ginger by-product was purchased from a local ginger farmer in Chiang Rai in Thailand, then ground, added to the JMS, and kept under anaerobic conditions at room temperature for 4-5 d. The fresh fermented mixture was then dried in a hot air oven at 50°C for 1-2 d. Finally, it was again ground. Regarding the FCP preparation, the corncobs were purchased from a local corn farmer in Chiang Rai in Thailand, sliced, soaked into the remaining JMS after it was used for DFG production, dried, and then milled.

Birds, management and diets

Forty 53-week-old Sonia strain hens were assigned based on their egg production rate and body weight, and placed into 4 groups of 10 birds each as follows: the control group was fed a basal diet (Table 1), and the other groups were fed the basal diet supplemented with DFG (Table 2) at a level of 50ppm (the 50ppm DFG group), with DFG at 50ppm and FCP at 250ppm (the 50ppm DFG + 250ppm FCP group), and with FCP at a level of 500ppm (the 500ppm FCP group). The hens were placed in individual wire cages (533 cm^2 /bird) with individual feed-troughs placed outside at an average environmental temperature of 28°C (with a 16-h photoperiod). This experiment was carried out over two phases: one from 53-61 weeks of age (for 56 days) and the other from 62-71 weeks of age (for 63 days). Feed and water were provided ad libitum during the feeding period.

Performance and egg quality

Egg production was recorded daily and feed consumption was measured weekly throughout the experiment. In both phases, eggs from each group were collected biweekly to measure egg weight, shell-breaking strength, shell thickness, shell weight, albumen weight, yolk weight, yolk color and haugh units. First, egg weight was measured using an electronic digital balance. Shell-breaking strength was measured using an eggshell strength instrument (accuracy: 0.1 kg/cm^2, Fujihira Industry Co., Ltd.). Haugh units and egg yolk color were determined using an egg multi-tester instrument (EMT 7300, Tohoku Rhythm Co. Ltd., Japan). Egg yolk and eggshell were measured using an electronic digital balance. Shell thickness was measured using a dial thickness gauge (Peacock, Tokyo, Japan) at three locations on the egg (air cell, equator and sharp end) after the shell membrane was removed from the shell. The mean of the three values was recorded as shell thickness per egg.

Item	Value
Ingredients	
Maize	610
Milo	10
Soybean meal	160
Rapeseed meal	40
Gluten meal	30
Fish meal	30
Rice bran	10
Animal fat	13
Calcium carbonate	85.7
Dicalcium phosphate	4
Salt	2
Paprika	0.3
Concentrate mixture[1]	5
Nutrient content	
Crude protein	170
Metabolizable energy (MJ/kg)	11.93
Crude fat	30
Crude fiber	60
Crude ash	140
Lysine	9.6
Methionine	7.5
Calcium	31
Phosphorus, available	4.5

Table 1: Composition and nutrient content of the basal diet fed to laying hens (g/kg diet).

[1]Concentrate mixture including (per kg of diet): retinyl acetate, 2106 μg; cholecalciferol, 35 μg; DL-α-tocopherol acetate, 12.5 mg; menadione, 1.5 mg; thiamine, 2.6 mg; riboflavin, 2.7 mg; pyridoxine, 6 mg; cobalamine, 9 μg; biotin, 0.2 mg; folic acid, 0.5 mg; pantothenic acid, 15 mg; niacin, 22 mg; choline, 1000 mg; iodine, 1.05 mg; manganese, 50 mg; iron, 160 mg; zinc, 70 mg; copper, 8 mg.

Blood collection

Blood samples were collected from 7 hens per group at the end of phase 2 of the feeding experiment. Hens were bled from the wing vein using heparin as an anti-clotting agent. Blood samples were centrifuged at 2,000 × g for 10 min, and plasma samples were stored at -20°C until assayed. Triglyceride (TG), High-Density Lipoprotein cholesterol (HDL), and Low-Density Lipoprotein cholesterol (LDL)

Items	(%)
Dry matter	87.44
Crude protein	7.29
Crude fat	6.51
Crude fiber	22.1
Crude ash	13.4
Calcium	0.46
Phosphorus	0.84
Gross energy (kcal/kg)*	3,908

Table 2: Proximate composition of Dried Fermented Ginger (DFG).

*Analyzed according to the AOAC [12].

All parameters of DFG samples were determined in triplicate [3,4].

concentrations were determined using a commercial HDL and LDL/VLDL cholesterol assay kit (Cells Biolabs, Inc., USA).

Statistical Analysis

The data from the experimental groups were statistically analyzed using one-way Analysis Of Variance (ANOVA) in the SPSS statistical software package (version 10.0 for Windows; SPSS, Inc., Chicago, IL). Significant differences among the treatments were determined with Duncan's multiple range test. Statistical significance was accepted at $P<0.05$.

The experiment was performed in accordance with the guidelines and rules of care and use of laboratory animals in experimentation established by Kagawa University in Japan. The experimental procedure was approved by the Animal Research Committee of Kagawa University, Japan (authorization number: 28).

Results

Feed intake, hen-day egg production and egg mass did not differ among the groups during phase 1 (53 to 61 weeks of age) or 2 (62 to 71 weeks of age) (Table 3). Compared with the control, during phase 1, shell-breaking strength, shell thickness, shell weight, albumen weight, yolk weight, yolk color and haugh unit did not differ in the 50ppm DFG group, and the shell-breaking strength, shell thickness, yolk weight and haugh unit did not differ in the 50ppm DFG + 250ppm FCP and 500ppm FCP groups (Table 4). The shell weight, albumen weight and yolk color increased in the 50ppm DFG + 250ppm FCP and 500ppm FCP groups ($P<0.05$); these values for the shell weight, albumen weight and yolk color were similar between the 50ppm DFG + 250ppm FCP and 500ppm FCP groups. During phase 2, these values did not differ among the groups, except that the albumen weights were heavier in the 50ppm DFG + 250ppm FCP group than in the 500ppm FCP group ($P<0.05$).

The blood plasma concentration of TG and LDL, showing the ratio to the index of 100 in the control, in laying hens during phase 2 (62-71 weeks of age) did not differ among the groups, but the HDL index was higher in 500ppm FCP group than that of the control ($P<0.05$); the concentration of HDL did not differ among the experimental groups (Table 5).

Discussion

In hen breeding, it is important to raise production performance effectively while keeping feed costs low. In our previous study using hens fed dietary 10,000-50,000ppm (1-5%) DFG diets, an 8-9% higher egg production rate, a 6-12% higher egg mass production (g/bird/day), and a 6-7% higher egg white weight were observed [3]. However, as the high purchase price of ginger was not cost-effective for businesses, it was necessary to develop another cheaper yet suitable feed ingredient fermented with JMS liquid to substitute for ginger.

The majority of corncobs remain unused as agricultural waste in fields and factories, and only half of these corncob resources might be used as compost [13], in mushroom cultivation [14,15], and as a feed ingredient for swamp buffalo [16] and cows [17].

Consequently, 24,500 and 9,000 tons of corncob are dumped or burnt in the Chiang Rai and Chiang Mai regions respectively, without be used effectively. Such corncob residues result in environmental contamination. As the transaction price (15 USD per ton) of corncob is cheaper than ginger, corncob was tried as a candidate source of a cheap agricultural by-product that could be used as a substitute for expensive ginger. The present low dietary level of 50ppm DFG did not improve egg production performance and egg quality, as in the previous hens fed dietary 10,000-50,000ppm DFG diets [3]. When the egg mass of the control was expressed as an index of 100, the egg mass index in phase 1 was better, by adding 250ppm FCP to 50ppm DFG (102, 50ppm DFG + 250ppm FCP group), than the control, and best in the 500ppm FCP group (106). The egg mass index in phase 2 was 1007 in the 50ppm DFG + 250ppm FCP group and 105 in the 500ppm FCP group. The egg quality ratio of the 50ppm DFG + 250ppm FCP and 500ppm FCP groups was higher than 100 in the control during phases 1 and 2, except for the shell-breaking strength and shell thickness during phase 1 and the yolk color during phase 2; the Haugh unit of the 50ppm DFG + 250ppm FCP group in phase 2 was similar to that of the control. These results suggest that levels of 50ppm DFG + 250ppm FCP and 500ppm FCP can improve egg performance. The DFG is composed of many kinds of nutrients (Table 2), and the JMS contains natural microorganisms, such as lactic acid bacteria, yeast fungus, photosynthetic bacteria, ray fungus, hyperthermal bacteria, and Aspergillus and *Bacillus subtilis* [3]. These substances might become packed in the pores of the corncob, arrive in the intestine without receiving the effects of stomach acid, flow out from the pores into the intestinal lumen, and thus might have an effect on intestinal function, resulting in improved egg performance in the 50ppm DFG + 250ppm FCP and 500ppm FCP groups. The phenomenon of substances packed by charcoal pores was demonstrated using electron microscopic [18].

In blood plasma concentration, when the levels of TG, HDL and LDL of the control were expressed as an index of 100, TG and HDL were higher in the experimental groups than in the control ($P<0.05$). The ratio of LDL was lower in the 50ppm DFG and 50ppm DFG + 250ppm FCP groups. The lipid for egg yolk is well known to be synthesized in the liver, and the dietary conjugated linoleic acid was incorporated in TG in the egg yolk in White Leghorn hens [19]. The present experimental groups showed elevated TG values, suggesting that DFG and FCP can stimulate egg production as shown the present egg mass, which is higher than the control. The HDL (good cholesterol) removes cholesterol from foam cells in the medial smooth muscles of the artery by inhibiting the oxidation of LDL, opposing atherosclerosis directly [20]. The present experimental groups showed increased HDL values, suggesting that DFG and FCP can elevate blood HDL. On the contrary, in cases where the LDL (bad cholesterol) is high, the LDL accumulates in the artery wall, and is oxidized and taken up by foam cells in the medial smooth muscles of the artery, leading to the development and progression of atherosclerosis [20]. Polyunsaturated fatty acids, in place of saturated fatty acids or carbohydrates, lowered the plasma LDL concentration [21]. The present LDL was also decreased in the 50ppm DFG and 50ppm DFG + 250ppm FCP groups, suggesting that a low level of DFG and FCP can decrease blood LDL. These blood results also indicate that the DFG and FCP are good feed ingredients for chicken health.

Conclusion

Taking every factor into consideration, dietary 50ppm DFG + 250ppm FCP and 500ppm FCP could improve egg production and blood TG, HDL and LDL levels. These results suggest that even low concentrations of DFG can be used as feed supplements for layer hens by adding FCP, and that even corncob of low commercial value can also be used as a feed supplement by fermenting with JMS. Furthermore, the DFG and FCP can be used as a functional feed that

Items	Level of substance (ppm)				
	Control	50ppm DFG	50ppm DFG + 250ppm FCP	500ppm FCP	p-value
Phase 1 (53 to 61 wk of age)					
Feed intake, g/day	87.47±11.62	85.85±10.39	85.30±13.67	89.71±10.66	0.83
Ratio for 100 index of control	100	98	98	103	
Hen-day egg production, %	91.42±9.98	87.14±10.54	88.88±9.52	92.85±7.52	0.54
Ratio for 100 index of control	100	95	97	102	
Egg mass, g/d	51.85±5.92	49.02±6.59	52.76±6.56	55.08±4.52	0.17
Ratio for 100 index of control	100	95	102	106	
Phase 2 (62 to 71 wk of age)					
Feed intake, g/day	113.62±11.92	108.05±8.14	109.57±11.41	108.94±10.32	0.65
Ratio for 100 index of control	100	95	96	96	
Hen-day egg production, %	94.28±7.37	94.28±7.37	95.71±6.90	95.71±6.90	0.94
Ratio for 100 index of control	100	100	102	102	
Egg mass, g/d	59.24±3.48	60.05±6.84	63.58±4.60	62.32±6.16	0.27
Ratio for 100 index of control	100	101	107	105	

Table 3: Effects of dietary basal diet with 0 (Control), 50ppm DFG, 50ppm DFG + 250ppm FCP and 500ppm FCP on egg production performance and ratio to 100 index of the control during phases 1 (53-61 weeks of age) and 2 (62-71 weeks of age) (Mean ± SE, n = 10).
DFG: Dried Fermented Ginger; FCP: Fermented Corncob Powder

Items	Level of substance (ppm)				
	Control	50ppm DFG	50ppm DFG +250ppm FCP	500ppm FCP	p-value
Phase 1 (53 to 61 wk of age)					
Shell-breaking strength, kg/cm^2	4.07±0.50	3.59±0.38	4.05±0.81	3.64±0.46	0.14
Ratio for 100 index of control	100	88	100	89	
Shell thickness, mm	0.36±0.01	0.36±0.02	0.36±0.02	0.36±0.02	0.89
Ratio for 100 index of control	100	100	100	100	
Shell weight, g	5.70±0.34^b	5.76±0.54^b	6.26±0.35^a	6.21±0.35^a	0.01
Ratio for 100 index of control	100	101	110	109	
Albumen weight, g	34.55±2.70^c	34.98±2.87^{bc}	38.04±4.31^{ab}	38.47±2.67^a	0.02
Ratio for 100 index of control	100	101	110	111	
Yolk weight, g	15.26±1.41	15.94±1.42	15.87±1.06	16.72±0.58	0.1
Ratio for 100 index of control	100	104	104	110	
Yolk color	11.62±0.51^b	11.60±0.51^b	12.22±0.66^a	12.00±0.00^{ab}	0.03
Ratio for 100 index of control	100	100	105	103	
Haugh unit	84.35±7.37	80.54±5.31	86.44±12.09	89.40±9.38	0.19
Ratio for 100 index of control	100	95	102	106	
Phase 2 (62 to 71 wk of age)					
Shell-breaking strength, kg/cm^2	3.63±1.02	3.50±0.70	3.86±0.52	4.26±0.48	0.12
Ratio for 100 index of control	100	96	106	117	
Shell thickness, mm	0.38±0.02	0.37±0.03	0.39±0.02	0.39±0.02	0.46
Ratio for 100 index of control	100	97	103	103	
Shell weight, g	6.70±0.65	6.61±0.63	7.09±0.39	7.17±0.52	0.08
Ratio for 100 index of control	100	99	106	107	
Albumen weight, g	38.40±1.82^b	39.48±3.93^{ab}	42.17±2.27^a	41.82±2.22^a	0.01
Ratio for 100 index of control	100	103	110	109	
Yolk weight, g	16.76±1.02	17.75±1.49	18.08±1.18	17.72±0.97	0.13
Ratio for 100 index of control	100	106	108	106	
Yolk color	12.33±0.50	12.30±0.48	12.10±0.56	12.28±0.48	0.74
Ratio for 100 index of control	100	100	98	100	
Haugh unit	85.03±5.47	84.47±4.83	84.96±3.06	86.70±8.62	0.88
Ratio for 100 index of control	100	99	100	102	

Table 4: Effects of dietary basal diet with 0 (Control), 50ppm DFG, 50ppm DFG + 250ppm FCP and 500ppm FCP on egg quality and ratio for 100 index of control during phase 1 (53-61 weeks of age) and 2 (62-71 weeks of age) (Mean ± SE, n = 10).
$^{a-c}$Means within a row with different superscripts are significantly different (P<0.05)
DFG: Dried Fermented Ginger; FCP: Fermented Corncob Powder

Items	Level of substance (ppm)				
	Control	**50ppm DFG**	**50ppm DFG +250ppm FCPs**	**500ppm FCP**	**p-value**
TG (mg/dl)					
Ratio for 100 index of control	100	110	163	110	0.14
HDL (mg/dl)					
Ratio for 100 index of control	100	131	130	164	0.03
LDL (mg/dl)					
Ratio for 100 index of control	100	93	83	101	0.86

Table 5: Effects of dietary basal diet with 0 (Control), 50ppm DFG, 50ppm DFG + 250ppm FCP and 500ppm FCP on blood plasma concentration of Triglyceride (TG), High-Density Lipoprotein Cholesterol (HDL), Low-Density Lipoprotein Cholesterol (LDL) showing ratio for 100 index of control in laying hens during 2 (62-71 weeks of age) (Mean ± SE, n = 7).

DFG: Dried Fermented Ginger; FCP: Fermented Corncob Powder

augments chicken health without inducing environmental contamination.

Declaration interest

The authors state no conflict of interest, and are responsible for the content and writing of this article.

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