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SOME BIOCHEMICAL EFFECTS OF GREEN TEA POLYPHENOLS UNDER HEAT STRESS IN BROILER CHICKENS

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Running Title: Polyphenols and heat stress

ABSTRACT

The present experiment was carried out to shed more light on the possible role of tea polyphenols in alleviating the negative effects of heat stress on broiler performance and oxidative status. Broiler chickens were raised under two different environmental temperatures (25 and 34 °C) for 3 days starting from 24 days of age. Each environmental group was further subdivided into two treatments (control and polyphenol, n = 16). The birds were tube-fed a same a mount of diet containing 0 (control) or 0.5% polyphenols (polyphenon 60®) which were extracted from green tea. The results showed that polyphenols treated birds gained more weight but not significantly than the control and thus the feed conversion ratio tended to be lower in polyphenols groups in both environments. Plasma corticosterone (CTC) level was markedly increased in the high temperature and the polyphenols significantly reduced this effect. The abdominal fat was decreased significantly by the polyphenols in the high temperature and, interestingly, a same trend was obtained for hepatic thiobarbituric acid reactive substance (TBRAS), which is an index for lipid peroxidation. Plasma glucose level was increased by the high temperature but not by the polyphenols.

INTRODUCTION

Polyphenols are found in many plants such as fruits, vegetables and tea. Tea (*Camellia sinensis*), especially green tea is proved to be a good source of polyphenols, which are known as catechins. Catechins are water-soluble polyhydroxylated flavonoids. The common catechins, which are found in tea, are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)- epigallocatechin gallate (EGCG), which differ in the number and position of the hydroxyl groups in the molecule (Douglas *et al.*, 1997; Ninomiya *et al.*, 1997; Matthew & Douglas 1997).

Since it has been observed that tea polyphenols (PP) have protection against cancer, coronary heart disease, inflammation, lipid oxidation and oxidative stress (Katiya & Mukhtar, 1995; Miura *et al.*, 2000; Tang, *et al.*, 2000, Ozercan, *et al.*, 2008, Almajano *et al.*, 2008).

When animals are subjected to stress conditions e.g. (transport, electric shocks, fasting, low or high ambient temperature), the hypothalamus-pituitary-adrenal axis is activated and glucocorticoid are released from the adrenal cortex (Klemcke *et al.*, 1989). The excessive levels of glucocorticoids result in catabolic effects on skeletal muscle (Tomas *et al.*, 1979; Odedra *et* *al.*, 1983). The high level of glucocorticoid decreases the rate of synthesis (Hayashi *et al.*, 1992) and increases the rate of breakdown of myofibrillar proteins, which leads to muscle wasting and growth retardation (Ohtsuka *et al.*, 1992; Higuchi *et al.*, 1996). Thus, adrenal function intensely influences the performance of meat animals.

Previous results (Eid *et al.*, 2003) showed that PP could minimize the CTC-induced growth inhibition, hyperlipidemia and oxidative stress. The objective of this study is to research how tea PP affects performance and oxidative status in broiler chickens under heat stress, which intensely affects poultry production.

MATERIALS AND METHODS

Animals and schedule

Broiler chickens (Cobb strain) provided with water and a commercial starter diet (20% crude protein and metabolizable energy *ME* 3,250 Kcal/Kg) *ad libitum* for the first 23 days. On day 24, birds were divided into two environmental groups (25 and 34°C) with similar body weight. Each environmental group was further subdivided into two treatments (control and polyphenol, n = 16). The birds were maintained at 14h light : 10h dark cycle. The relative humidity was 50-70% throughout the experiment.

Experimental diets and feeding

Chicks were tube fed the same amount of diets containing 0 (control) or 0.5% PP, three times a day to ensure that every individual bird had the same amount of the diets. Polyphenol was applied as POLYPHENON 60® purchased from Sigma Chemicals Co (St. Louis, MO, USA), which was extracted from green tea and contains a mixture of catechins (Table 1). The composition of the basal diet (CP 30% and ME 3,113 kcal/kg) is shown in Table 2. The heat treatment started from day 24 to day 27 of age.

Measurements and chemical analysis

At the end of the experimental period body weight was recorded then all the birds were slaughtered. Blood samples were collected into heparinized test tubes, quickly centrifuged at 5,900 xg for 10 min to separate plasma, and stored at -20°C until analysis. The birds were dissected to remove the abdominal fat and the liver.

Plasma CTC concentration: Plasma CTC concentration was measured by a commercial kit (ICN Biomedicals, Inc., California, USA). This assay is as sensitive as to detect 0.05 ng/ml in the plasma.

Lipid peroxidation: Lipid peroxidation in the liver was assessed as the concentration of thiobarbituric acid reactive substance (TBARS) (Ohkawa, *et al.*, 1979; Richard, *et al.*, 1992). TBRAS, in particular, malondiadehyde (MDA), is a product of the oxidative degradation of polyunsaturated fatty acids, and thus used as index of oxidative stress.

Plasma glucose level: Plasma glucose level was measured by the method of Somogyi (1952).

Table 1. Chemical Composition of POLYPHENON 60

Component	%
(–) – Epicatechin	7.0
(–) – Epigallocatechin	20.2
(–) – Epigallocatechin gallate	28.5
(–) – Epicatechin gallate	8.2

Table 2. Composition of basal diet

Ingredients	%
Ground yellow corn	61.70
Dehydrated alfalfa	3.25
Purified soybean protein	28.4
Mineral mixture*	3.31
Vitamin mixture *	0.16
L-Lysine	0.40
DL-Methionine	0.50
Cellelose	2.28
Determined analysis	
Crude Protein, %	30.00
ME, Kcal/Kg	3,113

* Contained (/Kg): $CaCO_3$ 210 g, $CaHPO_4$ 660 g, NaCl 113g, $MnSO_4$.4-5 H_2O 6,600 g, $ZnSO_4.7H_2O$ 4,000 g, $FeSO_4$ 6,145 g, $CuSO_4.7H_2O$ 233 mg, $NaIO_3$ 16 mg, H_2SeO_3 6mg.

* Contained (/Kg): retinol 1,750,000 IU, cholecalciferol 200,000 IU, riboflavin 2.5 g, thiamine 1 g, pyridoxine 0.5 g, cyanocobalamin 10 mg, pantothenic acid 4 g, nicotinic acid 10 g, menadione 250mg, folic acid 200mg, choline chloride 300 mg, biotin 25 mg, DL-α-tocopheryl acetate 2.5 g, sucrose 978 g.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) using the General Liner Model procedure of the statistical analysis system software package (SAS Institute Inc., Cary, NC, USA, 1988) with Duncan's multiple-range test. A P value <0.05 was considered to be statistically significant.

RESULTS

The data in Fig. 1 represent body weight gain from 24-27 day old. PP treated birds gained more weight than the control birds, but not significantly. The high temperature treated birds significantly gained more weight than birds kept under 25°C. Fig. 2 shows the effect of the environmental temperature on feed conversion ratio. The feed conversion ratio was significantly higher in 25°C group. PP has no significant effect comparing with the control group in both the environmental groups. The effect of environmental temperature on the relative weight of the abdominal fat is presented in Fig. 3. The abdominal fat deposition significantly increased in the control group in 34°C treatment. PP has no significant effect in increasing the abdominal fat deposition in both the environmental groups. Fig.4 shows the effect of environmental temperature on CTC level in the plasma. The plasma level of CTC was markedly increased in 34°C environment, and PP markedly reduced this elevation. Fig. 5 shows the effect of environmental temperature on plasma glucose level. The plasma glucose level tended to be increase by the high temperature but PP had no significant effect on plasma glucose. Fig. 6 shows the effect of environmental temperature on the hepatic TBARS level. The high temperature increased liver TBARS, and PP significantly reduced this effect of temperature.

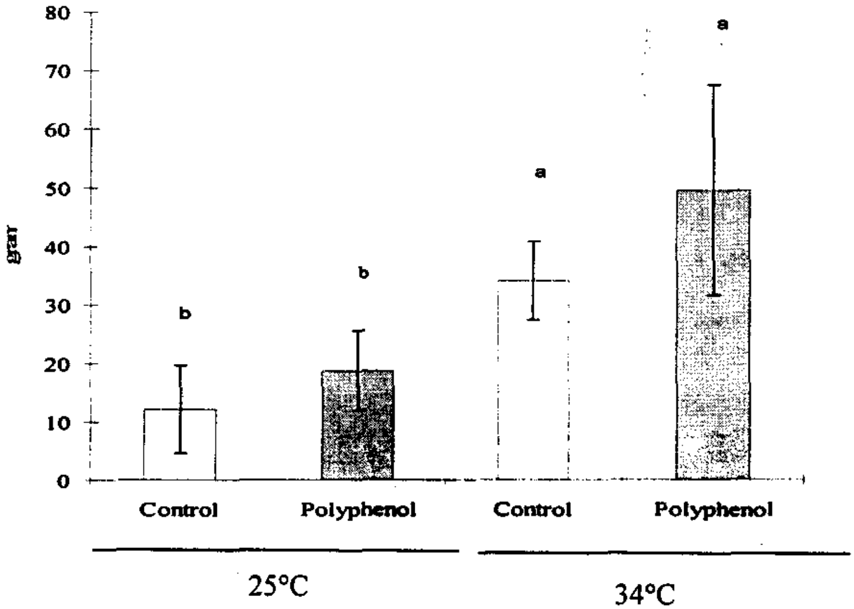

Fig. 1. Effect of environmental temperature on body weight gain in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$).

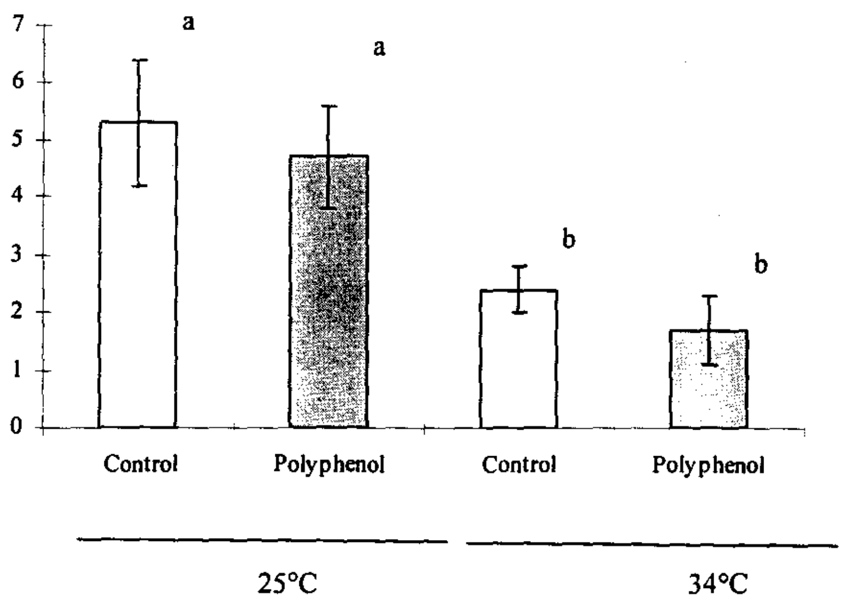

Fig. 2. Effect of environmental temperature on feed conversion ratio in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$).

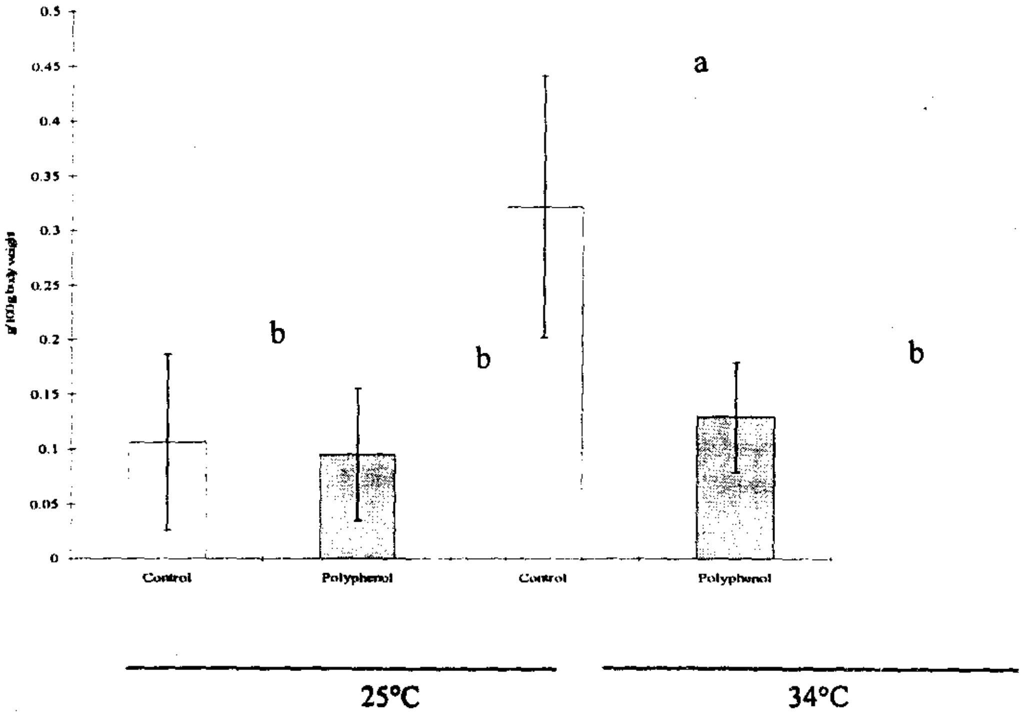

Fig. 3. Effect of environmental temperature on relative weight of the abdominal fat in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$)

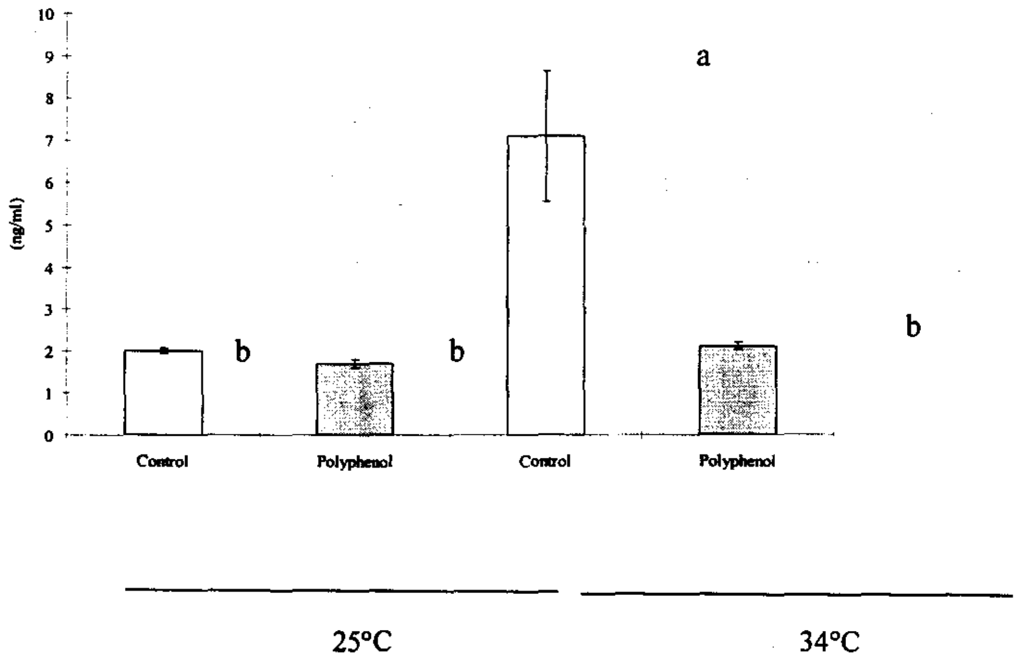

Fig. 4. Effect of environmental temperature plasma CTC level in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$).

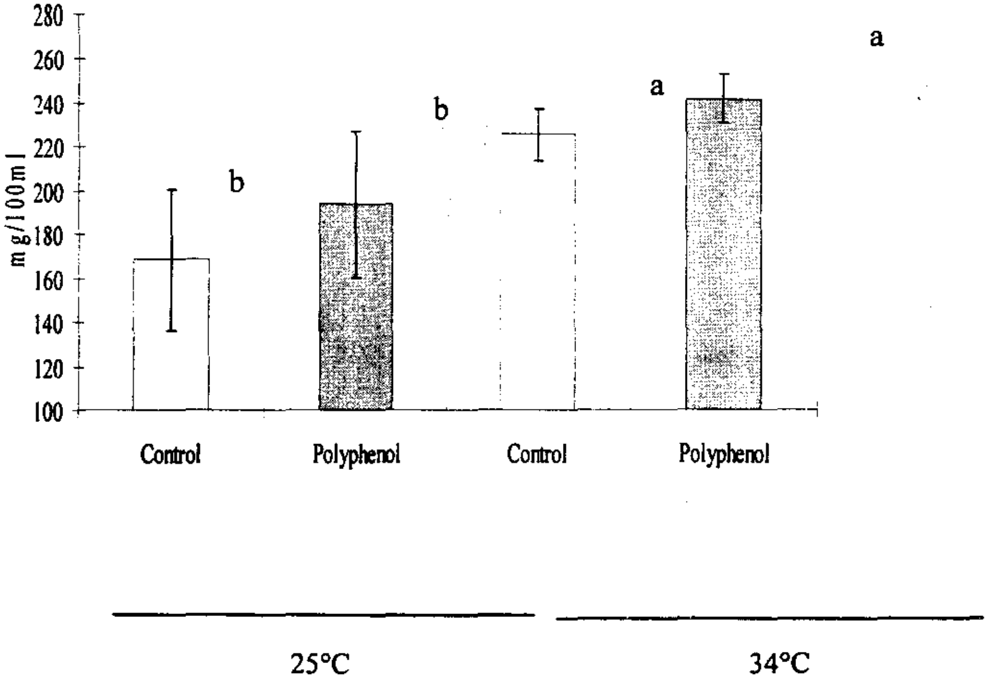

Fig. 5. Effect of environmental temperature plasma glucose level in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$).

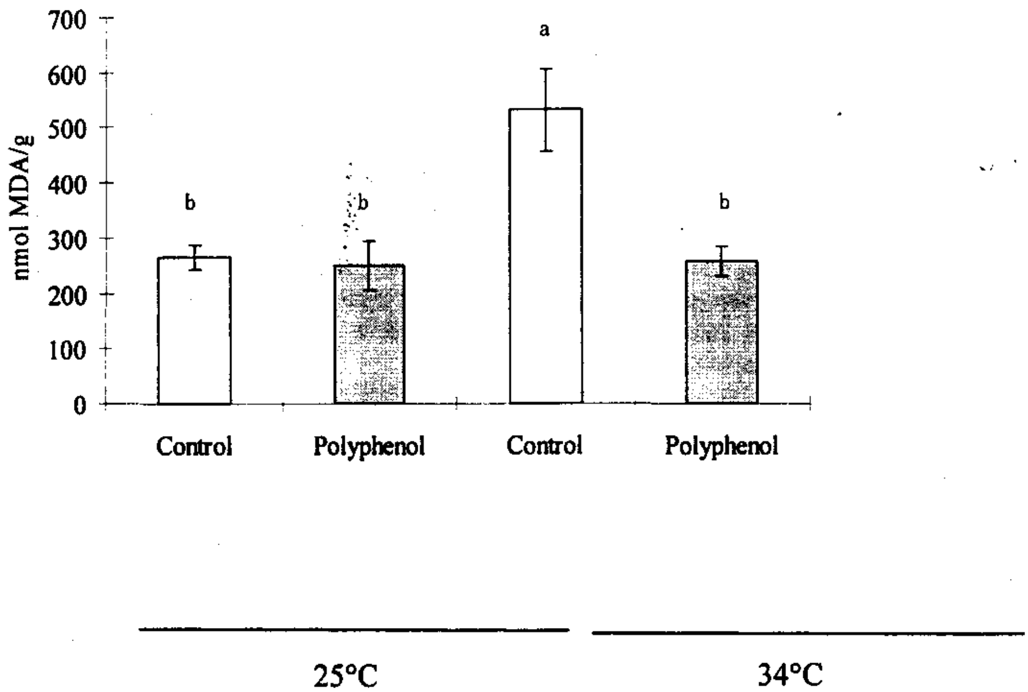

Fig. 6. Effect of environmental temperature on hepatic TBARS level in broiler chickens. Values are expressed as means ± standard deviation (STD); means with different superscript are significantly different from each other ($P < 0.05$).

DISCUSSION

In this study, we investigated how tea PP affects performance and oxidative status in broiler chickens under heat stress, which intensely affects poultry production. The PP treated birds gained more weight (Fig. 1) but not significantly than the control. However, this increase does not due to the increase in food consumption because all the birds were tube-fed with the same amount of diet. Thus, the feed conversion ratio (Fig. 2) tended to be lower in PP groups in both environments. Previous study (Eid *et al.*, 2003) shows alleviated of growth inhibition and impaired feed conversion due to CTC by PP treatment. This could be explained by the effect of PP in reducing the skeletal muscle proteins damaged by active oxygen and normalized the ratio between muscle protein synthesis and breakdown (Hayashi *et al.*, 1994). The birds in 34°C group gained weight more than the birds in 25°C group. That's due to the basic metabolizable energy is very low in 34°C group comparing with 25°C group.

The abdominal fat deposition was less in PP group than the control in the 34°C environment (Fig. 3). The plasma level of CTC (Fig. 4) was markedly increased in 34°C environment showing that the environment was a stress condition. Tea catechin may have an inhibitory effect on intestinal lipid absorption followed by a decrease of abdominal fat content. Tea catechin may also change the formation of micell that mediates re-absorption of bile acid (Biswas & Wakita 2001, Khan & Mukhtar 2007). In addition, it was observed in the present study that an increase in plasma CTC concentration due to heat treatment was significantly inhibited by PP treatment. This is because PP may change CTC metabolism according to its effect on lipid absorption and metabolism, which strongly interfere with steroid synthesis (Biswas & Wakita 2001). However, we do not have data at the present time to emphasis this assumption.

The plasma glucose level (Fig. 5) tended to be increase by the high temperature but PP had no significant effect on plasma glucose. It is well known that under stress condition, the level of glucose is increased in order to serve adequate amount of glucose to the sensitive organs such as heart and brain.

The high temperature increased liver TBARS (Fig. 6.), and PP significantly reduced this effect of temperature. Ohtsuka *et al.,* (1998) and Taniguchi *et al.,* (1999) showed that dietary CTC induced elevation of plasma TBARS levels in rats and broilers. Tomita *et. al.,* (1995) showed that a long term administration of tea polyphenol significantly suppressed plasma TBARS and triglyceride in rats. It is known that catechins were much more effective than BHT, vitamin E or vitamin C in inhibiting the Cu^{2+} mediated oxidative modification of low density lipoprotein (LDL) (Tomita *et al.*, 1995).

The present study showed that high environmental temperature increased plasma CTC by about 3-fold, and due to this effect on CTC the fat deposition and plasma glucose might be increased. It was also shown in the present study that the effects of the stress were minimized by PP. Thus, by using PP, it may be possible to improve performance of the birds under high environmental temperature.

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بعض التأثيرات البيوكميائية للبوليفينولات المستخلصة من الشاى الأخضر على دجاج التسمين المعرض للضغط الحرارى.

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أجريت هذه الدراسة للاشارة الى دور البوليفينولات المستخلصة من الشاى الأخضر فى تقليل التأثيرات السلبية الناتجة من الضغوط الحرارية على كفائة دجاج التسمين. قسمت كتاكيت التسمين المستخدمة فى هذه التجربة الى مجموعتين: عرضت الأولى الى ٢٥ ° م فى حين عرضت الثانية الى ٣٤ ° م لمدة ثلاثة أيام بداية من عمر ٢٤ يوم. ثم قسمت كل مجموعة الى تحت مجموعتين (ن = ١٦)، حيث قدم لكليهما عن طريق أنبوبة تغذية نفس الكمية من العليقة المحتوية على صفر % من البوليفينولات المستخلصة من الشاى الأخضر لاحداهما وعلى ٠,٥ % للأخرى.

ولقد أوضحت النتائج أن الطيور التى عوملت بالبوليفينولات قد حققت معدل نمو أعلى ولكن بصورة غير معنوية، وكذلك فان معامل الكفائة الغذائية قد انخفض فى المجاميع المعاملة بالبوليفينولات سواء مع ٢٥ ° م أو مع ٣٤ ° م. كما أظهرت النتائج زيادة واضحة فى مستوى الكورتيكوستيرون فى حالة درجة الحرارة المرتفعة (٣٤ ° م) ، والذى انخفض معنويا فى الطيور المعاملة بالبوليفينولات. كذلك فقد انخفض كلا من دهن البطن وال TBARS فى الطيور المعاملة بالبوليفينولات والمعرضة الى الحرارة المرتفعة مقارنة بقرينتها التى لم يقدم لها البوليفينولات. أما عن مستوى الجلوكوز فى البلازما فقد ارتفع فى حالة الجرارة المرتقعة الا أنه لم يتأثر معنويا باضافة البوليفينولات.