# Influences of Long-Term Feeding of Japanese Green Tea Powder on Laying Performance and Egg Quality in Hens[a]

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**ABSTRACT :** Influences of Japanese green tea powder (GTP) supplementation to commercial diet on laying performance and egg quality were studied by using 60 laying hens. The experimental diet with or without 0.6% GTP was given *ad libitum* to the birds during the period from 6 to 71 weeks of age. The birds started egg production from 21 wk of age regardless GTP feeding. Body weight, feed intake, egg weight tended to decrease with GTP supplementation, while egg production rate tended to increase. Haugh unit score was significantly increased with GTP, which accompanied with the increased albumen height. These were observed almost throughout the laying period over 50 wk. Gel proportion in thick albumen was decreased as storage time is prolonged, then higher values of the gel proportion were recorded in the eggs from GTP group. Thick albumen of the eggs from GTP-fed layers had more carbohydrate than that from control layers. All these indicate physical stability of thick albumen in the eggs from GTP group. Thiobarbituric acid content of egg yolk tended to remain lower in the eggs from GTP group during 5-10 days of storage at room temperature. Levels of egg yolk cholesterol and yolk lipid were significantly reduced by GTP feeding. There were no significant differences in eggshell weight, shell thickness and shell strength between the two groups. Thyroid gland and liver from hens slaughtered at 71 wk of age did not differ in weight irrespective of GTP feeding. The present results suggest that GTP could modify components of edible part of egg, leading to the characteristics favourable to consumers such as high durability of thick albumen and less cholesterol in yolk, without altering general performance of the layers throughout this year round experiment. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 980-985)*

**Key Words :** Green Tea Powder, Egg, Haugh Unit, Albumen, Cholesterol, Layer

## INTRODUCTION

Tea plant is a kind of evergreen laurel tree, taxonomically classified as Camellia sinensis. The tea plant grows widely from tropical to temperate regions in Asia and is closely associated with people's daily life, since tea leaves have been utilized for beverages and medicines. Major tasty components of Japanese green tea powder (GTP) are polyphenols, caffeins and several amino acids including theanine, of which the polyphenols have various biological roles as represented by a preventive effect on dental caries (Sakanaka et al., 1989), an inhibitory effect on lipid oxidation (Okuda et al., 1983) and arteriosclerosis (Kimura et al., 1983), and a depressive effect on renal hypertension (Yokozawa et al., 1994). The green tea also has some anti-mutagenic (Kada et al., 1985) and anti-hepatotoxic (Hikono et al., 1985) effects. Environmental carcinogenesis and mortality of cancer have negative relationship with intake of Japanese green tea (Oguni et al., 1981). Catechins and other flavonols, main components of Japanese green tea, have been shown to be effective as anti-oxidants (Matsuzaki and Hara, 1985). Thus, most of the researches on green tea have been concerned with human health, though its implication on domestic animals and their products is still scant.

The tea polyphenols have a deodorizing effect on pig feces, possibly modifying fecal metabolite composition (Hara et al., 1995). Yamane et al. (1999) reported that feeding of tea extracts to laying hens improved egg quality in a quite short-term (6 wk) experiment. However, more practical application study should be performed in order to evaluate the green tea as a feed additive for layers at commercial level. The present study was aimed at evaluating the effects of a year round, long-term feeding of GTP on performance of laying hens and some physical and chemical characteristics of their eggs.

## MATERIALS AND METHODS

### Birds and management

White Leghorn female chicks of one day old were purchased from a local commercial hatchery and housed in an electrically heated battery brooder equipped with wire mash floor. The birds were reared for 6 weeks under ad libitum feeding of a commercial starter diet (Hyper Chick Early, Tokai Feed Manufacturing Co., Ltd) containing 21.0% CP and 2950 kcal ME /kg. Then, all the birds were weighed and divided randomly into two groups with 5

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replications (6 birds in each group, 60 birds in total). They were reared individually in a wire fencing cage with normal lighting schedule. One group of birds (control) were fed with the following commercial diets. Hyper Chick Middle (18.0% CP and 2800 kcal ME/kg) and Hyper Layer (17.0% CP and 2830 kcal ME/kg), both from the above supplier, were given during the period of 6-10 and 11-71 wk of age, respectively. The other group of birds were fed with the same diets in the same schedule but supplemented with 0.6% GTP which was donated by Depatment of Tea-Rasing, Agricultural Research Center, Mie Prefectural Science and Technology Promotion Center, Japan. GTP was mixed with the above commercial diets before supplied to the birds. All the birds of each group were freely accessible to the respective diets and water throughout the experiment.

**Physical measurements**

The feed consumption and body weight were measured every five weeks during the period from 6 to 71 weeks of age. Production profiles in laying period were recorded as mean egg weight and egg production rate. Shell thickness was measured at three positions of each shell using a micrometer (Mitutory, Tokyo). Shell breaking force was monitored by a machine (Fujihara Co. Model). Height and weight of albumen and yolk were also measured after separation of each part from a whole egg. Gel and sol portions of thick albumen were separated by centrifuging at 59,000 xg for an hour (Hitachi Koki, P65 AT 1000), and the precipitates (gel) and the suparnatant (sol) were weighed. Thyroids and livers of the birds killed at 71 wk of age were collected and weighed.

**Chemical analyses**

Yolk cholesterol was photometrically assayed through a Killiani reaction (Yonemura, 1973) after extracting with ethanol/acetone (50/50) in a rotator at 37℃ for 24 hours. Total lipid of yolk, extracted by the same organic solvent, was also determined. Thiobarbituric acid (TBA) of egg yolk were assayed by the method of Sinnhuber and Yu (1977), using diluted and homogenated yolk as a material. Activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in liver tissue were photometrically assayed (Yonemura, 1973), using supernatant of tissue homogenate and pyruvate as crude enzyme solution and standard, respectively. Carbohydrate and protein present in thin albumen, whole thick albumen and sol portion of thick albumen were determined according to the methods of Morris (1948) and Lowry et al. (1951), respectively. All the chemicals used were of reagent grade (Nacalai Tesque, Japan).

Changes with time in several physical and chemical parameters of the eggs stored at room temperature for 5 and 10 days were monitored at 26, 31, 36, 41, 46, 51, 56, 61 and 71th weeks of age. The examined parameters were Haugh Unit (HU) score, egg albumen height, egg albumen weight, yolk weight, shell thickness, shell breaking force, and thiobarbituric acid (TBA).

**Statistical analysis**

The data were expressed as the mean and standard error. Statistical comparison was made with Student's t-test.

## RESULTS

Body weight, feed intake, egg weight and egg production rate are presented in table 1. The birds in both groups started egg production from 21 wk of age. Throughout the laying period over 50 wk, dietary GTP did not significantly affect body weight, egg weight and egg production rate ($p>0.05$). However, feed intake was decreased with GTP feeding at 26 and 56 wk ($p<0.05$).

HU score and albumen height tended to be higher in GTP-fed group when the eggs were fresh, and both the parameters became consistently higher in GTP-fed group when the eggs were stored for 5-10 d at room temperature (table 2). As well, these were observed at almost all ages examined (table 2).

Table 3 shows cholesterol and total lipid contents in yolk. GTP feeding consistently lowered yolk cholesterol and total lipid, though there was an exception for each parameter (56 wk for cholesterol and 41 wk for total lipid).

Changes with storage time and GTP feeding in physical and chemical parameters determining egg quality are shown in table 4. Proportions of thin albumen and of sol of thick albumen were increased as the storage time is prolonged, while proportion of gel of thick albumen was decreased. Thin albumen proportion in whole albumen tended to be lower in GTP group whether the eggs were fresh or stored. Of thick albumen, sol proportion tended to be lower, while gel proportion tended to be higher when GTP was fed. These were consistent in all the eggs that were fresh or stored. There were no notable changes with storage time and GTP feeding in other parameters, excepting that TBA concentration in yolk remained lower in GTP group after 5-10 d storage of the eggs.

Carbohydrate and protein concentrations in thin and thick albumen and in sol portion of thick albumen are given in table 5. GTP feeding increased carbohydrate concentration in all three fractions except those at 61 wk of age, while protein concentration did not show any consistent changes with GTP.

**Table 1.** Effects of Japanese green tea powder on body weight, feed intake, egg weight and egg production rate

| Age (weeks) | Body weight (g) | | Feed intake (g/d) | | Egg | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Weight (g) | | Production rate (%) | |
| | Control | GTP | Control | GTP | Control | GTP | Control | GTP |
| 6 | 446±4 | 446±3 | - | - | - | - | - | - |
| 11 | 845±9 | 835±14 | 48.9±0.5 | 49.4±0.7 | - | - | - | - |
| 16 | 1150±15 | 1146±15 | 63.2±2.2 | 63.5±1.8 | - | - | - | - |
| 21 | 1393±26 | 1370±18 | 112.8±2.1 | 110.5±1.0 | 48±3 | 48±1 | 45.3±0.8 | 46.8±2.1 |
| 26 | 1494±22 | 1477±26 | 126.0±1.1 | 112.3±0.7*** | 58±1 | 57±1 | 84.7±1.2 | 85.7±3.8 |
| 31 | 1561±27 | 1533±26 | 105.8±1.4 | 105.5±2.6 | 64±4 | 60±1 | 89.2±5.0 | 90.6±0.5 |
| 36 | 1595±29 | 1514±56 | 115.6±3.3 | 110.8±9.1 | 62±1 | 60±1 | 95.1±1.1 | 96.1±0.7 |
| 41 | 1568±34 | 1537±27 | 118.4±5.3 | 108.6±2.4 | 65±1 | 61±2 | 91.6±1.0 | 94.1±0.5 |
| 46 | 1585±35 | 1541±33 | 116.4±3.2 | 112.7±1.4 | 61±2 | 63±1 | 86.2±1.3 | 90.2±0.7 |
| 51 | 1586±35 | 1513±30 | 122.8±3.5 | 105.1±6.8 | 64±1 | 63±2 | 86.7±3.0 | 85.7±1.0 |
| 56 | 1498±40 | 1443±30 | 100.7±0.5 | 95.8±1.6* | 64±1 | 62±1 | 71.4±1.0 | 74.6±1.5 |
| 61 | 1559±28 | 1489±16 | 103.0±3.6 | 99.2±1.3 | 64±2 | 61±1 | 73.8±1.2 | 75.9±1.1 |
| 66 | 1550±37 | 1513±33 | 106.4±2.3 | 103.7±0.8 | 65±1 | 64±2 | 75.7±1.1 | 77.4±0.8 |
| 71 | 1571±25 | 1520±24 | 107.9±0.6 | 104.1±0.6 | 63±1 | 61±1 | 78.1±2.1 | 81.8±1.0 |

Values represent mean±SE of 30 birds for body weight (n=30) and of 5 replications each consisting for other parameters (n=5).
* p<0.05, *** p<0.01.

**Table 2.** Effects of Japanese green tea powder on haugh unit score and albumen height of eggs stored for different period

| Age (weeks) | Parameters | Storage period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 d | | 5 d | | 10 d | |
| | | Control | GTP | Control | GTP | Control | GTP |
| 26 | Haugh unit | 72±1 | 82±1*** | 51±2 | 60±2** | 35±2 | 52±2*** |
| 31 | | 92±2 | 98±1* | 78±1 | 82±1* | 71±1 | 76±2* |
| 36 | | 97±1 | 99±0 | 78±2 | 87±0* | 73±2 | 77±1* |
| 41 | | 96±2 | 98±1 | 79±2 | 84±1* | 73±2 | 77±1* |
| 46 | | 99±1 | 99±1 | 79±1 | 83±3 | 70±1 | 76±1* |
| 51 | | 94±1 | 97±1 | 83±2 | 88±1* | 78±2 | 82±1*** |
| 56 | | 98±1 | 99±0 | 81±2 | 88±1** | 75±2 | 88±1** |
| 61 | | 92±2 | 95±2 | 68±1 | 75±2 | 40±1 | 51±3** |
| 71 | | 94±2 | 99±0 | 71±3 | 79±1* | 67±3 | 81±2*** |
| 26 | Albumen height (mm) | 5.31±0.12 | 6.88±0.14*** | 3.33±0.10 | 4.23±0.15*** | 2.32±0.11 | 3.40±0.19*** |
| 31 | | 9.82±0.43 | 10.00±0.61 | 5.50±0.42 | 6.90±0.37 | 4.98±0.32 | 5.53±0.94 |
| 36 | | 9.11±0.37 | 9.59±0.12 | 5.74±0.54 | 7.24±0.25*** | 5.03±0.24 | 5.63±0.24 |
| 41 | | 9.52±0.46 | 10.00±0.49 | 5.99±0.20 | 6.88±0.30* | 5.24±0.33 | 5.74±0.21 |
| 46 | | 9.00±0.41 | 9.94±0.21 | 5.87±0.36 | 6.53±0.24* | 4.86±0.41 | 5.62±0.41 |
| 51 | | 8.91±0.30 | 9.80±0.32 | 7.11±0.31 | 7.72±0.18** | 6.25±0.18 | 6.78±0.68 |
| 56 | | 10.00±0.33 | 10.00±0.13 | 6.78±0.28 | 8.02±0.31** | 5.68±0.27 | 6.68±0.20** |
| 61 | | 10.00±0.11 | 10.00±0.11 | 4.88±0.30 | 5.90±0.26* | 2.94±0.28 | 3.60±0.23 |
| 71 | | 9.27±0.38 | 10.00±0.13* | 5.77±0.31 | 6.63±0.16* | 6.22±0.19 | 6.71±0.26 |

Each value represents mean±SE (n=33).
* p<0.05, ** p<0.01, *** p<0.001.
Eggs were stored at room temperature for 0, 5 and 10 days.

Thyroid and liver were not significantly different in size between the two groups. Also, GOT and GPT activities in liver homogenate did not show any notable change with GTP feeding (table 6).

## DISCUSSION

GTP is unlikely to affect greatly laying performance and egg shell profiles (tables 1 and 4), while it is

**Table 3.** Effects of Japanese green tea powder on cholesterol and total lipid concentrations in egg yolk

| Age (weeks) | Cholesterol (mg/g wet egg) | | Total lipid (mg/g wet yolk) | |
|---|---|---|---|---|
| | Control | GTP | Control | GTP |
| 26 | 15.5±0.5 | 11.8±0.6*** | 327.1±6.0 | 309.3±8.0 |
| 31 | 18.7±1.3 | 14.8±0.4* | 311.0±4.3 | 303.0±8.0 |
| 36 | 19.0±0.6 | 18.0±0.2 | 346.0±2.0 | 330.4±3.5** |
| 41 | 14.3±0.9 | 11.1±0.4* | 324.2±2.6 | 351.0±5.4** |
| 46 | 13.4±0.4 | 12.4±0.8 | 360.1±6.2 | 357.5±9.0 |
| 51 | 16.1±0.5 | 12.6±0.4*** | 346.5±5.0 | 361.0±6.4 |
| 56 | 14.1±0.2 | 16.8±0.3*** | 393.9±1.4 | 330.0±1.4*** |
| 61 | 19.3±0.4 | 16.4±0.3*** | 318.9±4.8 | 312.0±4.5 |
| 71 | 18.5±0.20 | 17.7±0.5 | 346.0±3.9 | 330.0±4.2 |

Each value represents mean±SE (n=20).
* p<0.05, ** p<0.01, *** p<0.001.

**Table 4.** Effects of Japanese green tea powder on physical and chemical properties of eggs stored for different period

| Parameters | Storage period | | | | | |
|---|---|---|---|---|---|---|
| | 0 d | | 5 d | | 10 d | |
| | Control | GTP | Control | GTP | Control | GTP |
| Whole egg (g) | 62.61±1.28 | 62.50±1.05 | 62.30±1.66 | 61.37±1.59 | 63.23±1.19 | 61.05±1.00 |
| Yolk (% of whole egg) | 26.23±0.52 | 27.98±0.63* | 27.09±1.03 | 28.82±0.63 | 27.31±0.80 | 26.99±0.6* |
| Albumen (% of whole egg) | 63.52±0.50 | 61.33±0.72* | 62.27±0.82 | 61.61±0.75 | 61.87±1.34 | 63.10±0.53 |
| Thin albumen (% of whole albumen) | 15.19±0.30 | 14.47±0.98 | 18.83±0.38 | 17.06±0.30* | 20.97±0.79 | 19.79±0.35 |
| Thick albumen (% of whole albumen) | | | | | | |
| Gel (% of thick albumen) | 48.00±3.00 | 54.00±3.00 | 32.00±2.00 | 38.00±2.00* | 15.00±2.00 | 18.00±3.00 |
| Sol (% of thick albumen) | 52.00±3.00 | 46.00±3.00 | 68.00±2.00 | 62.00±2.00* | 85.00±2.00 | 82.00±3.00 |
| Shell (% of whole egg) | 10.25±0.17 | 10.69±0.23 | 10.64±0.37 | 9.57±0.30 | 10.82±0.20 | 9.91±0.35 |
| Shell breaking strength ($kg/cm^2$) | 4.95±0.37 | 5.00±0.31 | 3.04±0.29 | 3.80±0.26 | 3.32±0.22 | 3.74±0.34 |
| Shell thickness (mm) | 0.350±0.001 | 0.350±0.001 | 0.350±0.001 | 0.350±0.001 | 0.350±0.001 | 0.350±0.001 |
| TBA (nmol/ml yolk) | 68.70±2.09 | 70.31±4.81 | 77.34±5.40 | 77.34±5.40 | 60.70±8.94 | 55.75±3.80 |

Each value represents mean±SE (n=99), except for the value of TBA (n=10).
TBA was determined using eggs collected at 41 wk of age.
Other parameters were determined at 26, 31, 36, 41, 46, 51, 56 and 61 wk of age.
* p<0.05; Eggs were stored at room temperature for 0, 5 and 10 days.

**Table 5.** Effects of Japanese green tea powder on carbohydrate and protein contents of fractioned albumen

| Age (weeks) | Albumen fraction | | Carbohydrate (mg/g) | | Protein (mg/g) | |
|---|---|---|---|---|---|---|
| | | | Control | GTP | Control | GTP |
| 31 | Thin albumen | | 2.36±0.04 | 2.73±0.05*** | 29.58±1.60 | 31.16±1.03 |
| | Thick albumen | (total) | 2.16±0.04 | 2.63±0.07*** | 46.08±2.22 | 43.82±2.43 |
| | | (sol) | 2.37±0.02 | 3.31±0.07*** | 41.33±0.88 | 36.56±1.06** |
| 41 | Thin albumen | | 1.92±0.10 | 2.58±0.15** | 35.99±0.40 | 33.15±0.35*** |
| | Thick albumen | (total) | 2.02±0.15 | 2.52±0.04* | 41.70±0.37 | 42.11±0.66 |
| | | (sol) | 2.79±0.09 | 3.32±0.09*** | 35.76±0.45 | 40.61±0.54 |
| 51 | Thin albumen | | 2.72±0.03 | 3.93±0.09*** | 39.89±0.36 | 34.00±0.32 |
| | Thick albumen | (total) | 3.06±0.08 | 4.17±0.09*** | 42.53±0.40 | 41.08±0.40 |
| | | (sol) | 2.45±0.16 | 3.41±0.55*** | 32.68±0.14 | 42.29±0.54 |
| 61 | Thin albumen | | 2.40±0.02 | 2.56±0.09 | 38.50±0.24 | 34.30±0.68 |
| | Thick albumen | (total) | 2.59±0.08 | 2.14±0.09*** | 40.17±0.36 | 41.43±0.38 |
| | | (sol) | 2.87±0.16 | 3.12±0.05 | 33.27±0.18 | 41.11±0.33 |

Each value represents mean±SE (n=20).
* p<0.05, ** p<0.01, *** p<0.001.

**Table 6.** Effects of Japanese green tea powder on thyroid and liver of hens slaughtered at 71 wk of age

| Parameters | Control | GTP |
|---|---|---|
| Thyroid gland (mg/100 g BW) | 6.91 ± 0.76 | 7.77 ± 0.95 |
| Liver weight (g/100 g BW) | 2.59 ± 0.27 | 2.75 ± 0.12 |
| Liver cholesterol (mg/100g liver) | 353 ± 19.23 | 330.83 ± 17.85 |
| Glutamate oxaloacetate transminase (n mol pyruvate/ml liver homoginate/30 minutes) | 27.62 ± 0.56 | 27.58 ± 0.47 |
| Glutamate pyruvate transminase (as above) | 15.04 ± 0.62 | 14.11 ± 0.62 |

Each value represents mean ± SE (n=12).

proved to change physical and chemical properties of egg albumen and yolk toward the direction favorable to consumers in this long term feeding experiment (tables 2, 3 and 4).

HU score was improved with GTP feeding, in particular remained higher in GTP-fed layers during the storage period. This was accompanied with a higher albumen height, suggesting a higher physical stability of egg albumen when GTP was fed. A higher HU score has been reported in a short term (6 wk) feeding of green tea extract to layers (Yamane et al., 1999). This was confirmed to be consistent in the present year round feeding study as well. This physically durable albumen is also explained by a higher gel and a lower sol proportions in thick albumen from GTP-fed layers when the eggs were stored for 5-10 d at room temperature (table 4).

Thinning of thick albumen can be essentially described by degradation (deglycosylation) of β-ovomucin rich in carbohydrate, which causes an increased sol portion of thick albumen followed by an increase of thin albumen proportion, both accompanying with a higher carbohydrate content (Kato et al., 1970). Robinson and Monsey (1972) also pointed out that thinning of thick albumen was caused by changes in physical and chemical properties of ovomucin complex, i.e. breakdown of hexose-rich β-ovomucin forming an ionic complex with lysozyme, leads to less viscosity of thick albumen and more carbohydrate in sol portion of thick albumen. Although we do not have any supportive data about these, GTP feeding allowed production of thick albumen richer in carbohydrate (table 5). This would be caused by a higher β-ovomucin content of thick albumen in relation to GTP feeding. Presence of more β-ovomucin could enhance thick albumen durability. In addition, since polyphenols have a propensity to form complexes with proteins and polysaccharides (Bravo, 1998), β-ovomucin supporting the albumen durability could be protected from deglycosylation if the polyphenols were there. This also excuses maintenance of a higher proportion of gel in thick albumen (table 4), a higher albumen height and HU score (table 2), all of which are favorable properties as food at commercial level.

Other advantageous profiles of the eggs from GTP-fed layers are less lipid and cholesterol in egg yolk (table 3). Green tea containing high catechin may have an inhibitory effect on intestinal absorption of lipid (Ikeda et al., 1992). This may prevent an excessive accumulation of lipid in liver and other tissues including egg yolk. The reduction in tissue cholesterol may be also explained by a negative effect of tea catechin on formation of micell (Muramatsu et al., 1986) that meadiates reabsorption of bile acids. Such increase of unabsorbable bile acids may also lead to reduction in liver cholesterol, which would turn out to induce reduction in yolk cholesterol.

TBA is widely used to estimate the degree of oxidation in various biological materials. Serum TBA level is known to be reduced by tea extract feeding (Yamane et al., 1999), which suggests antioxidative effect of tea catechins. TBA in fresh egg yolk was at a similar level between the two groups, though the level in GTP-fed group tended to remain lower during the storage. This supports the above efficacy of tea catechins and may suggest possible transfer of the catechins into egg yolk.

Thyroid and liver were sampled in order to examine if a long term feeding of GTP has harmful effects on main organs regulating nutrition and growth of the layers. GTP did not greatly affect sizes of these organs, and GPT and GOT in liver homogenate were at similar levels in activity. These results indicate that GTP feeding over a year is unlikely to have adverse effects on these organs and their functions. To conclude these, however, further endocrinological studies remain to be attempted.

In conclusion, a year round long term feeding of GTP to laying hens has favorable effects on egg quality such as thick albumen stability without adverse effect on general laying performance. GTP could modify lipid profiles of egg yolk and possibly body tissues as well. All these changes are reasonably considered to be influenced mainly or partly by catechins in GTP.

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