

**RESPONSES OF LAYING HENS TO DIFFERENT LEVELS OF AMOXICILLIN, HOT PEPPER OR GREEN TEA AND THEIR EFFECTS ON PRODUCTIVE PERFORMANCE, EGG QUALITY AND CHEMICAL COMPOSITION OF YOLK AND BLOOD PLASMA CONSTITUENTS**

By

**M. A. Al-Harhi**

King Abdulaziz Univ., Fac. of Meteorology, Envir. and Arid Land Agric., Saudi Arabia

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**Abstract:** *The objective of this work was to study the impact of adding different levels of Amoxicillin (20 and 40 mg/kg), hot pepper (0.1, 0.2 and 0.3%) and green tea (0.1, 0.2, 0.3 and 0.4%) to laying hen diets on productive performance, egg quality, and chemical composition of yolk and plasma. One hundred and fifty Hisex laying hens were fed the experimental diets from 20 to 44 wks of age. The control diet was fed without the aforementioned levels of Amoxicillin, hot pepper and green tea. Hens were divided into 10 experimental groups. Each diet was fed to three replicates of 5 Hisex laying hens. Results could be summarized as follows:*

- 1- Addition of 0.1% of green tea significantly improved egg weight by 7.3%, also Amoxicillin, 0.2 and 0.3% of hot pepper and 0.2 and 0.4% of green tea also significantly improved egg weight, but by less extent.*
- 2- Addition of 0.2% hot pepper or green tea yielded significantly better egg production and egg mass than the control group and being equal potent as.*
- 3- Adding 0.2% of green tea to hen diets resulted in higher oviduct weight than the control group and this connected with increasing rate of laying of this group.*
- 4- It was found that diet supplemented with 0.2% of hot pepper significantly improved shell thickness as compared to the control group.*
- 5- Yolk color of fresh eggs laid at 24 wks of age was significantly improved by included hot pepper and green tea in the laying diets and linearly increased with increasing their levels.*
- 6- Amoxicillin, hot pepper and green tea significantly decreased the loss in weight of eggs of 40 wks old hens stored under normal (room) environmental condition compared to the control group.*

- 7- *Green tea significantly improved yolk color and albumen height of eggs of 40 wks old hens stored under normal (room) environmental condition compared to the control diet.*
- 8- *Amoxicillin, hot pepper and green tea significantly increased yolk height of eggs of 40 wks old hens stored under refrigerating condition compared to the control group.*
- 9- *Amoxicillin at 20mg and any level of hot pepper and 0.3 and 0.4% of green tea significantly decreased yolk lipids of eggs of 24 wks old hens stored in the refrigerator as compared to the control groups.*
- 10- *Yolk cholesterol was significantly decreased linearly with increasing the level of hot pepper and green tea in the laying diets of eggs collected at 24 wks of age and stored in refrigerator.*
- 11- *Yolk lipids and cholesterol of 24 wks old hens of eggs stored in air conditioning were lower of hot pepper and green tea supplemented group, while yolk protein was significantly higher of groups fed diets supplemented with 0.2% of hot pepper and green tea as well as 0.1 and 0.3% of green tea as compared to the control group.*
- 12- *Yolk lipid increased significantly at any level of hot pepper and green tea was supplemented to the diets of laying hens, while cholesterol was significantly lower of fresh eggs of 40 wks old hens fed diets containing 0.3% hot pepper.*
- 13- *Yolk cholesterol increased significantly of eggs of 40 wks old hens stored for 15 days in refrigerator when 40 mg of antibiotic and 0.2% of hot pepper were included.*
- 14- *Plasma lipids decreased significantly when hot pepper was supplemented at any tested level compared to the control group, and the same was shown when 0.1% of green tea was included in the diets for laying hens.*
- 15- *Plasma cholesterol decreased significantly when 40 mg of antibiotic, and any tested level of hot pepper or green tea was included in the diets for laying hens.*
- 16- *Plasma AST and ALT was not affected by Amoxicillin inclusion and hot pepper and green tea in the diets for laying hens, indicating no adverse effects on liver and intestinal function.*

It was concluded that 0.20% of hot pepper or green tea could serve as non conventional feed additive in the diets for laying hens. Plasma total

## Hot Pepper, Green Tea, Amoxicillin, Laying Hens, Egg Quality, Cholesterol

lipids and cholesterol significantly decreased when hot pepper and green tea were supplemented without adverse effect on liver and intestinal functions. Also, there were evidences indicating that hot pepper and green tea decreased yolk cholesterol and can decrease the losses in the weight of eggs stored under room temperature.

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## INTRODUCTION

Some research works have been conducted lately about the using of non conventional feed additives as growth enhancers in broiler nutrition (El-Husseiny et al., 2002; Al-Harathi, 2002a and b). However, little have been carried out with laying hens perhaps due to the better adaptability and maturity of gastrointestinal tract of laying hens as compared to those of broiler chicks. Mechanisms of feed additives may include improving nutrient digestibility, controlling of pathogenic micro-organisms and facilitating for a favorable intestinal microbial balance, and enhancing absorption of calorogenic nutrients across the gut wall through increasing its absorption capacity (Nelson *et al.*, 1963).

Improving the rate of laying and feed conversion ratio (FCR) as a result of addition of different feed additives to layer diets were observed (Miles *et al.*, 1985; Guerrero and Hoyos, 1991; Nahashon *et al.*, 1993; Dorgham *et al.*, 1994 and Fayek *et al.*, 1995). Due to the growing public concerns about residuals of antibiotics in animal products (Heitzman, 1986), and despite the lack of empirical evidence, many restrictions and bans have been placed on the use of antibiotics in livestock feeding in Europe. Therefore, many scientists are searching alternatives to antibiotics for commercial use in animal nutrition (El-Husseiny *et al.*, 2002).

Hot or chili pepper (Paprika) has terpenoid compound capsaicin which has antibacterial proprieties. It contains 14.12% crude protein CP, 15.91% crude lipid, 23.02% crude fibre, and 7.48% ash and 31.87% total soluble sugars. Also it is a good source of vitamins C and E, and pro-vitamin A (El-Aidy, 1981; Cowan, 1999). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption (Cowan, 1999). A terpenoid constituent, capsaicin, has a wide range of biological activities in humans, affecting the nervous, cardiovascular, and digestive system and finding use as an analgesic (Virus and Gebhart, 1979 and Cordell and Araujo, 1993). Although, possibly detrimental to the human gastric mucosa, capsaicin is also bactericidal to *Helicobacter pylori* (Jones et al., 1997). Vogt *et al.* (1989), El-Husseiny *et al.* (2002) and Al-Harathi (2002b) found that broiler chicks fed hot pepper

containing-diet had improved growth and FCR. The positive effect of hot pepper may be due to its stimulant, carminative, digestion and antimicrobial properties (Jones *et al.*, 1997, El-Husseiny *et al.*, 2002).

Green tea leaves (*Camellia sinensis*) contain antioxidative catechins consisting of various flavan or polyphenols (Miura *et al.*, 2001; Varilek *et al.*, 2001). Tea catechins have a variety of pharmacologic effects, *i.e.* antioxidative (Lin *et al.*, 1996), antimutagenic (Jain *et al.*, 1989), anticarcinogenic (Sano *et al.*, 1999), antimicrobial (Yoshino *et al.*, 1996), hypolipidemic effects (Yoshino *et al.*, 1996), and anti-inflammatory effects (Varilek *et al.*, 2001). Recently Miura *et al.* (2001) reported that ingestion of green tea improved growth of mice and did not affect plasma cholesterol and triglyceride concentrations however; plasma peroxides were reduced indicating that *in vivo* oxidative state is improved by tea ingestion. Moreover, aortic cholesterol and triglyceride contents were 27 and 50% lower, respectively in tea group than in the control group. Thus, the aim of this study was to investigate the impact of hot pepper and green tea as non traditional feed additives as compared to Amoxicillin on laying hen performance, egg quality, and yolk composition as well as plasma constituents.

## MATERIALS AND METHODS

### **Birds, Housing and Management:**

This experiment was conducted at King Abdulaziz University, Faculty of Meteorology Environmental and Arid Land Agriculture. Hisex laying hens were housed in cages under similar managerial and hygienic conditions. A mash laying diet and water were offered *ad libitum* from tube feeders and automatic nipple drinkers, respectively with a 16 *hrs* lighting program. Diets (Table 1) were formulated based on NRC (1994) tables of feedstuffs and requirements for laying hens. Laying hens were randomly distributed to the experimental groups with keeping approximately equal initial live body weight and rate of laying at the time of initiation of the experimental. Number of dead birds was recorded daily.

### **Experimental Design:**

The objective of this work was to study the impact of different levels of Amoxicillin (20 and 40 mg/kg, of feed), hot pepper (0.1, 0.2 and 0.3%) and green tea (0.1, 0.2, 0.3 and 0.4%, of feed) on laying performance, egg quality, chemical composition of yolk and plasma constituents. Hens were fed the experimental diets from 20 to 44 wks of age, control diet was fed

without the aforementioned dosages of Amoxicillin, hot pepper and green tea. There were 10 experimental treatments. Each treatment was represented by 3 replicates of 5 hens each, as a total of 150 hens.

### **Measurements:**

Laying hens were weighed at 20, and 44 wk of age, and daily egg production, egg weight, egg mass, was recorded and percent hen day egg production (HD) was calculated. At the end of the trial, 3 females of each treatment were randomly chosen from each treatment, weighed after fasted for 12 hours, slaughtered, and only the oviduct was separated and weighed.

Eggs laid on one day were used to determine egg quality at 24 wk, and 40 wk of age. Eggs were weighed and egg weight (EW) was recorded and then eggs were broken out and shell thickness (mm), shell weight (SW), yolk weight, height and diameter and albumen weight, and height were recorded. Shells were washed, air dried, and weighed to the nearest 0.1 g. Yolk and Albumen height was measured using a tripod micrometer reading to the nearest 0.01 mm. Yolk color was measured using Hoffmann La Roche yolk color fan. Egg and yolk diameter were measured using caliber. The eggs used for measuring egg quality at 40 wks of age were stored for 15 days in normal (room) environmental condition, or in a refrigerator (-4°C). Visual examination of albumen condition was carried out to score the condition of albumen as watery, normal, good and excellent.

Yolk samples of eggs used for determination of egg quality at 24 and 40 wks of age and stored under different environmental conditions were chemically analyzed for moisture, protein, ether extract and ash on fresh basis according to AOAC (1990). Cholesterol content as (mg/100g fresh weight) of yolk was determined according to Rutkowski and Krygier (1979).

In order to study the effect of spices and Amoxicillin on plasma constituents of laying hens, blood samples were collected from five hens/treatment in heparinized tube at 40 week of age. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Concentrations of plasma total protein (Weichselbaum, 1946; Henry *et al.* 1974), total lipid (Chabrol and Charonnat, 1973), total cholesterol (Watson, 1960), ALT and AST (Retiman and Frankel, 1957), Ca (Sendroy, 1944), P (Gomorri, 1942) were determined.

### **Statistical Analysis:**

Data were analyzed using the GLM procedure of SAS® (SAS Institute, 1985) using nested design with types of additives (antibiotic, hot pepper vs. green tea) and level within each type with keeping the control diet that was fed diet without such additives as mean effects. Duncan's New Multiple Range Test was used to test mean differences at  $P \leq 0.05$  (Duncan, 1955).

## **RESULTS AND DISCUSSION**

### **Effect of different levels of Amoxicillin, hot pepper and green tea on laying hen performance and oviduct weight:**

Results shown in Table 2, indicated that Amoxicillin at 20 or 40 mg, hot pepper at 0.2, and 0.3%, and green tea at 0.1, 0.2, 0.3 and 0.4% significantly increased egg weight as compared to the control group. However, the greatest increase in egg weight (7.3%) was recorded by group fed diet supplemented with 0.1% of green tea. Meanwhile, there was no significant effect of antibiotic, hot pepper and green tea on egg weight as compared to the control group when data were pooled over levels.

Rate of laying was significantly improved when antibiotic was added at 40 mg, or with addition of 0.1 or 0.2% of hot pepper. The same was also observed when green tea was added to the layer diet at 0.2, 0.3 and 0.4%. Meanwhile, 0.2% of hot pepper or green tea recorded the highest increase in laying rate, 13.4, 12.6 %, respectively as compared to the control group. Irrespective of level of feed additives, Amoxicillin, hot pepper and green tea significantly improved laying rate by 5, 5.2, and 5.4%, respectively as compared to the control group.

Egg mass was significantly higher for groups fed diets supplemented with 40 mg of Amoxicillin, 0.1, 0.2 and 0.3% of hot pepper and 0.1, 0.2, 0.3 and 0.4% of green tea as compared to the control group and group fed diet supplemented with 20 mg of antibiotic (Table 2). In general, groups fed diets supplemented with 40 mg of Amoxicillin, and 0.2% of hot pepper or green tea yielded the greatest improvement in egg mass, 12.7, 17.1 and 15% respectively compared to the control group. When data were pooled over levels, results indicated that either antibiotic, hot pepper, and green tea yield significantly higher egg mass of 7.3, 6.9 and 8.4%, respectively than the control group. The improvements in egg production and egg mass due to feeding Amoxicillin, hot pepper and green tea could be attributed to their antimicrobial, antioxidant and improving nutrient utilization (Nelson, 1963;

Grieve, 1995; Jones *et al.*, 1997; El-Husseiny *et al.* 2002, Yoshino *et al.* 1996; Miura *et al.*, 2001).

Also, Miles *et al.* (1985), Guerrero and Hoyos (1991), Nahashon *et al.* (1993), Dorgham *et al.* (1994), and Fayek *et al.* (1995) reported that laying performance was improved in response to diet supplementation with feed additives including antibiotic, enzymes and direct fed microbial. Also, results from broiler research (El-Husseiny *et al.*, 2002, and Al-Harthi, 2002 a and b) indicated that hot pepper was as an effective agent as antibiotic for improving growth performance of broilers.

Oviduct weight was significantly affected by types and level within types of feed additives (Table 2). Results indicated that feeding diet supplemented with 20 or 40 mg of Amoxicillin, 0.1, and 0.2% of hot pepper, and 0.1, 0.2 and 0.3% of green tea significantly increased oviduct weight as compared to the control group. On the other hand, group fed diet supplemented with 0.4% of green tea had significantly lower oviduct weight than the control group. Meanwhile, group fed diet supplemented with 0.3% hot pepper had similar oviduct weight to that of the control group. The highest oviduct weight was recorded by group fed diet supplemented with 0.2% of green tea and hot pepper, and which had higher laying rate of these particular groups (Table 2).

It could be concluded that 0.2% of hot pepper or green tea is effective as Amoxicillin in improving laying rate and egg mass of laying hens, indicating that natural feed additives such as hot pepper or green tea could be used as alternative to the antibiotic Amoxicillin in laying hens diets.

**Effect of different levels of Amoxicillin, hot pepper and green tea on the quality of fresh eggs and those stored under different environmental conditions:**

Data for egg quality measured at 24 wks of age as influenced by different levels of Amoxicillin, hot pepper and green tea are shown in Table (3). There were insignificant interactions between levels of feed additives and types of feed additives on egg weight, shell weight, yolk, albumen weight, and length of egg. Also, there were insignificant effects of types of feed additives as independent variables on these criteria except for egg weight.

Width of eggs was significantly higher of groups fed diets supplemented with 40 mg of antibiotic, 0.2% of hot pepper, and 0.2, 0.3 and



0.4% of green tea as compared to the control group. Irrespective of levels of feed additives, egg width was significantly higher for group fed diets supplemented with antibiotic, hot pepper, and green tea than the control group. Also, group fed diet supplemented with 0.2% of hot pepper had significantly higher shell thickness than control group or groups fed diets supplemented with 20 or 40 mg Amoxicillin.

Yolk color was improved significantly by feeding diets supplemented with 40 mg of antibiotic, and linearly increased by increasing the level of hot pepper and green tea. Moreover, yolk color of groups fed hot pepper or green tea supplemented-diets was significantly higher than the control and antibiotic supplemented-groups, regardless of level of feed additives. The improvements in yolk color of hot pepper and green tea supplemented groups may be due to pigment contents of hot pepper and green tea as well as the antioxidative activity of green tea (Miura *et al.*, 2001).

Quality of the eggs stored in a refrigerator for 15 days as affected by different levels and types of feed additives are shown in Table (4). Results indicated that percentage of loss in egg weight was only significantly higher in the group fed the diet supplemented with 0.3% of green tea than all other experimental groups. Also, group fed diet supplemented with 0.1 or 0.4% green tea showed significantly higher percentage loss in weight of eggs than those fed diet supplemented with 20 or 40 mg Amoxicillin. It seems that addition of Amoxicillin at 20 or 40 mg resulted in the lowest rate in loss of weight of eggs stored in refrigerator. Regardless of level of feed additives, green tea yield the highest losses in egg weight compared with other groups, whereas, Amoxicillin showed the lowest value, with no significant differences between the control group and hot pepper supplemented ones. This may be due to the antimicrobial action of Amoxicillin which could limit both germ positive and negative bacteria.

Yolk color of groups supplemented with 0.3 or 0.4% of green tea was significantly higher as compared to all other experimental groups. On the other hand, group fed diet supplemented with 20 mg of Amoxicillin or with 0.1% or 0.2% of hot pepper and 0.1% of green tea had significantly lower yolk color than the control group (Table 4). There were no significant differences in yolk color among groups fed diets supplemented with 40 mg of antibiotic, 0.3% hot pepper or 0.2% of green tea.

Haugh unit was not affected by types of feed additives or the level with each additives, indicating that antibiotic, hot pepper or green tea had no additive effects on the albumen quality of eggs stored under refrigeration.

Visual examination of albumen indicated that all groups displayed better condition of albumen except for group fed diet supplemented with 0.4 of green tea which appears watery (Table 4).

**Effect of different levels of Amoxicillin, hot pepper and green tea on the chemical composition and cholesterol content of yolk of eggs and that stored for 15 days in the refrigerator:**

Data for chemical composition and cholesterol of eggs stored in a refrigerator of 24 wks of old hens as influenced by different levels of Amoxicillin, hot pepper and green tea are shown in Table (5). There was a significant interaction between level of feed additives and types of feed additives on percentage moisture, protein, lipids, ash percentage and cholesterol (mg/ 100g.).

Results indicated that moisture percentage was significantly increased when 20 mg of Amoxicillin was supplemented. On the other hand, it was significantly decreased when 40 mg of the same antibiotic was added as compared to the control group. Only yolk protein percentage was significantly decreased in the group fed the 20 mg Amoxicillin (Table 5). Feeding diets supplemented with 20 mg of antibiotic, 0.1, 0.2 and 0.3% of hot pepper, and 0.3 and 0.4% of green tea resulted in significant decrease in yolk lipids compared to the control group. On the other hand, feeding diets supplemented with 20 or 40 mg of Amoxicillin, and increasing the supplementation level of hot pepper or green tea decreased linearly yolk cholesterol significantly as compared to the control diet. Irrespective of level of feed additives, feeding Amoxicillin and hot pepper supplemented-diets decreased yolk lipids, while antibiotic, hot pepper and green tea resulted in also significant decrease in yolk cholesterol and yolk ash (Table 5).

Irrespective of level of feed additives, it was found that Amoxicillin, hot pepper and green tea resulted in insignificantly higher yolk moisture as compared to the control group. Feeding Amoxicillin, hot pepper and green tea supplemented-diets decreased yolk lipids significantly compared to the control group,

The decrease in yolk lipids of hot pepper fed-groups and cholesterol of hot pepper and green tea supplemented-groups of eggs of 24 wks old hens is interesting findings for public health benefits. These results are in agreement with those of Saito *et al.* (1999) who suggested that a single high

dose of treatment of capsaicin may inhibit the absorption of lipid from the gastrointestinal tract. Also, Yoshioka et al. (1998) indicated that capsaicin enhanced energy metabolism by increasing the catecholamine secretion of the adrenal medulla, mainly through activation of the central nervous system. Regarding the effect of green tea Miura *et al.* (2001) observed that green tea decreased aortic cholesterol and triglyceride contents by 27 and 50% compared to the control group, and these may be through the potent antioxidative activity of the tea.

Data for chemical composition and cholesterol of fresh eggs of 40 wks of old hens as influenced by different levels of Amoxicillin, hot pepper and green tea are shown in Table (6). There was significant interaction between level of feed additives and types of feed additives on percentage of moisture, lipids, ash and cholesterol. On the other hand, feeding diets supplemented with 40 mg of Amoxicillin, 0.1 and 0.2% of hot pepper or 0.2 and 0.4% of green tea resulted in higher yolk cholesterol than the control group as well as 20 mg of antibiotic, 0.1 and 0.3% of green tea. Results also indicated that feeding 0.3% hot pepper containing-diet significantly decreased yolk cholesterol compared with all experimental groups (Table 6).

Data for chemical composition and cholesterol of yolks of eggs stored in refrigerator of 40 wks of old hens as influenced by different levels of Amoxicillin, hot pepper and green tea are shown in Table (7). There was significant interaction between level of feed additives and types of feed additives on percentage of moisture, lipids, ash and cholesterol of yolk.

Irrespective of level of feed additives, results indicated that Amoxicillin and hot pepper resulted in significantly higher percentage yolk moisture, ash, and cholesterol. However, lipids percentage of yolk was significantly decreased compared to the control group of group fed diets supplemented with antibiotic and hot pepper. Also, green tea supplemented diets resulted in significantly higher percentage yolk ash than the control group (Table 7).

#### **Effect of different levels of Amoxicillin, hot pepper and green tea on plasma constituents of laying hens:**

Data for plasma constituents of 40-weeks old laying hens as affected by different levels of Amoxicillin, hot pepper and green tea are displayed in Table (8). There were significant interactions between Amoxicillin and natural feed additives on plasma total lipids, and cholesterol of laying hens. Results indicated that plasma total lipids was significantly decreased when hens were fed diets supplemented with 0.1, 0.2 and 0.3% of hot pepper or

0.1% of green tea. Regardless of level of feed additives, results indicated that hot pepper significantly decreased plasma total lipids by 4.2% only when compared to the control group (Table 8).

It is interesting to report that addition of 40 mg of Amoxicillin or supplementation with any levels of hot pepper or green tea significantly decreased plasma cholesterol of 40-wk old laying hens as compared to the control group and 20 mg amoxicillin supplemented-diet. Plasma cholesterol was significantly decreased when antibiotic, hot pepper and green tea were fed when compared to the control group, irrespective of level of feed additives, with hot pepper being more effective 15.2% vs. 8.0% and 10.7% of groups fed diets supplemented with Amoxicillin and green tea, respectively. These results are in agreement with those reported by El-Husseiny *et al.* (2002) who found that total lipids and cholesterol level in plasma of broilers fed hot pepper-supplemented feed was lower than the those fed the control diet. Also, some researchers indicated that hot pepper had hypoglycemic or hypocholesterolemic effect (Matsuo *et al.*, 1996). Also, results by Saito *et al.* (1999) suggested that a single high dose of capsaicin may inhibit the absorption of lipid from the gastrointestinal tract. Also, the decrease in plasma cholesterol of green tea fed group are in general agreement with those reported by Miura *et al.* (2001) who showed that tea ingestion did not influence plasma cholesterol or triglyceride concentrations of mice, however, aortic cholesterol and triglyceride and plasma lipid peroxides were reduced in the tea group, suggesting potent antioxidative activity of tea.

Plasma AST and ALT were not significantly affected by different types of levels of feed additives (Table 8), showing no adverse effect Amoxicillin, hot pepper and green pepper on liver and intestinal functions. These results disagree with those reported by El-Husseiny *et al.* (2002) who found that hot pepper increased plasma GOT and GPT compared to the control group, and this may be due to the higher level of 1% used by these authors. On the other side, the detrimental effect of capsaicin on gastric mucosa was reported by (Jones *et al.*, 1997).

It is concluded that hot pepper and green tea significantly decreased plasma lipids and cholesterol and had no negative effect on liver and intestinal functions. The decrease in plasma lipid and cholesterol of hot pepper supplemented groups might be beneficial for human health.

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**Table (1): Composition and calculated analyses of the experimental diet**

Ingredients, %	Laying diet
Yellow corn	66.33
Soybean meal (48%CP)	24.20
Limestone	7.50
Dicalcium phosphate	1.32
Vit+Min mix <sup>1</sup>	0.25
NaCl	0.25
DL-methionine	0.15
Total	100.0
Calculated values	
ME kcal/kg diet	2775
Crude protein,%	17.26
Methionine,%	0.44
TSAA,%	0.71
Lysine,%	0.83
Ca,%	3.20
Available P, %	0.36

<sup>1</sup>Vitamins and minerals mixture provide per kilogram of diet: vitamin A (as all-trans-retinyl acetate); 12000 IU; vitamin E (all rac- $\alpha$ -tocopheryl acetate); 10 IU; k<sub>3</sub> 3mg; Vit.D<sub>3</sub>, 2200 ICU; riboflavin, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; choline chloride, 500 mg; vitamin B<sub>12</sub>, 10 $\mu$ g; vitamin B<sub>6</sub>, 1.5 mg; thiamine (as thiamine mononitrate); 2.2 mg; folic acid, 1 mg; D-biotin, 50 $\mu$ g. Trace mineral (milligrams per kilogram of diet) : Mn, 55; Zn, 50; Fe, 30;Cu, 10; Se, .1 and Ethoxyquin 3mg.

Hot Pepper, Green Tea, Amoxicillin, Laying Hens, Egg Quality, Cholesterol

**Table (2): Effect of different levels of Amoxicillin, hot pepper and green tea fed to hens from 20 – 44 wks on egg production traits and body weight and oviduct weight**

Treatment	Egg weight, g	Egg production,%	Egg mass. kg	Initial body weight, g	Final body weight, g	Oviduct weight, g
Additive × level						
Control	59.0 <sup>c</sup>	80.3 <sup>c</sup>	8.53 <sup>c</sup>	1542	1630 <sup>ab</sup>	90.4 <sup>f</sup>
Amoxicillin mg/kg						
20						
40	60.7 <sup>b</sup>	79.4 <sup>c</sup>	8.66 <sup>c</sup>	1545	1613 <sup>b</sup>	94.7 <sup>c</sup>
Hot pepper,%						
0.1	60.0 <sup>b</sup>	89.1 <sup>a</sup>	9.61 <sup>a</sup>	1551	1634 <sup>ab</sup>	94.1 <sup>e</sup>
0.2	58.6 <sup>c</sup>	83.3 <sup>b</sup>	8.79 <sup>b</sup>	1513	1634 <sup>ab</sup>	107.7 <sup>c</sup>
0.3	61.0 <sup>b</sup>	91.1 <sup>a</sup>	9.99 <sup>a</sup>	1555	1642 <sup>ab</sup>	118.3 <sup>b</sup>
Green tea,%						
0.1	60.3 <sup>b</sup>	79.8 <sup>c</sup>	8.66 <sup>b</sup>	1538	1638 <sup>ab</sup>	88.6 <sup>f</sup>
0.2	63.3 <sup>a</sup>	78.3 <sup>c</sup>	8.92 <sup>b</sup>	1542	1623 <sup>b</sup>	103.1 <sup>d</sup>
0.3	60.3 <sup>b</sup>	90.4 <sup>a</sup>	9.81 <sup>a</sup>	1554	1616 <sup>b</sup>	106.6 <sup>a</sup>
0.4	60.1 <sup>b</sup>	87.6 <sup>a</sup>	9.39 <sup>ab</sup>	1512	1667 <sup>a</sup>	105.5 <sup>c</sup>
	60.0 <sup>b</sup>	82.5 <sup>b</sup>	8.92 <sup>b</sup>	1537	1648 <sup>ab</sup>	83.1 <sup>g</sup>
Additive effect						
Control	59.0	80.3 <sup>b</sup>	8.53 <sup>b</sup>	1542	1630	90.4 <sup>b</sup>
Amoxicillin	60.3	84.3 <sup>a</sup>	9.15 <sup>a</sup>	1548	1623	94.4 <sup>b</sup>
Hot pepper	59.9	84.5 <sup>a</sup>	9.12 <sup>a</sup>	1535	1638	104.9 <sup>ab</sup>
Green tea	60.9	84.6 <sup>a</sup>	9.25 <sup>a</sup>	1536	1614	119.5 <sup>a</sup>
SEM	3.35	2.88	0.53	43.1	39.6	33.30
ANOVA						
Additive × level	**	***	***	NS	NS	***
Additive	*	**	**	NS	NS	**

\*<sup>c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

**Table (3): Effect of different levels of Amoxicillin, hot pepper and green tea on egg quality of fresh eggs determined at 24 wk of age**

	Egg weight, g	Egg length, cm	Egg width, cm	Shell thickness, mm	Shell weight, g	Yolk weight, g	Yolk color
Additive × level							
Control	50.6	5.34	4.00 <sup>b</sup>	0.33 <sup>b</sup>	6.43	13.13	6.33 <sup>d</sup>
Amoxicillin mg/kg							
20	51.7	5.56	4.23 <sup>ab</sup>	0.33 <sup>b</sup>	6.23	13.03	6.33 <sup>d</sup>
40	51.5	5.76	4.40 <sup>a</sup>	0.33 <sup>b</sup>	6.33	13.13	7.00 <sup>c</sup>
Hot pepper,%							
0.1	51.9	5.53	4.20 <sup>ab</sup>	0.34 <sup>ab</sup>	6.36	12.56	7.00 <sup>c</sup>
0.2	51.7	5.53	4.30 <sup>a</sup>	0.35 <sup>a</sup>	6.33	12.43	7.33 <sup>b</sup>
0.3	52.1	5.40	4.23 <sup>ab</sup>	0.34 <sup>ab</sup>	6.36	12.63	8.15 <sup>ab</sup>
Green tea,%							
0.1	51.8	5.40	4.20 <sup>ab</sup>	0.34 <sup>ab</sup>	6.36	12.66	7.34 <sup>b</sup>
0.2	52.3	5.73	4.33 <sup>a</sup>	0.34 <sup>ab</sup>	6.40	12.70	8.00 <sup>b</sup>
0.3	51.9	5.66	4.30 <sup>a</sup>	0.34 <sup>ab</sup>	6.46	12.73	8.30 <sup>ab</sup>
0.4	52.9	5.67	4.33 <sup>a</sup>	0.34 <sup>ab</sup>	6.46	13.30	9.00 <sup>a</sup>
Additive effect							
Control	50.60 <sup>b</sup>	5.34	4.00 <sup>b</sup>	0.33 <sup>b</sup>	6.43	13.13	6.33 <sup>b</sup>
Amoxicillin	51.50 <sup>ab</sup>	5.66	4.31 <sup>a</sup>	0.33 <sup>b</sup>	6.28	13.08	6.66 <sup>b</sup>
Hot pepper	51.90 <sup>ab</sup>	5.48	4.24 <sup>a</sup>	0.34 <sup>ab</sup>	6.35	12.54	7.55 <sup>a</sup>
Green tea	52.24 <sup>a</sup>	5.61	4.29 <sup>a</sup>	0.35 <sup>a</sup>	6.42	12.58	8.13 <sup>a</sup>
SEM	1.14	0.27	0.13	0.007	0.26	0.59	0.66
ANOVA							
Additive × level	NS	NS	***	***	NS	NS	**
Additive	**	NS	**	**	NS	NS	**

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

**Table (4): Effect of different levels of Amoxicillin, hot pepper and green tea on egg quality of eggs collected at 40 wks of age and kept in refrigerator for 15 days**

Treatment	Egg weight loss,%	Yolk color	Haugh unit	Albumen condition
<b>Additive × level</b>				
Control	2.71 <sup>bc</sup>	5.67 <sup>b</sup>	78.3	Normal
Amoxicillin mg/kg				
20	1.80 <sup>c</sup>	4.34 <sup>c</sup>	76.6	Excellent
40	1.75 <sup>c</sup>	5.00 <sup>b</sup>	78.0	Excellent
Hot pepper,%				
0.1	2.61 <sup>bc</sup>	4.00 <sup>c</sup>	83.3	Excellent
0.2	2.77 <sup>bc</sup>	4.00 <sup>c</sup>	84.3	Excellent
0.3	2.05 <sup>bc</sup>	5.00 <sup>b</sup>	70.6	Excellent
Green tea,%				
0.1	3.79 <sup>b</sup>	4.00 <sup>c</sup>	83.3	Excellent
0.2	2.56 <sup>bc</sup>	5.67 <sup>b</sup>	86.6	Excellent
0.3	4.90 <sup>a</sup>	6.63 <sup>a</sup>	80.0	Excellent
0.4	3.88 <sup>b</sup>	6.65 <sup>a</sup>	76.6	Watery
<b>Additive effect</b>				
Control	2.71 <sup>b</sup>	5.67 <sup>a</sup>	78.3	ND
Amoxicillin	1.78 <sup>c</sup>	4.66 <sup>ab</sup>	77.3	ND
Hot pepper	2.48 <sup>b</sup>	4.33 <sup>b</sup>	79.4	ND
Green tea	3.78 <sup>a</sup>	5.75 <sup>a</sup>	81.6	ND
SEM	0.12	0.40	8.65	ND
<b>ANOVA</b>				
Additive × level	***	***	NS	ND
Additive	**	***	NS	ND

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant



**Table (5): Effect of different levels of Amoxicillin, hot pepper and green tea on chemical composition of yolks of eggs collected at 24 wks of age and kept in refrigerator for 15 days**

Treatment	Moisture %	Protein,%	Total lipids%	Ash%	Cholesterol mg/100g
<b>Additive × level</b>					
Control	50.89 <sup>b</sup>	15.50 <sup>a</sup>	29.84 <sup>a</sup>	1.55 <sup>a</sup>	1.79 <sup>a</sup>
Amoxicillin mg/kg					
20	52.67 <sup>a</sup>	14.77 <sup>b</sup>	27.95 <sup>c</sup>	1.46 <sup>b</sup>	1.20 <sup>c</sup>
40	50.10 <sup>c</sup>	15.92 <sup>a</sup>	29.67 <sup>a</sup>	1.53 <sup>a</sup>	1.33 <sup>d</sup>
Hot pepper,%					
0.1	51.43 <sup>ab</sup>	15.57 <sup>a</sup>	28.87 <sup>b</sup>	1.47 <sup>b</sup>	1.41 <sup>c</sup>
0.2	51.92 <sup>ab</sup>	15.56 <sup>a</sup>	29.16 <sup>b</sup>	1.50 <sup>ab</sup>	1.32 <sup>d</sup>
0.3	50.65 <sup>b</sup>	15.39 <sup>a</sup>	28.66 <sup>b</sup>	1.54 <sup>a</sup>	1.26 <sup>e</sup>
Green tea,%					
0.1	50.49 <sup>b</sup>	15.62 <sup>a</sup>	30.05 <sup>a</sup>	1.50 <sup>ab</sup>	1.42 <sup>b</sup>
0.2	50.70 <sup>b</sup>	15.51 <sup>a</sup>	29.96 <sup>a</sup>	1.51 <sup>ab</sup>	1.19 <sup>e</sup>
0.3	51.62 <sup>ab</sup>	15.72 <sup>a</sup>	29.27 <sup>b</sup>	1.49 <sup>ab</sup>	1.14 <sup>f</sup>
0.4	51.64 <sup>ab</sup>	15.71 <sup>a</sup>	29.28 <sup>b</sup>	1.47 <sup>ab</sup>	1.12 <sup>f</sup>
<b>Additive effect</b>					
Control	50.89	15.50 <sup>ab</sup>	29.84 <sup>a</sup>	1.55 <sup>a</sup>	1.79 <sup>a</sup>
Amoxicillin	51.38	15.34 <sup>b</sup>	28.81 <sup>b</sup>	1.49 <sup>c</sup>	1.27 <sup>c</sup>
Hot pepper	51.30	15.50 <sup>ab</sup>	28.89 <sup>b</sup>	1.51 <sup>b</sup>	1.33 <sup>b</sup>
Green tea	51.10	15.64 <sup>a</sup>	29.63 <sup>a</sup>	1.49 <sup>c</sup>	1.22 <sup>c</sup>
SEM	5.55	0.83	3.85	0.008	0.33
<b>ANOVA</b>					
Additive × level	***	***	**	**	**
Additive	NS	**	**	*	**

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

Hot Pepper, Green Tea, Amoxicillin, Laying Hens, Egg Quality, Cholesterol

**Table (6): Effect of different levels of Amoxicillin, hot pepper and green tea on chemical composition of yolks from fresh eggs collected at 40 wks of age**

Treatment	Moisture %	Protein, %	Total lipids%	Ash%	Cholesterol mg/100 g
<b>Additive × level</b>					
Control	50.56 <sup>b</sup>	20.18 <sup>a</sup>	27.1 <sup>c</sup>	1.45 <sup>b</sup>	1.316 <sup>c</sup>
Amoxicillin mg/kg					
20	51.90 <sup>ab</sup>	18.78 <sup>ab</sup>	27.6 <sup>b</sup>	1.75 <sup>a</sup>	1.375 <sup>c</sup>
40	51.24 <sup>b</sup>	18.63 <sup>ab</sup>	28.3 <sup>ab</sup>	1.75 <sup>a</sup>	1.550 <sup>a</sup>
Hot pepper, %					
0.1	52.03 <sup>a</sup>	18.38 <sup>b</sup>	28.0 <sup>b</sup>	1.56 <sup>ab</sup>	1.566 <sup>a</sup>
0.2	51.97 <sup>a</sup>	18.17 <sup>b</sup>	28.8 <sup>ab</sup>	1.59 <sup>ab</sup>	1.471 <sup>b</sup>
0.3	51.73 <sup>ab</sup>	18.23 <sup>b</sup>	28.2 <sup>ab</sup>	1.75 <sup>a</sup>	1.263 <sup>d</sup>
Green tea, %					
0.1	51.36 <sup>b</sup>	18.60 <sup>ab</sup>	29.9 <sup>a</sup>	1.80 <sup>a</sup>	1.380 <sup>c</sup>
0.2	52.25 <sup>a</sup>	18.21 <sup>b</sup>	27.8 <sup>b</sup>	1.54 <sup>ab</sup>	1.454 <sup>b</sup>
0.3	51.94 <sup>ab</sup>	18.16 <sup>b</sup>	28.7 <sup>ab</sup>	1.71 <sup>a</sup>	1.368 <sup>c</sup>
0.4	51.23 <sup>c</sup>	18.33 <sup>b</sup>	28.4 <sup>ab</sup>	1.65 <sup>ab</sup>	1.444 <sup>b</sup>
<b>Additive effect</b>					
Control	50.56 <sup>b</sup>	20.18 <sup>a</sup>	27.1 <sup>b</sup>	1.45 <sup>c</sup>	1.316 <sup>b</sup>
Amoxicillin	51.57 <sup>a</sup>	18.71 <sup>b</sup>	27.95 <sup>ab</sup>	1.75 <sup>a</sup>	1.460 <sup>a</sup>
Hot pepper	51.91 <sup>a</sup>	18.26 <sup>b</sup>	28.33 <sup>a</sup>	1.63 <sup>b</sup>	1.433 <sup>a</sup>
Green tea	51.69 <sup>a</sup>	18.22 <sup>b</sup>	28.70 <sup>a</sup>	1.67 <sup>b</sup>	1.412 <sup>ab</sup>
SEM	1.05	0.95	0.88	0.13	0.16
<b>ANOVA</b>					
Additive × level	**	NS	***	***	***
Additive	***	NS	**	**	**

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

**Table (7): Effect of different levels of Amoxicillin, hot pepper and green tea on chemical composition of yolks from eggs collected at 40 wks of age and kept in refrigerator for 15 days**

	Moisture %	Protein,%	Total lipids%	Ash%	Cholesterol mg/ 100g
<b>Additive × level</b>					
Control	50.37 <sup>b</sup>	18.47	28.8 <sup>ab</sup>	1.53 <sup>b</sup>	1.282 <sup>b</sup>
Amoxicillin mg/kg					
20	51.28 <sup>a</sup>	18.36	28.1 <sup>b</sup>	1.62 <sup>a</sup>	1.333 <sup>ab</sup>
40	51.02 <sup>ab</sup>	18.79	28.9 <sup>ab</sup>	1.68 <sup>a</sup>	1.395 <sup>a</sup>
Hot pepper,%					
0.1	50.83 <sup>ab</sup>	18.80	28.4 <sup>b</sup>	1.70 <sup>a</sup>	1.409 <sup>a</sup>
0.2	51.44 <sup>a</sup>	18.28	28.9 <sup>ab</sup>	1.68 <sup>a</sup>	1.405 <sup>a</sup>
0.3	51.40 <sup>a</sup>	18.38	28.0 <sup>b</sup>	1.60 <sup>b</sup>	1.270 <sup>b</sup>
Green tea,%					
0.1	50.62 <sup>ab</sup>	18.57	29.7 <sup>a</sup>	1.80 <sup>a</sup>	1.375 <sup>ab</sup>
0.2	50.71 <sup>ab</sup>	18.44	28.3 <sup>b</sup>	1.70 <sup>a</sup>	1.288 <sup>b</sup>
0.3	51.52 <sup>a</sup>	18.71	28.1 <sup>b</sup>	1.75 <sup>a</sup>	1.274 <sup>b</sup>
0.4	50.37 <sup>b</sup>	18.48	28.9 <sup>ab</sup>	1.52 <sup>b</sup>	1.299 <sup>b</sup>
<b>Additive effect</b>					
Control	50.37 <sup>b</sup>	18.47	28.80 <sup>a</sup>	1.53 <sup>b</sup>	1.282 <sup>b</sup>
Amoxicillin	51.15 <sup>a</sup>	18.57	28.50 <sup>b</sup>	1.65 <sup>a</sup>	1.364 <sup>a</sup>
Hot pepper	51.22 <sup>a</sup>	18.48	28.43 <sup>b</sup>	1.66 <sup>a</sup>	1.361 <sup>a</sup>
Green tea	50.80 <sup>ab</sup>	18.55	28.75 <sup>a</sup>	1.69 <sup>a</sup>	1.309 <sup>ab</sup>
SEM	1.12	0.95	1.06	0.13	0.13
<b>ANOVA</b>					
Additive × level	**	NS	***	***	***
Additive	***	NS	**	**	**

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts

are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

Hot Pepper, Green Tea, Amoxicillin, Laying Hens, Egg Quality, Cholesterol

**Table (8): Effect of different levels of Amoxicillin, hot pepper and green tea on plasma total protein, total lipids, Ca, P (mg/100 ml), cholesterol, AST and ALT collected at 40 wks of age of laying hens**

Treatment	Total protein (g/100ml)	Total lipid (g/L)	Ca (mg/100 ml)	P (mg/100 ml)	Cholesterol (mg/100 ml)	AST (u/100ml)	ALT (u/100ml)
<b>Additive x level</b>							
Control	4.07	7.06 <sup>a</sup>	21.86	8.96	162.0 <sup>a</sup>	121.3	24.3
Amoxicillin mg/kg	3.92	6.92 <sup>ab</sup>	21.76	9.03	150.3 <sup>ab</sup>	121.3	22.0
20	4.13	6.90 <sup>ab</sup>	22.46	9.53	147.6 <sup>b</sup>	122.6	24.3
40							
Hot pepper,%	4.09	6.76 <sup>b</sup>	21.73	9.50	136.0 <sup>b</sup>	122.3	22.3
0.1	4.15	6.79 <sup>b</sup>	22.06	9.91	136.3 <sup>b</sup>	122.3	22.0
0.2	4.08	6.75 <sup>b</sup>	21.36	9.23	140.0 <sup>b</sup>	123.6	24.0
0.3							
0.4	4.01	6.77 <sup>b</sup>	20.83	8.76	143.0 <sup>b</sup>	125.0	22.0
Green tea,%	3.89	6.91 <sup>ab</sup>	20.66	9.03	143.3 <sup>b</sup>	123.6	21.6
0.1	4.00	6.95 <sup>ab</sup>	21.76	9.13	147.3 <sup>b</sup>	121.6	24.0
0.2	3.91	7.03 <sup>a</sup>	21.50	9.20	145.0 <sup>b</sup>	123.0	23.0
0.3							
0.4							
<b>Additive effect</b>							
Control	4.07	7.06 <sup>a</sup>	21.86	8.96	162.0 <sup>a</sup>	121.3	24.3
Amoxicillin	4.02	6.91 <sup>ab</sup>	22.11	9.28	149.0 <sup>b</sup>	122.0	23.2
Hot pepper	4.11	6.76 <sup>b</sup>	21.72	9.54	137.4 <sup>c</sup>	122.7	22.7
Green tea	3.95	6.92 <sup>ab</sup>	21.19	9.53	144.6 <sup>b</sup>	123.0	22.7
SEM	0.14	0.11	1.16	1.38	6.84	2.02	1.61
<b>ANOVA</b>							
Additive x level	NS	**	NS	NS	**	NS	NS
Additive	NS	***	NS	NS	NS	NS	NS

<sup>a-c</sup> means within the same column within the same treatments not bearing similar superscripts are significantly different (P<0.05).

\*P<0.05; \*\* P<0.01; P<0.001

NS not significant

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