

## Application of green tea waste with fibrolytic enzyme and phytase on layer performance and egg quality

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### ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh suplementasi limbah teh hijau, enzim fibrolitik (xilanase dan selulase) dan fitase pada ransum petelur terhadap performans dan kualitas telur. Percobaan ditugaskan dalam pengaturan faktorial  $2 \times 3$  dengan desain acak lengkap. Faktor A terdiri dari dua jenis diet: a) diet basal tanpa limbah teh hijau dan b) diet basal dengan 1% limbah teh hijau. Faktor B terdiri dari tiga level enzim: a) level 0X, tanpa suplementasi enzim; b) level 1X, kombinasi enzim fibrolitik, 24.000 FAXU xilanase dan 100 ECU selulase, dan 1.000 FTU fitase; dan c) level 2X, kombinasi enzim fibrolitik, 48.000 FAXU xilanase dan 200 ECU selulase, dan 2.000 FTU fitase. Seratus sembilan puluh dua ekor ayam ras Roman Brown, umur 52 minggu, dibagi secara acak ke dalam enam kelompok dan empat ulangan. Ayam petelur diberi makan makanan komersial dengan limbah teh hijau dan enzim fibrolitik ditambahkan di atas seperti yang ditugaskan dalam pengobatan. Hasil penelitian menunjukkan bahwa limbah teh hijau meningkatkan produksi telur, massa telur, konversi pakan, dan Haugh unit secara nyata ( $p < 0,05$ ). Selain itu, limbah teh hijau cenderung mempengaruhi ( $p = 0,07$ ) nilai TBA. Selanjutnya, tingkat enzim menurun secara signifikan ( $p < 0,05$ ) asupan pakan dan nilai TBA. Suplementasi 1% limbah teh hijau dan enzim 2X dapat meningkatkan konversi pakan dan sifat antioksidan telur.

*Kata kunci: Ampas teh hijau, Xylanase, Selulase, Fitase, Kinerja ayam petelur.*

### ABSTRACT

This study aimed to investigate the effect of green tea waste, fibrolytic enzymes (xylanase and cellulase) and phytase supplementation in layer diets on performance and egg quality. The experiment was assigned in a  $2 \times 3$  factorial arrangement by completely randomized design. Factor A consisted of two types of diets: a) a basal diet without any green tea waste and b) a basal diet with 1% of green tea waste. Factor B consisted of three levels of enzymes: a) level 0X, no enzyme supplementa-

tion; b) level 1X, a combination of fibrolytic enzymes, 24,000 FAXU xylanase and 100 ECU cellulase, and 1,000 FTU phytase; and c) level 2X, combination of fibrolytic enzymes, 48,000 FAXU xylanase and 200 ECU cellulase, and 2,000 FTU phytase. One hundred and ninety-two Roman Brown hens, aged 52 weeks, were randomly distributed to six groups and four replications. Layer hens were fed commercial diets with green tea waste and fibrolytic enzymes added on top as assigned in the treatment. The results showed that green tea waste significantly increased ( $p < 0.05$ ) egg production, egg mass, feed conversion, and Haugh unit. In addition, green tea waste tended to affect ( $p = 0.07$ ) TBAs value. Furthermore, the enzyme level significantly decreased ( $p < 0.05$ ) feed intake and TBAs value. In conclusion, the supplementation of 1% of green tea waste and 2X enzymes can improve feed conversion and the antioxidative property of eggs.

*Keywords: Green tea waste, Xylanase, Cellulase, Phytase, Layer performance.*

## INTRODUCTION

Green tea waste is a by-product from the tea beverage industry. In waste management, some kinds of tea wastes are used in composting materials but most of them are disposed as industrial waste. In the long run, it can cause environmental pollution.

Green Tea (*Camellia sinensis*), a kind of herb, contains proteins, amino acids, carbohydrates, vitamins, minerals and polyphenols, such as catechins, tannin, etc. Polyphenols have antioxidant properties to reduce oxidation in the animal body which causes cancer and inflammation (Yamamoto *et al.*, 1997; Miyazawa, 2000; Trompezinski *et al.*, 2003). In addition, tannin in tea also has antibacterial activities that reduce a large number of bacteria (Li and Liu, 1999). Considered as a by-product, green tea waste may have potential as animal feed after it has been through the beverage production process since it still contains proteins, carbohydrates, and phenol compounds (Tsubaki *et al.*, 2008; Toh *et al.*, 2010). The chemical compositions of green tea waste contain 22-35% of crude protein, 19.5-62.03 % of crude fiber, 29.9-31.0% of Neutral Detergent Fiber (NDF), 22.4-24.1% of Acid Detergent Fiber (ADF), 11.39% of phenolics, 5.95-9.23% of tannins (Yang *et al.*, 2003; Kondo *et al.*, 2004a, b; Nishida *et al.*, 2006), and 9.18% of DPPH scavenging activity (IC50) (Bunyapraphatsara and Prapinsara, 2007). Previous studies suggest that green tea waste could be used as a source of antioxidants or nutrients in animal feed for broilers and laying hens (Yang, 2003a, b), pigs (Ko *et al.*, 2008), cows (Kendo *et al.*, 2004b) and cattle (Nishida *et al.*, 2006). However, tea has a high level of fiber, which consists of celluloses, hemicelluloses, pectin and oligosaccharides, etc. Poultry does not have

enzymes for digesting insoluble polysaccharides especially non starch polysaccharides such as celluloses, hemicelluloses, pectin and it also lacks endogenous phytase. Therefore, supplementation of fibrolytic enzymes in chicken diet can improve the digestibility and feed efficiency (Yu *et al.*, 2004; Khattak *et al.*, 2006). The final product of fibrolytic enzyme digestion is sugar, which is the source of energy for animals. (Bedford, 2000; Woyengo *et al.*, 2011). There are many fibrolytic enzymes used in animal feeds, such as cellulase, xylanase and hemicellulase, which are produced by various microorganisms. The digestibility efficiency of each enzyme is different, so it must be applied appropriately. In addition, plants have phytate bound to some minerals, sugar and amino acids. Phytate decreases the digestion and utilization of nutrients. Phytase supplementation can increase the digestibility of other minerals, amino acids and energy, which are bound to phytic acid (Ravindran *et al.*, 2001; Ravindran *et al.*, 2006). The objectives of this experiment was to investigate the effect of fibrolytic enzymes and phytase enzyme supplementation in green tea waste diets on layer performance and egg quality, which helps add the value of green tea waste from the tea beverage industry and keep waste to the minimum.

## MATERIALS AND METHODS

### Green Tea Waste

Green tea waste (GTW) from the green tea beverage industry (in Phra Nakhon Si Ayutthaya province, Thailand) was dried at 60 °C for 6-8 hr. and ground to powder. The chemical compositions of GTW were analyzed according to AOAC (1990) included 16.62% of crude proteins, 2.06% of crude lipids, 37.77% of crude

fibers and 4841.80 Kcal of gross energy. There were also 0.01% of tannin analyzed by the FAO/IAEA method (2000), 56.56% of DPPH scavenging analyzed by the method of Roche *et al.* (2005), and 203.43 mg./kg. of carotenoid analyzed by the method of Foss *et al.* (1984). The green tea waste's nutritive values and active compounds were investigated at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

### Enzyme

A combination of two types of enzymes in liquid form (AB Vista Asia Pte. Ltd., Singapore), fibrolytic enzymes (xylanase and cellulase) and phytase enzyme were used in this study. The application of these enzymes for chickens were level 1X fibrolytic enzymes equal to 24,000 FAXU xylanase and 100 ECU cellulase, level 1X phytase equal to 1,000 FTU and level 2X fibrolytic enzymes equal to 48,000 FAXU xylanase and 200 ECU cellulase, level 2X phytase equal to 2,000 FTU. One unit of FAXU equals to one unit of xylanase activities as analyzed by the ABVista in-house method. One unit of ECU equals to one unit of cellulase activities as analyzed by the ABVista in-house method.

### Experimental Diet

The experimental diet was commercial diet composed of corn, soybean and other raw materials (from Laemthong Corporation Company) which consisted of 18.12% of crude protein, 4.69% of crude fat, 12.79% of crude fiber and 3751.14 Kcal of gross energy (Table 1). All supplementations in the diet like GTW and a combination of fibrolytic enzymes and phytase were added on top of the feed.

### Animal: Layer Hens

One hundred and ninety two Roman Brown hens purchased from a layer hen farm in Nakornnayok province was quarantined in the farm of Agricultural Technology Faculty, Valaya Alongkorn Rajabhat University, under the Royal Patronage, Pathum Thani province. Layer hens were stocked in a trail condition and fed a control diet including vitamins in water for 14 days before the experiment began.

### Experimental Design

The experiment was assigned in a 2×3 factorial arrangement by completely randomized design. Factor A consisted of two types of diets: a) a basal diet without any green tea waste (GTW0) and b) a basal diet with 1% of green tea waste (GTW1). Factor B consisted of three levels of enzymes: a) level 0X, no enzyme supplementation (E0); b) level 1X, a combination of fibrolytic enzymes, 24,000 FAXU xylanase and 100 ECU cellulase, and 1,000 FTU phytase (E1); and c) level 2X, a combination of fibrolytic enzymes, 48,000 FAXU xylanase and 200 ECU cellulase, and 2,000 FTU phytase (E2).

The research was divided into six groups; each group contained 4 replicates, with 8 hens (aged 52 weeks) in each replicate. The experimental diets were as follows: T1, a basal diet without any green tea waste and enzyme 0X (GTW0E0); T2, a basal diet that contained 1X enzymes (GTW0E1); T3, a basal diet that contained 2X enzymes (GTW0E2); T4, a basal diet that contained 1% of GTW without enzyme (GTW1E0); T5, a basal diet that contained 1% of GTW and 1X enzymes (GTW1E1); and T6, a basal diet that contained 1% of GTW and 2X enzymes (GTW1E2). The GTW and a combination of fibrolytic enzyme and phytase supple-

Table 1. Enzyme activity of experimental diets

Green tea waste (%)	Enzyme level	Treatment	Phytase Activity (FTU/kg)	Xylanase Activity (FAXU/kg)	Cellulase Activity (ECU/kg)
GTW0	E0	GTW0E0	312	12000	2700
	E1	GTW0E1	1410	38100	2400
	E2	GTW0E2	2260	57500	2300
GTW1	E0	GTW1E0	359	13900	2100
	E1	GTW1E1	1200	38100	2000
	E2	GTW1E2	2070	61000	3200

GTW = Green tea waste; E = Enzyme; GTW0 = Non supplementation with green tea waste; GTW1 = Supplementation with green waste 1%; E0 = Non supplementation with enzyme; E1 = Supplementation with enzyme 1X; E2 = Supplementation with enzyme 2X

mentation was added on top of the feed.

### Experimental Condition

The Layer hens were reared in the evaporated housing at temperature  $28 \pm 1^\circ\text{C}$ , and supplied with drinking water as well as 16 hr. daylight intensity. The experimental diet were distributed ad libitum to the hens in mash form and topped up with GTW and enzyme. The trial period was 84 days.

### Data Collection

The study was divided into three periods of 28 days, including data collection and the calculation of variables of performance and egg quality. The layer performance was observed in terms of feed intake (g/h/d), egg production (%), egg weight (g), egg mass (g), and feed conversion (feed consumption per kilogram of eggs). Six eggs per replication (24 eggs /treatment) were collected randomly during the last three days of each period. Egg quality was evaluated in terms of egg weight, yolk color by using a yolk color fan (Roche, Switzerland), Haugh unit by using height gauge (TSS-QCD instrument, England), egg shell thickness by taking measurement using micrometer (395-541-30 BMD-25DM, Mitutoya, Japan), specific egg gravity by immersing the eggs in salt solutions of specific gravities and counting the number of floating and sinking eggs, and thiobarbituric acid reactive substance values (TBAs) by using the method of Cherian *et al.* (1996) and Botsoglou *et al.* (1994).

### Enzyme Activity

Enzyme activity of the experimental diets was analyzed by the ABvista in-house method. The enzyme phytase was conducted on Quantum Blue in-feed (wet chemistry method); the enzyme xylanase was determined on Econase XT; the enzyme cellulase was analyzed on Vista Pre-T (wet chemistry method) (the ABvista in-house method, 2018). (Table1)

### Efficacy of Exogenous Enzyme Supplementation

The efficacy of exogenous enzyme supplementation on experimental diets was studied by *in vitro* digestibility of the experimental diets, following the modified method of Bisswanger (2011). The fine ground diet was incubated in the buffer (pH 6.5) for 6 hours. Then the reaction was stopped by immersing the diet in water bath at  $100^\circ\text{C}$  for 5 minutes. The reducing sugar in

term of glucose was determined for carbohydrate digestion products by dinitrosalicylic acid (DNS). The amino acid content was determined by ninhydrin and the soluble protein content was determined by biuret test for the protein digestion products. The amount of phosphate was determined by an assay mixture for phosphorus digestion products. All enzyme assays and *in vitro* digestibility were studied at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

### Statistical Analysis

The data of this research were analyzed by analysis of variance. Duncan's New Multiple Range test was used to compare the mean difference. The alphabetical notation was applied to determine differences at the 95% confidence interval ( $\alpha$  0.05).

## RESULTS AND DISCUSSION

### Efficacy of Exogenous Enzyme Supplementation in Layer Diets

The efficacy of exogenous enzyme supplementation on the experimental diet digestibility is detailed in Table 2. The reducing sugar presented the efficacy of fibrolytic enzymes for improving carbohydrate digestion. The phosphate demonstrated the efficacy of phytase enzyme for improving phytate digestion. The amino acid and soluble protein exhibited the efficacy of both enzymes for improving protein digestion. The results showed that the supplementation of GTW in layer diets (GTW1E0, GTW1E1 and GTW1E2) exhibited poor ( $p < 0.05$ ) carbohydrate, amino acids and phosphorus digestibility compared to the basal diet without GTW (GTW0E0, GTW0E1 and GTW0E2). The supplementation of phytase, cellulase and xylanase enzyme in diet without GTW (GTW0E1 and GTW0E2) showed significantly higher level of phosphorus ( $p < 0.05$ ), carbohydrate and protein digestion in terms of amino acids higher ( $p < 0.05$ ) than the diets with GTW supplementation. Moreover, the layer diets with GTW supplementation (GTW1E0, GTW1E1 and GTW1E2) showed no significant differences ( $p > 0.05$ ) on phosphorus and protein digestion.

Woyengo *et al.* (2011) reported that fibrolytic enzymes were effective in releasing reducing sugar, amino acids and other nutrients

Table2. Efficacy of exogenous enzyme supplementation in layer diets

Green tea waste (%)	Enzyme level	Treatment	Amino acid (g/kg)	Protein (g/kg)	Reducing sugar (g/kg)	Phosphorus (g/kg)
GTW0	E0	GTW0E0	0.629 <sup>b</sup>	0.274	2.783 <sup>a</sup>	0.003 <sup>a</sup>
			±0.01	±0.03	±0.18	±0.001
	E1	GTW0E1	0.650 <sup>b</sup>	0.297	2.333 <sup>a</sup>	0.004 <sup>a</sup>
			±0.04	±0.06	±0.22	±0.001
	E2	GTW0E2	0.733 <sup>a</sup>	0.321	1.983 <sup>b</sup>	0.003 <sup>a</sup>
			±0.05	±0.04	±0.57	±0.001
GTW1	E0	GTW1E0	0.517 <sup>c</sup>	0.251	1.090 <sup>c</sup>	0.001 <sup>b</sup>
			±0.02	±0.03	±0.07	±0.0003
	E1	GTW1E1	0.530 <sup>c</sup>	0.292	1.299 <sup>c</sup>	0.002 <sup>b</sup>
			±0.03	±0.04	±0.07	±0.0002
	E2	GTW1E2	0.556 <sup>c</sup>	0.326	1.345 <sup>c</sup>	0.002 <sup>b</sup>
			±0.06	±0.09	±0.01	±0.0001
P-value	E		0.0153	0.1560	0.0315	0.6233
	GTW		0.0001	0.7512	0.0001	0.0001
	E x GTW		0.2929	0.8836	0.0030	0.0594

Mean values within the same column with difference superscripts are significantly different ( $P < 0.05$ ).

Data are expressed with mean  $\pm$ SD. GTW = Green tea waste; E = Enzyme; GTW0 = Non supplementation with green tea waste; GTW1 = Supplementation with green waste 1%; E0 = Non supplementation with enzyme; E1 = Supplementation with enzyme 1X; E2 = Supplementation with enzyme 2X

by means of hydrolyzing non starch polysaccharides in plant cell walls. In addition, carbohydrase can also increase the efficacy of phytase by increasing the accessibility of phytase to phytic acid and absorption of nutrients released by phytase. Phytase supplementation can increase the digestibility of other minerals, amino acids and energy which are bound with phytic acid (Ravindran *et al.*, 2001; Ravindran *et al.*, 2006). The highest amino acid digestibility in the GTW0E2 group showed the synergism between supplemental fibrolytic enzymes (xylanase and cellulase) and phytase on amino acid utilization. The reducing sugar presented the efficacy of fibrolytic enzymes on fiber digestion to release sugar as energy for animals (Bedford, 2000). In the diets without GTW that contained 12.79% of fiber, supplemental fibrolytic enzyme or carbohydrase and phytase seemed to have a low impact on carbohydrate digestibility due to GTW0E0 and GTW0E1 groups had the same reducing sugar level. However, the GTW0E2 group had a lower level of sugar when compared to GTW0E0 and GTW0E1 groups, although this group of diets was supplemented with a high level of enzymes. These may come from a wide range of standard deviation ( $1.983 \pm 0.57$ g./kg.) compared to GTW0E0

( $2.783 \pm 0.18$ g./kg.) and GTW0E1 ( $2.333 \pm 0.22$  g./kg.). The *in vitro* phosphorus digestibility in the basal diets without GTW (GTW0E0, GTW0E1 and GTW0E2) both with and without phytase enzymes demonstrated a significantly higher efficacy ( $p < 0.05$ ) than GTW diets (GTW1E0, GTW1E1 and GTW1E2). The phytase enzyme worked on phytate digestibility and liberated more phosphorus (Woyengo *et al.*, 2011). GTW decreased amino acid, carbohydrate and phosphorus digestibility in GTW1E0, GTW1E1 and GTW1E2, due to the anti-nutritional compound of GTW. The present study revealed that GTW contained 0.01% of tannin. Tannin is a complex group of polyphenolic compounds. They are naturally- occurring plant polyphenols that combine with protein and other polymers such as cellulose, hemicellulose and pectin to form stable complexes (Mangan, 1988; Krueger *et al.*, 2010), and reduced the digestibility of protein and carbohydrate like starch and cell wall particularly in protein digestion (Mueller-Harvey, 2006; Bravo, 1998; Jansman, 1993). Supplementation of fibrolytic enzymes and phytase in GTW diets did not improve the digestibility of nutrients as much as diets without GTW. However, enzyme supplementation in the GTW1E0, GTW1E1 and GTW1E2 groups enhanced the nutritional di-

Table 3. Production performance of layer fed exogenous enzyme and green tea waste supplementation diets

Green tea waste (%)	Enzyme level	Treatment	Egg Production (%)	Egg wt.(g)	Egg mass (g)	Feed intake (g/day)	FCR	Feed cost/kg egg (baht/egg)
GTW0	E0	GTW0E0	80.21 <sup>b</sup> ±1.55	57.69±1.67	46.32 <sup>b</sup> ±2.16	92.14 <sup>a</sup> ±3.18	2.00 <sup>a</sup> ±0.12	26.67 <sup>a</sup> ±1.66
	E1	GTW0E1	79.84 <sup>b</sup> ±1.45	58.19±1.27	46.16 <sup>b</sup> ±1.48	90.05 <sup>ab</sup> ±1.84	1.95 <sup>ab</sup> ±0.05	26.16 <sup>ab</sup> ±0.71
	E2	GTW0E2	79.72 <sup>b</sup> ±1.86	57.64±0.91	45.95 <sup>b</sup> ±1.58	89.90 <sup>ab</sup> ±3.58	1.96 <sup>ab</sup> ±0.08	26.66 <sup>a</sup> ±1.66
GTW1	E0	GTW1E0	81.02 <sup>b</sup> ±3.59	58.03±2.44	46.26 <sup>b</sup> ±1.66	93.37 <sup>a</sup> ±4.66	1.98 <sup>a</sup> ±0.14	26.40 <sup>ab</sup> ±2.11
	E1	GTW1E1	84.55 <sup>a</sup> ±1.22	57.93±0.55	49.01 <sup>ab</sup> ±0.50	89.16 <sup>ab</sup> ±3.11	1.83 <sup>b</sup> ±0.56	24.58 <sup>ab</sup> ±0.81
	E2	GTW1E2	81.85 <sup>ab</sup> ±2.56	58.38±2.06	47.74 <sup>ab</sup> ±0.65	85.81 <sup>b</sup> ±1.88	1.80 <sup>c</sup> ±0.05	24.43 <sup>b</sup> ±0.62
P-value	E		0.3118	0.9688	0.1650	0.0209	0.0482	0.1789
	GTW		0.0109	0.6871	0.0275	0.3523	0.0137	0.0182
	E x GTW		0.2240	0.8287	0.2120	0.2710	0.3237	0.3204

Mean values within the same column with difference superscripts are significantly different (P<0.05). Data are expressed with mean±SD.

GTW = Green tea waste; E = Enzyme; GTW0 = Non supplementation with green tea waste; GTW1 = Supplementation with green waste 1%; E0 = Non supplementation with enzyme; E1 = Supplementation with enzyme 1X; E2 = Supplementation with enzyme 2X.

Table 4. Egg quality of layer fed exogenous enzyme and green tea waste supplementation diets

Green tea waste (%)	Enzyme level	Treatment	Hu	Yolk color	Shell thickness (mm.)	Specific gravity	TBAS (mg./kg.)
GTW0	E0	GTW0E0	82.06 <sup>b</sup> ±2.02	9.82±0.51	0.326±0.02	1.082 <sup>a</sup> ±0.001	0.030 <sup>a</sup> ±0.001
	E1	GTW0E1	81.91 <sup>b</sup> ±4.47	9.70±0.09	0.319±0.01	1.081 <sup>a</sup> ±0.001	0.026 <sup>ab</sup> ±0.002
	E2	GTW0E2	82.73 <sup>ab</sup> ±3.84	9.78±0.34	0.316±0.01	1.082 <sup>a</sup> ±0.003	0.024 <sup>bc</sup> ±0.002
GTW1	E0	GTW1E0	85.55 <sup>ab</sup> ±2.97	9.86±0.28	0.312±0.01	1.079 <sup>ab</sup> ±0.002	0.027 <sup>ab</sup> ±0.003
	E1	GTW1E1	83.74 <sup>ab</sup> ±1.03	9.71±0.37	0.330±0.02	1.082 <sup>a</sup> ±0.002	0.025 <sup>ab</sup> ±0.005
	E2	GTW1E2	86.40 <sup>b</sup> ±2.39	9.90±0.51	0.300±0.002	1.077 <sup>b</sup> ±0.001	0.021 <sup>c</sup> ±0.007
P-value	E		0.5226	0.7044	0.0897	0.1477	0.0011
	GTW		0.0253	0.7067	0.2717	0.0032	0.0709
	E x GTW		0.8007	0.9534	0.1156	0.0232	0.0915

Mean values within the same column with difference superscripts are significantly different (P<0.05). Data are expressed with mean ±SD.

GTW = Green tea waste; E = Enzyme; GTW0 = Non supplementation with green tea waste; GTW1 = Supplementation with green waste 1%; E0 = Non supplementation with enzyme; E1 = Supplementation with enzyme 1X; E2 = Supplementation with enzyme 2X.

gestibility of layer diets.

### Egg Production Performance

Egg production performance is detailed in Table 3. There was no interaction effect on egg production performance. Egg production, egg weight, egg mass and feed cost/kg of eggs were not significantly influenced by the enzymes but green tea waste had an effect on egg production, egg mass, feed conversion and feed cost/kg of eggs ( $p < 0.05$ ). A high rate of egg production and egg mass were observed in the GTW1E1 group, followed by the GTW1E2 group. Feed cost/kg of eggs was significantly low ( $p < 0.05$ ) in the GTW1E2 group followed by the GTW1E1 group. Feed intake was not affected ( $p > 0.05$ ) by the GTW amount but by enzyme concentration ( $p < 0.05$ ). Layer hen fed E1 and E2 in the GTW0E1, GTW0E2, GTW1E1 and GTW1E2 groups had lower ( $p < 0.05$ ) feed intake than layer hen fed diets without enzyme supplementation (E0) in the GTW0E0 and GTW1E0 groups. Feed conversion ratio (FCR) was significantly affected by both GTW and enzyme level. The low FCR ( $p < 0.05$ ) was present in the GTW1E2 group, followed by the GTW1E1, GTW0E1 and GTW0E2 groups.

The results are in line with previous research. The high level of green tea by-products (4.00% and 6.00%) promoted egg production (Yang *et al.*, 2003). Al-Harhi (2004) and Abdo *et al.* (2010) also found that the addition of green tea or its aqueous extract resulted in improvement in egg production, egg mass and feed conversion values. Yang *et al.* (2003) reported that feed conversion in broilers fed with green tea by products were slightly lower in the diets supplemented with 1% and 2% of green tea by products. These results are probably due to the availability of nutritive values of GTW like tannin, an anti-nutritional factor in plant materials which can bind proteins precipitate proteins and inhibit bacterial growth (Sakanaka *et al.*, 2000), carotene and antioxidative properties. Condensed tannin is mainly polymerized products of flavan-3-ols (catechin) (Jansman, 1993). According to Karori *et al.* (2007) the chemical compositions of green tea are included a complex of polyphenols, alkaloid, amino acids, carbohydrates, protein, chlorophyll, volatile compound, minerals and other unidentified compounds. The major tea polyphenol compound is flavan-3-ols called catechins. The total catechins in green tea are 13.6 g/100g dry weight. They are good antioxi-

dant agents of free radical scavenger for promotion of health and prevention of diseases in humans and animals (Vuong *et al.*, 2011; Yamamoto *et al.*, 1997). Antioxidants or free radical scavengers help prevent and repair the cell damage caused by the free radicals. These promote animal health, resulting in layer performance. The high egg production and egg mass were observed in the GTW1E1 and GTW1E2 groups due to absorption of the polyphenol compound contents of GTW through the intestinal walls, which positively affected the digestive function, egg formation process and feed efficiency of hens.

Enzyme supplementation had an effect on feed intake and feed conversion. Similar results were witnessed by Geraert *et al.* (2003) who founded that the supplementation of xylanase, beta-glucanase and cellulase in layer diets based on wheat, barley or maize improved the digestibility of energy, protein and lipid. These results are reflected in improvement in feed conversion. Adding both glucanase and xylanase to maize/soy-based diets has been shown to improve feed conversion and ileal nutrition digestibility (Cowieson *et al.*, 2010). In addition, Viveros *et al.* (1994) and Jaroni *et al.* (1999) found that adding enzyme in animal diets improved intestinal morphology and integrity, which resulted in enhanced digestion and absorption of dietary components. However, some previous studies founded that supplementation of exogenous plant cell walls, glucanase and xylanase, showed no effect on improving nutritive values of alfalfa-containing diets in layer hens. Such supplementation could not significantly improve layer performance (Mourao *et al.*, 2006). Similar results were also witnessed this study. Supplementation of phytase, xylanase and cellulase exhibited a little improvement on layer production performance because nowadays, some enzymes especially fibrolytic enzymes such as xylanase and cellulase including phytase are already added in some commercial diets. Moreover, the physiology of layer hens' digestive tracts is better at digesting fibers than those of broilers, which in turn will absorb more nutrients. Hence, the concentration of enzyme supplementation in diets needs to be taken into consideration. It is clear that as for the GTW supplementation groups in this study, fibrolytic enzymes including xylanase, cellulase and phytase could digest fibers and phytate, release more nutrients of sugar from fibers, release proteins, amino acids and minerals from phytate (See Ta-

ble 2). Therefore, animals receive more energy and nutrients, which is demonstrated in the decreasing of layer feed intake in the GTW0E1, GTW0E2, GTW1E1 groups and especially in the GTW1E2 group that saw a lower FCR of layers.

### Egg Quality

Egg quality is presented in Table 4. The results of enzyme and GTW supplementation showed a significant effect ( $p < 0.05$ ) on egg quality. Supplemental enzymes and GTW significantly ( $p < 0.05$ ) improved Haugh unit (index of egg internal quality), specific gravities of eggs and the antioxidative property of eggs (i.e. reducing lipid oxidation). Haugh unit was significantly high in all GTW1 groups especially the GTW1E2 group and a high dosage of enzyme E2 presented a rather high Haugh unit than E1 and E0 groups. The specific gravity was low ( $p < 0.05$ ) in GTW1 group, with the lowest level ( $p < 0.05$ ) witnessed in GTW1E2 group. An antioxidant property of eggs was investigated in term of TBAs value (lipid oxidation value). TBAs value was significantly affected ( $p < 0.05$ ) by enzyme. TBAs value was lowest in the GTW1E2 group and the highest TBAs value was found in control (GTW0E0) group.

Supplementation of GTW in the diet significantly increased ( $p < 0.05$ ) Haugh unit. It was also found that lipid oxidation in term of thiobarbituric acid reactive substance value (TBAs) decreased in the GTW1E2 group. Generally, nutrients in layer diets do not appear to have any effects on Haugh unit (Naber, 1979), but certain natural antioxidants such as vitamin C, vitamin E and selenium benefit albumen quality in term of the antioxidant property (Keshavarz, 1996; Sahin *et al.*, 2003).

Green tea waste in the present experiment contained 0.01 % of tannin, 203.40 mg/kg of carotenoid and 56.56% of DPPH scavenging. These results are in line with those in Varilek *et al.* (2001) and also Wilhelm and Helmut (2003). They reported that the beneficial effects of green tea came from its contents of catechins, flavonoid, phenolic extracts and carotenoids. The active substances of green tea can act as a reducing agents, hydrogen donator, and superoxide radical scavengers. Carotenoids are high antioxidants for scavenging singlet oxygen molecules and peroxy radicals. This mechanism could be a possible mechanism for increasing Haugh unit due to the antioxidant effect from

tea polyphenols. This finding is in accordance with Abdo *et al.* (2010), who found that adding green tea powder in laying hen diets for 1 to 3 % or green tea extracts at 0.5 to 1.5 L/100 kg can reduced thiobarbituric acid values of egg yolk, implying its potential effect on egg quality parameters, especially during storage. Similar results are witnessed in AlHarthi (2014) who found that supplementing green tea for 1 g per 1 kg in layer diets can improve Haugh unit score (3.5%) of stored eggs when compared to the control group and green tea supplemented with vitamin E resulted in an improvement in Haugh unit score (2.4%) of fresh eggs and Haugh unit score (10.9%) of stored eggs. It is possible that transfer of the antioxidant constituents of GTW through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into eggs and reducing TBAs value. Egg specific gravity (indirect measurements of shell strength) was lowest in the GTW1E2 group ( $p < 0.05$ ), may lower nutrient availability, especially of calcium and phosphorus through the intestines during shell formation due to the use of GTW containing tannin. Tannin is a group of polyphenols which can bind protein then reduced digestibility of nutrients (Mueller-Harvey, 2006; Bravo, 1998; Jansman, 1993). Tannin also has a bacterial inhibition property (Sakanaka *et al.*, 2000). However, phytase supplementation has been shown to improve eggshell quality, and the effect of phytase supplementation depends on the levels of calcium and nonphytate phosphorus in the diet (Hatten *et al.*, 2001; Jamroz *et al.*, 2003; Lim *et al.*, 2003).

### CONCLUSION

Supplementation of 1 % GTW, 24,000 FAXU xylanase, 100 ECU cellulase and 1,000 FTU phytase (GTW1E1) in layer diet is recommended to improve egg performance as it increased egg production and egg mass. A high dosage of 1 % GTW supplemented with 48,000 FAXU xylanase, 200 ECU cellulase, and 2,000 FTU phytase (GTW1E2) in the layer diet is recommended for enhancing layer performance in terms of improving feed conversion by reducing feed intake, feed cost/kg of eggs, TBAs value, and increasing Haugh unit.



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