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Effects of Green Tea Powder Feed Supplement on Performance of Hens in the Late Stage of Laying

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Abstract: The effects of green tea powder on the levels of alpha-tocopherol in egg yolk, egg production and egg quality of laying hens were examined. Twenty 84-week-old laying hens were divided into 4 dietary groups of 5 hens each in the late stages of laying. Four levels of green tea powder (0% = control diet, 1%, 5% and 10%) were fed to the hens for 3 weeks. At the end of 3 weeks, all experimental diets were switched to the control diet (0% green tea powder) for 3 weeks. The first group was fed the controlled diet throughout the experimental period. The egg weight, egg mass and feed intake were the highest for the 0% and 1% green tea powder diets and the lowest for the 5% and 10% diets (P < 0.05). The rate of egg production and the intake of feed decreased significantly with an increase in the intake of green tea powder (P < 0.05). The weights of the hens who received up to 5% green tea powder in their diets decreased. However, the gain in the hens' weight was not influenced by the intake of 1% green tea powder diet. There were no significant differences in the yolk color fan score for the 4 treatment diets. The egg shell strength decreased with increasing green tea powder intake, while the alpha-tocopherol content of egg yolk of the hens fed the experimental diet increased with increasing green tea powder intake. The fatty acid composition of yolk was not affected by the diet. The results suggested that the transfer of alpha-tocopherol from dietary green tea powder to egg yolk was sensitive to the alpha-tocopherol intake and that green tea supplementation would support adequate egg production and egg weight when used up to a level of 1% in the diets of laying hens.

Key words: Green tea, feeding, alpha-tocopherol, laying hen, egg production

Introduction

In addition to various kinds of catechin, vitamins and caffeine, green tea has been noted for having many different physiological effects. To name a few, there have been numerous reports regarding its antioxidant, antiallergen and anti-viral properties, its role in controlling high cholesterol and blood sugar and its ability to prevent cancer (Muramatsu et al., 1986; Mukoyama et al., 1991; Matsumoto et al., 1993; Yoshino et al., 1994; Yamamoto, 2000, 2002). However, many of these reports were confirmed by experiments done on mice or rats. Till date, studies performed on domestic animals and fowls or on the effects of the components of green tea have not investigated in detail. Previously, it was reported that eggs produced by hens fed with enriched green tea powder had low cholesterol in egg yolks (Biswas and Wakita, 2000, Unganbayer et al., 2005). Moreover, it was reported that addition of green tea extract to hot water decreases the serum lipid concentration and the amount of fat in egg yolks (Yamane et al., 1999). Furthermore, vitamin E (as an alpha-tocopherol) is fat soluble and hardly dissolves in the regular green tea water that is consumed by us; however, when the hens consume green tea leaves orally: therefore, it is thought to accumulate within their bodies.

Hence, in this experiment we examined the benefits of

adding green tea leaves in the form of fine powder to hen feed. The effects of which were measured on the egg-laying performance and the concentration of alphatocopherol in egg yolks. In addition, egg weight suppression in the late stage of laying hen is very important and increases the marketability of the eggs. Then, it has been reported that green tea supplementation feeding decreased hens egg weight (Yamane et al., 1999). This study also examined the effects of green tea powder feed on suppression in the egg weight produced in the late stage of hens laying.

Materials and Methods

Experimental diet and feeding: Green tea (Yabukita) powder was provided by the Tokyo Metropolitan Agricultural Experiment Station (Tachikawa, Tokyo). Before adding it to the feed, the leaves were pulverized with a cyclone sample mill (UDY Co.) affixed with a 1.0 mm diameter screen. The green tea used in this experiment contained: 29.5 mg/100 g of alphatocopherol, 12.4% epicatechin and 2.2% caffeine.

The experiment was conducted in twenty 84-week-old egg-producing hens, each confined to their own individual cages and feeders. The experiment period lasted 42 days. Twenty hens were divided into 4 test groups. In the first 21 days, they received a different percentage of additive green tea powder feed in their

feeder for each: 0% (the control diet) 1%, 5% and 10%. In the next 21 days, all birds were fed the control diet. Water was provided ad libitum. Each bird was given 120g of feed daily. Fresh feed was provided on a daily base irrespective of the leftover feed. The leftover feed was retained only if it was not spoilt by moisture and was mixed by hands if left in the feeder.

Measurements: We investigated the following factors throughout the experiment: rate of egg production, egg weight, eggs mass, amount of feed consumed and feed efficiency. Every 7th day we weighed the amount of the leftover feed remaining and the weights of each individual hen.

Fifty six eggs were produced between 15-21 days of the experiment. The eggs were collected daily, refrigerated at 5°C and examined the following day. Each egg was weighed and the daily egg count (g/hen per day) and feed efficiency were calculated. The egg yolk color and Haugh Units (HU) were measured using the egg multi tester EMT-5200 (Robotmation Co., Ltd.) and the egg shells were measured by the spectrophotometer CM-500 (MINOLTA) to determine L*(Lightness), a*(Redness) and b*(Yellowness).

Chemical analysis: We measured the alpha-tocopherol content in the egg yolks obtained from eggs laid on (excluding day 0) days 1, 3, 6, 10, 15, 21, 22, 24, 27, 31 and 42. Three egg yolks from each test group were mixed and four mixtures were prepared in equal amounts and their alpha-tocopherol content was measured using HPLC. After saponification with potassium hydroxide, we performed our analysis on egg yolks that did not saponificate by extraction separation with a 1:9 solvent mixture of ethyl acetate and n-hexan. The conditions for HPLC analysis were: ULTRON VX-SIL (Shinwa Chemical Industries, Ltd.) was used as the column, 1000:5 solvent mixture of n-hexan and isopropyl alcohol was used for transport, flux 1.5 mL/min, column temperature 40°C and a fluorescence detector RF-550 (Shimadzu) detected a fluorescent brightness of (Ex. 298 nm, Em. 325 nm). In order to provide adequate time for the green tea to effect the egg yolk fatty acid composition, we began testing on the 20th day. We collected 3 eggs from each test group and separated the egg yolk from the egg white using a household egg separator. After measuring the yolks, we mixed the three yolks together for each test group. We extracted the egg yolk fat within 24 h of the egg being laid. We performed chloroform methanol extraction technique on the egg yolk fat according to the Bligh and Dyer (1959) method and methyl etherification of the lipid extraction according to the Christopherson and Glass (1969) method. Hitachi G-5000 gas chromatography technique was used to analyze the fatty acid composition using Crompack CP-Sil88 fused-silica capillary column (50m x 0.25mmi.d.,

0.2m). The column temperature was adjusted to 170°C. 190 with a 2°C/min rate of increase and helium was used as a carrier gas, which had a flow rate of 30 mL/min.

Statistical analysis: All data were analyzed by one-way analysis of variance and treatment means were compared using Tukey's multiple range test. Statistical significance was considered P < 0.05.

Results and Discussion

Productivity performance: The results of egg production for the first 21 days when the hens consumed the feed enriched with green tea is given in Table 1. There was no significant difference between the results of the control group and the 1% group regarding the rate of egg production, egg weight, eggs mass, feed intake and feed efficiency. Moreover, we did not find a significant difference between the 5% and 10% groups; however, a significant difference between the control and 1% groups versus the 5% and 10% groups, respectively (P < 0.05) was observed. The rate of egg production, the egg mass, the feed efficiency was decreased with increased green tea powder supplement levels. Fig. 1 illustrates the weekly feed intake of the hens. In the first week, we could not detect a significant difference between the four test groups. However, until the 3rd week, the feed intake for the 5% and 10% groups decreased remarkably and there was a significant difference for weeks 2 and 3 (P < 0.05). In particular, of the 120 g/hen per day (100%) of feed provided to the hens, in the 3rd week the 10% diet group only consumed 75 g/hen per day of it (62.5%). From day 22 to day 42 when all the hens received the control diet with no green tea, the 5% and 10% groups increased their food consumption remarkably and then there was no difference in the performance of feed intake. As illustrated in Table 1, the rate of egg production and the feed efficiency were significantly decreased with increased green tea powder supplement levels (P<0.05). Fig. 2 illustrates the weekly rate of egg production of the hens. We observed a significant difference between the rate of egg production in the control group versus those in the 5% and 10% groups by the 2nd week. The 1% group hardly showed any changes, but the 5% and 10% groups showed a continuous decrease in the rate of egg production till the time they received feed enriched with green tea powder. In particular, the rate of egg production for the 10% group on the week 3rd of the experiment period was just 10.7%, whereas that of the control group was 74.3%, a point difference of 63.6. Regarding feed efficiency, the egg mass and rate of egg production decreased as the proportion of green tea in their feed increased. Based on this, we found the control group was twice as superior as the 10% group (Table 1). From these results, it is

Table 1: Effeect of green tea powder on laying performance1

| Item | Green tea powder level (%) | | | | |
|--------------------------------|----------------------------|--------|-------------------|-------------------|------|
| | 0 | 1 | 5 | 10 | SEM |
| Hen-day egg production (%) | 74.3° | 58.1° | 40.2b | 33.3 ^b | 2.32 |
| Egg weight (g) | 69.3° | 71.9ª | 59.9b | 60.6⁵ | 0.41 |
| Egg mass (g/hen/day) | 51.6ª | 41.8ª | 24.3b | 20.2 ^b | 1.55 |
| Feed intake (g/day) | 120.0° | 119.8ª | 97.8⁵ | 93.8⁵ | 3.67 |
| Feed efficiency (g feed/g egg) | 43.0° | 34.9ª | 24.9 ^b | 21.5 ^b | 0.01 |

¹Data = means value form 1 to 21 days. ^{a-b} means in raw without a common superscripts are different (p<0.05). SEM = standerd error of the mean.

Table 2: Caffine, total epicatechine and alpha-tocopherol intakes from the experiental diet1

| | Green tea powder level (%) | | | | |
|--|----------------------------|--------|--------|---------|--|
| Item | 0 | 1 | 5 | 10 | |
| Caffine intake, mg per hen per day | 0.00 | 26.40 | 107.60 | 206.40 | |
| Total epictechin intake, mg per hen per day | 0.00 | 148.60 | 606.40 | 1163.10 | |
| alpha- tocopherol intake, mg per hen per day | 0.00 | 0.35 | 1.44 | 2.77 | |

¹Data = mean values from 1 to 21 days.

clear that, compared to the control group, the feed enriched with 5% or more of the green tea powder was significantly inferior. The feed consumption and the egg weight were not affected by the 1% enriched feed. It was also clear that a negative influence was hardly observed on their rate of egg production or the egg mass. Although, we did not find any significant difference between the control and the 1% groups with regards to egg mass, it was clear that the egg mass decreased as the proportion of green tea in the feed increased. Yamane et al. (1999) observed that feed consumption decreased when the feed was enriched with green tea extract, but it was unclear whether the cause of decrease was due to the bitterness of the catechin or some other component. Kaneko et al. (2005) reported that the broilers feed enriched with 2.5% used tea leaves, showed no significant difference between their control and 2.5% groups with respect to the weight of the hen or the amount of feed consumed. Therefore, the catechin or caffeine liquefied from green tea leaves by the hot water extraction process had an effect on the amount of feed consumed or the weight gained by the hens.

During our experiment, there was no significant difference in the weight of the hen between the control group, the 1% group and the 5% group. However, the weight of the hen in the 5% and 10% groups did decrease till 3 weeks. In particular, by the 3rd week there was a significant difference between the weight of hens in the 10% group versus those in the 1% group and the control group (P < 0.05). Conversely, after switching them back to the control feed, their weights recovered remarkably and after the 4th week there was no significant difference between them. A study by Biswas and Wakita (2001) in which they administered feed enriched with 0%, 0.5%, 0.75% and 1.5% green tea leaf powder to broiler chickens for 34 days reported no significant difference in the weight of the chicken for the

experiment groups receiving 0.1% enriched feed; in particular, the 0.5% group showed maximum consumption of feed. In our study, the trend for increase in weight gain of the 1% group changed compared with the control group (illustrated in Fig. 3). Kaneko *et al.* (2001) reported that after 10 weeks, the broiler chickens receiving 2.5% and 5% enriched green tea feeds had significantly lower body weights than the control group. Kaneko *et al.* (2000) also reported that a similar trend was found green tea leaf extract. Thus, summarizing the above points we anticipate that the water soluble components of the green tea may have an influence on the difference in birds weight gain, due to the presence of catechin and caffeine.

The green tea powder used in this study had 12.4% epicatechin and 2.2% caffeine. Our presumption is based on the weight gain trend of the 1% group from the 3rd week in which they received 120 g/hen per day of feed. In other words, the green tea caffeine of over 26.4 mg/day per chicken and epicatechin of more than 148.6 mg/day per chicken limit the amount of feed consumed. We hypothesized that consuming less than this improves the efficiency of digestion (Table 2). Hence, the weight of the 1% group which consumed the same 120 g/bird per day as the control group was greater than the control group.

A study by Yamane *et al.* (1999) reported that the fat content of egg yolks significantly decreased due to the caffeine and catechin content in the powder. It is estimated that along with this powder's capability of decreasing lipid in the blood, catechin obstructs the intestine's ability to absorb fat or suppresses the fat synthesis within the body.

The content of the catechin of 0.17% addition group of their study corresponds to the addition of the green tea powder of about 3% in our experiment and it corresponds to the green tea powder addition of about

Table 3: Effects of green tea powder on egg quality¹

| Item | Green tea powder level (%) | | | | |
|-------------------------------|----------------------------|-------------------|--------------------|--------------------|------|
| | 0 | 1 | 5 | 10 | SEM |
| Egg shell strenght (kg) | 3.8 | 3.0 | 2.9 | 2.8 | 0.14 |
| Egg shell thickness (x0.01mm) | 38.1° | 37.9ª | 36.0 ^{ab} | 33.0⁵ | 0.50 |
| Roche color fan value (1-15) | 8.9 | 8.8 | 9.0 | 9.0 | 0.08 |
| Haugh units | 57.0 ^{ab} | 61.6ª | 47.4 ^b | 46.4b | 1.54 |
| Lightness (L*) of egg shell | 69.1° | 70.9ab | 71.5⁵ | 69.4 ^{ab} | 0.36 |
| Redness (a*) of egg shell | 12.1ª | 10.3ab | 9.8⁵ | 10.2ªb | 0.32 |
| Yellowness (b*) of egg shell | 27.0° | 24.1 ^b | 24.5 ^{ab} | 25.1 ^{ab} | 0.38 |

¹Data = means value from 15 to 21 days. ^{ab}Means in a raw withuot a common superscripts are different (p<0.05). SEM = standerd error of the mean.

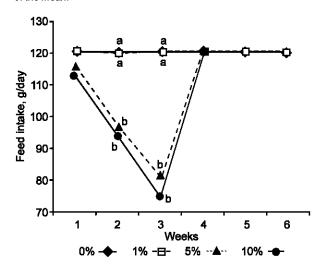


Fig. 1: Effect of supplemental green tea powder on the feed intake.

a-b; P<0.05. Addition period = 1 to 3 weeks.and no addition period = 4 to 6 weeks.

1% in caffeine. Because experiment by Yamane *et al.* (1999) showed no negative effects on egg production performance apart from egg weight, we think that the feed consumption was limited because the effects of the caffeine are stronger than that of catechin.

Egg quality: Table 3 showed our results for egg quality produced from day 15 to day 21. We did not find a significant difference between the treatment groups with regards to strength of the egg shell, but there was a trend of a decrease in shell strength associated with increase in proportion of green tea. Moreover, the thickness of the eggshell decreased as the proportion of green tea in the feed increased, with the 10% group showing the worst results. This was corroborated by a study by Unganbayar et al. (2005), which found that as the proportion of green tea powder in the feed increased the eggshells produced were weaker and thinner. With respect to the eggshell color, compared to the control group, the 5% group's eggs had the lowest L* and a* values (P < 0.05). The HU values were significantly lower in the case of birds fed with 5% diet and 10% diet than 1% diet (P < 0.05). There was no significant difference

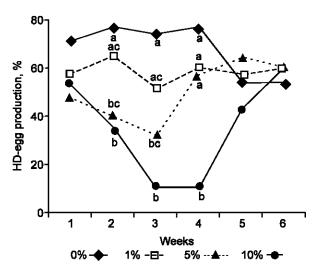


Fig. 2: Effect of supplemental green tea powder on the Hen day egg production.
a-b; P<0.05. Addition period = 1 to 3 weeks and no addition period = 4 to 6 weeks.

with regard to the egg yolk color; hence, we did not observe a correlation between the proportion of green tea in the feed and yolk color. Each test group had an average score from 8.8 to 9.0 on the Roche color fan score, with no significant difference between the groups. The carotenoids found in the bird feed can be divided into xanthophylls and carotene. The yellow color is mainly found in xanthophyll. The b* value (Yellowness) is determined by the crypt xanthin found in the corn used in standard feed of hen and lutein and zeazanthin found in the cone glutinous meal. To produce the additional value eggs with thick yolk color, the eggs should score high on the Roche color fan. Capsaicin extracted from naturally red paprika is used to strengthen the a* value (Redness). On the other hand, there is 13.29 mg% of beta-carotene in green tea leaves. According to Naber (1979), beta-carotene is an important precursor for Vitamin A, which upon colorization is categorized as a vellow. But the amount found in egg volks is just one tenth of the amount of xanthophylls; hence, its influence on color is reported to be insignificant. Thus, this study shows that the influence of green tea on an egg yolk's yellowness is non-existent or minute.

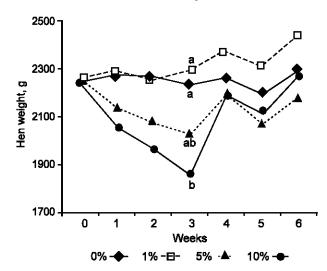


Fig. 3: Effect of supplemental green tea powder on the Hen weight.
a-b; P<0.05. Additional period = 1 to 3 and no additional period = 4 to 6 weeks

Fatty acid composition and alpha-tocopherol contents of egg yolk: In our experiment, the fatty acid composition of the egg yolks was not affected by the green tea powder. Meluzzi et al. (2000) verified that adding 0, 50, 100 and 200 ppm DL-alpha-tocopherol acetate to Hy-Line had virtually no effect on the fatty acid composition of egg yolks. The results of our study corroborate this. We noticed that the amount of alpha-tocopherol in the egg yolks is dependent on the amount of green tea powder (Fig. 4). The peaks for each test group were as follows. On day 21, the 1% group had 8.6 mg/100 g. On day 22, the 5% group had 10.3 mg/100 g. On day 24 the 10% group had 11.6 mg/100 g. However, each group's respective amounts began to decrease. According to Jiang et al. (1994), the amount of DL-alphatocopherol acetate added to the feed was directly correlated to the amount of vitamin E found in the egg yolks. Alam and Alam (1983) reported a negative correlation between the amount of alpha-tocopherol in rat liver, blood plasma and peroxide. Chen and Chang (1978) found that an increase of liver lipid peroxide correlated with a lack of vitamins C and E in guinea pigs. Biswas and Wakita (2001) reported that as a result of giving broiler chickens 0.5% green tea enriched feed for 34 days, the TBA density in their muscles significantly decreased (P < 0.05). Our study also suggested that the transfer of alpha-tocopherol from dietary green tea powder to egg yolk was sensitive to the alpha-tocopherol intake. And, during the 21 days when the green tea powder enriched feed was given, the alpha-tocopherol in the egg yolks continued to increase but did not plateau. Based on analysis of 39 points for the control group, the average was 5.3 mg/100 g. After changing to the control diet, it took the 1% group 1 day, the 5% group 6 days and the 10% group 15 days to attain the same level as or lower than the control

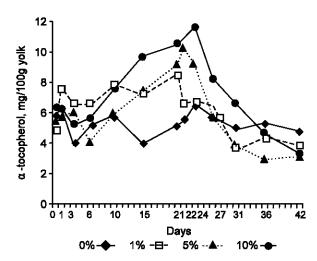


Fig. 4: Effect of supplemental green green tea powder on alpha-tocopherol contents of egg yolk. a-b; P<0.05. Additional period = 1 to 21 and no additional period = 22 to 42 days.

group. It is very interesting that compared to the 5 to 7 mg/100 g of alpha-tocopherol found at the beginning of the experiment, each of the 3 test groups showed a decrease of 3 to 4 mg/100 g. Thus, the results of our study suggest that green tea enriched feed leads to a suppression of egg weight in the late stage of laying. And we also thought that the alpha-tocopherol found in the egg yolks increased depending upon the amount of feed enriched with green tea powder or given period. Moreover, our study suggests that chicken feed enriched with 1% or less green tea leaf powder when consumed for more than 21 days has no negative effect and can actually increase the amount of vitamin E found in their eggs.

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