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Physiological, Hematological, Biochemical and Oxidative Stress Indices in Growing Rabbits fed on Green Tomato Powder Supplemented Diets

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Abstract

Dietary antioxidants used in livestock systems are considered the key to improving animal production. In a search for unconventional dietary supplements for improving animal health and production, the current study assumed to investigate the effect of two concentrations (10 or 20 g/kg diet) of green tomato powder (GTP) in the diet of New Zealand white growing rabbits on physiological, hematological, biochemical and antioxidant parameters. A total of 24 unsexed New Zealand white growing rabbits (45 days old) were randomly divided into three experimental groups (8 rabbits each). The first group considered as a control group (no additives). The second and the third group were received GTP in the diet by 10 or 20 g/kg diet, respectively during 8 weeks. GTP (10 or 20 g/kg diet) administration exhibited a substantial positive effect on growing rabbits, including strengthening RBCs, Hb%, PCV%, and serum protein concentration, reduce serum lipids, enlarge activity of antioxidant enzymes and decrease malondialdehyde (MDA) level as a reliable marker of lipid peroxidation. These findings suggest that GTP has a potential effect of improving the health and production of New Zealand white growing rabbits.

Keywords: *Antioxidant, biochemical, hematological, green tomato, rabbits.*

Introduction

Tomato is one of the most essential consumed vegetable food crops worldwide due to its content of biologically active compounds such as lycopene, phenolics, vitamin C, carotenoids, flavonoids, tocopherols, and others (Willcox *et al*., 2003; Shi *et al*., 2008 and Burda, 2014). These compounds have been known as protective compounds against diabetes, cancer and cardiovascular diseases (Burda, 2014). The green color of immature tomato fruits is due to the presence of chlorophyll a and b pigments which are degraded enzymatically during ripening and synthesis of yellow pigments such as β-carotene and xanthophylls which are accompanied by a loss of green color (De Sousa *et al*., 2014). It is well known that tomato fruits harvested at the green stage generally are not rich in vitamin E, ascorbic acid and lycopene as those collected at the red stage (Yahia *et al*., 2001 and Raffo *et al*., 2002), but are rich in green pigment chlorophyll, glycoalkaloids α-tomatine and dehydrotomatine which play an essential functional role in animal and human diets (Friedman *et al*., 2000 and Barrette, 2001). A diet rich in green tomato can reduces plasma cholesterol by the formation of tomatine cholesterol complex (Friedman *et al*., 2000) and protects against arteriosclerosis and cancer (Kozukue and Friedman, 2003).

Furthermore, tomatine-containing green tomato extract is known to strengthen the immune response of the human body against the infectious agents (Arena *et al*., 2018), and has the potential to inhibit human cancer cells (Friedman *et al*., 2009). Moreover, chlorophyll-containing green tomato is a chlorine pigment, which helps to cleanse the body of harmful toxins by its antioxidant properties. It was reported that the intake of chlorophyll maintains a healthy digestive system by increasing the beneficial bacteria in the gastrointestinal tract (Kopsell *et al*., 2005). In this context, the main objective of this study was to evaluate the effect of green tomato powder supplementation on physiological, hematological, biochemical, and serum oxidative stress parameters of growing rabbits.

Materials and Methods

Green tomato powder preparation

Green immature tomato fruits were purchased from a private commercial farm at El-Minya Governorate, Egypt. Then, fruits were washed, cleaned of impurities, and oven-dried on 50ºC until constant weight. Dry green tomato powder (GTP) was finely ground, sieved and stored at room temperature (25 ± 2 °C) until use.

Animals, study design and diets

A total of 24 unsexed New Zealand white rabbits (45 days old) were randomly allocated into 3 experimental groups (8 rabbits each), with dietary supplementation of 0, 10, or 20 g green tomato powder (GTP) /kg diet respectively, for 8 weeks. Animals were individually housed in the same managerial conditions in cages (35 × 40 × 40 cm) at the poultry farm site in the Department of Animal and Poultry Production, Faculty of Agriculture, Minia University. Feed and water were available *ad libitum* throughout the experimental period (8 weeks). The experimental diets contents were as follows; protein 16%, fiber 10%, energy 2550 kcal/kg, 44 % ground yellow corn, 15.5% wheat bran, 25% sugar beet pulp, 13.5% soybean meal (44% crude protein), 0.75% premix (vitamins and minerals), 0.5% sodium chloride, and 0.75% limestone. The basal diets were formulated and pelleted to content growing rabbits nutrient requirements, according to the National Research Council (NRC, 1977).

Physiological assessments

At the end of the experimental period, all experimental animals of all groups have fasted for 12 hrs, weighed, handily slaughtered and deskinned. Edible offal's (liver and kidneys) were considered separately and recorded. All organ weights were attributed to living body weights.

Hematological indices

Red blood cells (RBCs), hemoglobin concentration (Hb %), packed cell volume percentages (PCV %) and RBC indices (MCV, MCH, and MCHC) were estimated using a veterinary hematology analyzer (Vet-Scan HM5 Haematology System Abaxis Europe, UK).

Serum biochemical analyses

Blood samples were collected between 07:00–08:00 a.m. from the marginal ear vein in tubes, then centrifuged at 3000 rpm for 10 min, sera were collected and stored at −80°C until use for the biochemical analyses. The total serum protein and albumin concentrations were estimated using commercial kits (Bio-Med, Egypt). Globulin concentration and albumin /globulin (A/G) ratio were calculated. T80 UV Spectrophotometer UK was used in all analytical analysis.

Serum lipid profile, including triglycerides (TG) and total cholesterol (TC), was determined according to the manufacturer's prescript by commercial kits (Vitro Scient, Germany) using an enzymatic colorimetric method. HDL-cholesterol concentration was determined using the method designed by Lopes-Virella *et al*. (1997). Serum LDL-cholesterol and VLDL- cholesterol were calculated by Friedewald equations which altered by Ahmadi *et al*. (2008) as follows:

LDL (mg/dL) =

$$TC/1.19 + TG/1.9 - HDL/1.1 - 38$$

$$VLDL\text{-}c = Triacylglyceroles/5$$

Assessment of oxidative stress and lipid peroxidation biomarkers

Serum levels of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were estimated spectrophotometrically by commercial kits (BioMed chemical company, Cairo, Egypt). Additionally, serum malondialdehyde (MDA) was measured enzymatically using the method introduced by Satoh (1978).

Statistical analysis

Differences between the mean of eight replicates for three experimental groups were statistically analyzed by one-way ANOVA and Dunnett multiple comparisons test using GraphPad Prism software version 8.0.2 (263). Data are displayed as means ± standard error means (SEM). Results are considered significant at $p < 0.05$.

Results and Discussion

Physiological alterations

Data tabulated in Table 1, explained the effect of dietary 10 or 20 g GTP /kg diet on carcass traits. The results indicated that, GTP inclusion in diet of growing rabbits daily by 10 or 20 g / kg diet for 8 weeks significantly ($p < 0.05$) increases the live body weight (g), carcass weight (g) and carcass % by 13.78, 13.39, 35.50, 37.61, 25.19 and 27.61%, respectively compared to the control group. In contrast, 10 or 20 g GTP / kg diet administration significantly ($p < 0.05$) depleted liver weight (g) and liver % by 10.58, 10.10, 22.88 and 22.15%, respectively compared to rabbits fed on the control diet. Non-significant elevation was observed in kidney weighs induced by 10 g GTP / kg diet administration and in kidney % by 10 or 20 g GTP / kg diet administrations (Table 1).

Table 1. Carcass traits of New Zealand white growing rabbits fed diets containing 10 and 20g green tomato powder/kg diet for 8 weeks.

Items	Experimental treatments			*p-value*
	Control	10 g GTP/kg diet	20 g GTP/kg diet	
Live body weight (g)	2265 ± 2.55 [b]	2627 ± 4.62[a]	2615 ± 8.66 [a]	< 0.0001
Carcass weight (g)	1105 ± 2.55 [b]	1713 ± 4.62 [a]	1771 ± 11.74 [a]	< 0.0001
Carcass %	48.77 ± 0.16 [b]	65.19 ± 0.29 [a]	67.73 ± 0.42 [a]	< 0.0001
Liver weight (g)	61.49 ± 0.46 [b]	54.99 ± 0.47 [a]	55.28 ± 0.65 [a]	< 0.0001
Liver %	2.71 ± 0.02 [b]	2.09 ± 0.01 [a]	2.11 ± 0.01 [a]	< 0.0001
Kidney weight (g)	15.02 ± 0.46 [a]	17.77 ± 0.86 [a]	18.35[b] ± 1.30	0.0377
Kidney %	0.66 ± 0.01	0.67 ± 0.03	0.70 ±0.04	0.8106

Mean ± standard error of mean SEM, [a,b] different letters in a row differ significantly ($p < 0.05$).
Control = green tomato powder free diet; GPT = green tomato powder.

Carcass composition is one of the most critical aspects of animal production relating to food production (Silva and Cadavez, 2012). Therefore, the current investigation used GTP as an unconventional dietary supplement for the first time to improve animal production. Data exhibited that include both levels of GTP (10 or 20 g/ kg diet) in the diet of growing rabbits significantly ($p < 0.05$) elevated their live body weight, carcass weight and carcass percent (Table 1). The improvement of body and carcass weight by administration of GTP may be due to containing green tomatoes a several bioactive compounds including tomatine and chlorophyll that are known to boost the immune system against the infectious microbes (Arena *et al*., 2018), maintain a healthy digestive system via elevating the serviceable microorganisms in the gastrointestinal tract and clean the body of deleterious compounds by antioxidant properties (Kopsell *et al*., 2005).

Hematological changes

Hematological alterations in the blood of growing rabbits induced by adding two various levels of GTP to their diets are shown in Figure 1. Hematological estimated data revealed that, the RBCs, Hb% and PCV% were significantly ($p < 0.05$) elevated within the normal range (RBCs: 4.11-7.20 x 10^6/mm^3; Hb%:10.45- 16.81; PCV%: 38.41-51.50) up to (6.25 and 5.90 x 10^6/mm^3 for RBCs), (13.22 and 13.28 for Hb%) and (38.60 and 38.60 % for PCV%) by the administration of GTP (10 or 20 g / kg diet), respectively. In contrast, 10 and 20 g GTP / kg diet supplementation caused significant ($p < 0.05$) decreases in MCV, MCH and MCHC within the normal range (MCV(fl):58.42-72.80; MCH(pg): 19.45-27.83; MCHC(g/dl):28.54-36.29) by 11.56, 7.55, 23.69, 13.43, 13.80 and 6.16%, respectively compared to control group (Figure 1). These results, in partial agreement with the previous investigation conducted by Elwan *et al*. (2019), who found that there was a significant increase in RBCs induced by the addition of tomato powder to fattening rabbit diets.

As is well known, hematological indices are one of the most vital tools that reflect the physiological and nutritional status of the body (Doyle, 2006). Red blood cells which are also known as erythrocytes, act as a carrier of hemoglobin in the body (Chineke *et al*., 2006). Hemoglobin plays an important vital role in releasing energy by transporting oxygen to tissues for food oxidation (Isaac *et al*., 2013 and Soetan *et al*., 2013). Packed Cell Volume (PCV) is the proportion of red blood cells in the blood, which depends on the Hb concentration in red blood cells and it is implicated in the absorbed nutrients and transport of oxygen (Purves *et al*., 2003). Several blood indices including MCV, MCH and MCHC mentioned as useful indicators for evaluating in mammals the bone marrow capacity to produce RBCs (Chineke *et al*., 2006).

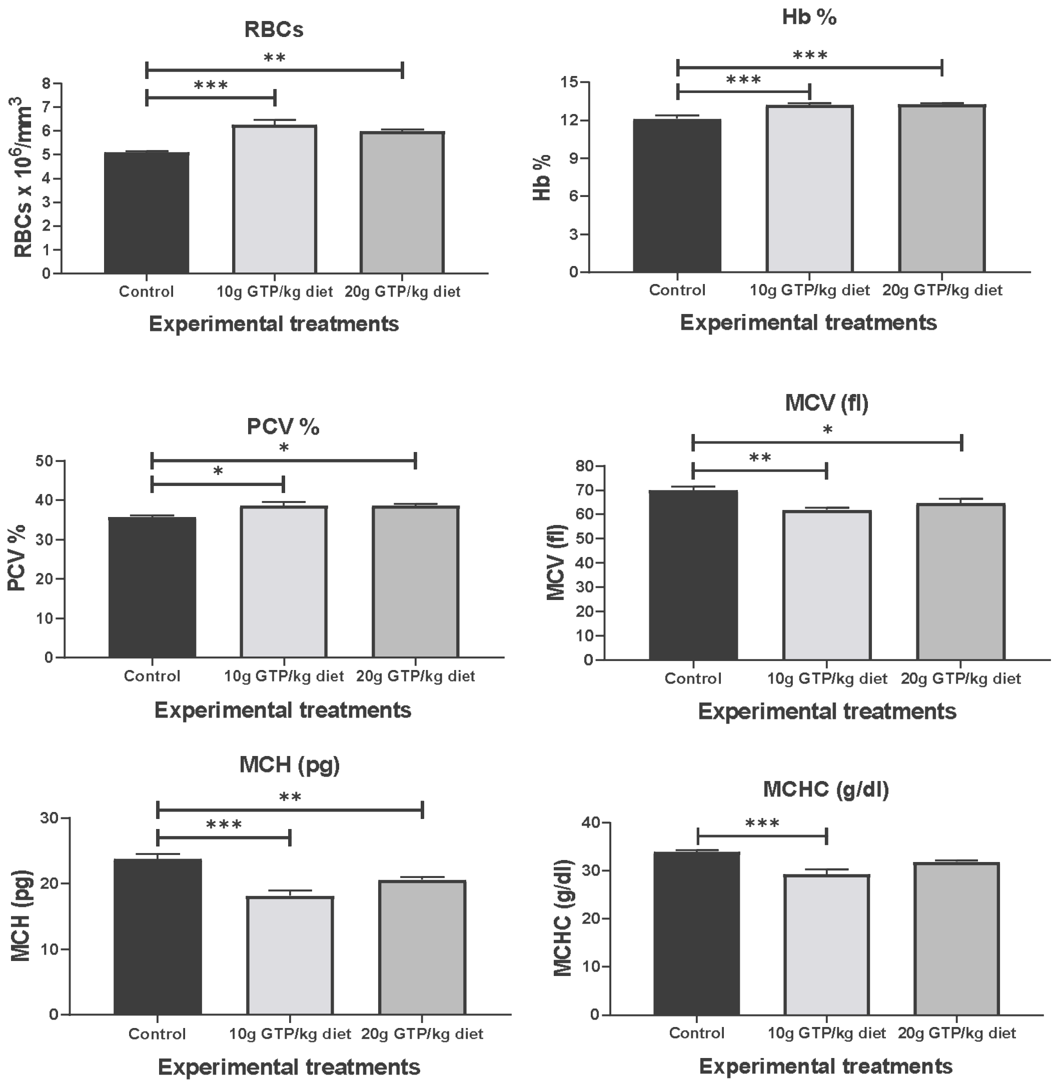

Fig (1): Hematological indices (Mean ± SEM) in growing rabbits fed diets containing two various concentrations of green tomato powder for 8 weeks.

*$P < 0.05$ significant differences, ** $P < 0.01$ highly significant differences and *** $P < 0.001$ very high significant differences compared to normal group. MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; Control = green tomato powder free-diet; GPT = green tomato powder.

The enlargement in several blood indices, including RBCs, Hb%, and PCV% in the current study induced by GTP administration, may be attributed to bioactive ingredients in GTP (tomatine and chlorophyll) which are indispensable for promoting blood formation. The similarity between hemoglobin and chlorophyll structure prompted meditation regarding the action of chlorophyll as a promoting agent for blood formation (Fan *et al*., 2019).

Biochemical indices

Serum proteins and lipid profile were estimated in the present study as an indicator for the alteration in liver functions in rabbits fed on GTP for 8 weeks. As shown in Table 2, rabbits were supplemented with 10 or 20 g GTP/ kg diet for 8 weeks had the higher ($p < 0.05$) serum total protein (6.27 and 6.63g/dl), albumin (3.62 and 3.83g/dl) and globulin (2.64 and 2.70 g/dl) within the normal range (Total protein: 4.19-10.65 g/dl; Albumin: 2.11-3.98; Globulin: 1.94-3.10 g/dl) compared to dietary control treatment. Otherwise, 10 or 20 g GTP /kg diet administration promoted a significant ($p < 0.05$) decrease in A/G ratio by 34.77 %.

Table 2. Serum proteins profile of New Zealand white growing rabbits fed diets containing 10 and 20g green tomato powder/kg diet for 8 weeks.

Items	Experimental treatments			*p-value*
	Control	10 g GTP/kg diet	20 g GTP/kg diet	
Total protein (g/dl)	5.12 ± 0.06 [b]	6.27 ± 0.08 [a]	6.63 ± 0.06 [a]	< 0.0001
Albumin (g/dl)	3.47 ± 0.04 [b]	3.62 ± 0.04[c]	3.83 ± 0.03 [a]	0.0415
Globulin (g/dl)	1.65 ± 0.02 [b]	2.64 ± 0.03 [a]	2.70 ± 0.02 [a]	< 0.0001
Albumin/Globulin Ratio	2.10 ± 0.02 [b]	1.37 ± 0.01 [a]	1.37 ± 0.01 [a]	< 0.0001

Mean ± standard error of mean SEM, [a,b,c] different letters in a row differ significantly ($p < 0.05$). Control = green tomato powder free diet; GPT = green tomato powder.

It is well known that; serum protein profile reflects the health and nutritional status of the body. Hepatocytes create serum proteins. The main discernment of their divides into albumin and globulins. Albumin plays an important role in fat metabolism by binding with fatty acids and maintaining them in soluble form in plasma (Tothova *et al*., 2016). Whereas the main role of the globulin in the body is to consist of the immunoglobulins that are produced by the immune system (Eckersall, 2008). The obtained data revealed that, GTP supplementation exhibited a substantial effect on serum total proteins level and their fractions by increasing their concentrations in the serum after induced feeding rabbits to two different levels of GTP (10 or 20 g/ kg diet) for 8 weeks. The important bioactive constituents in GTP such as chlorophyll may be responsible for this magnification in serum protein concentrations due to their antioxidant activity (Durga devi and Banu, 2015), which can reduce the protein oxidation (Katerji *et al*., 2019).

The effect of active constituents in GTP on serum lipid profile serum triglycerides, cholesterol, and lipoproteins including high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein cholesterol (VLDL-c) concentrations were determined and data are presented in Table 3. Tabulated data regarding serum lipid profile (Table 3) exhibited that, rabbits fed a diet containing a high amount of green tomato powder (20 g GTP/kg diet) had the lowest values ($p < 0.05$) for serum triglycerides, cholesterol, LDL-c and VLDL-c compared to others fed diets containing the most economical level 10 g GTP/ kg diet, and the control diet. Otherwise, rabbits fed on the control diet had the lowest ($p < 0.05$) level for HDL-c compared to those fed supplemented diets (GTP 10 or 20 g/ kg diet).

Table 3. Serum lipid profile of New Zealand white growing rabbits fed diets containing 10 and 20g green tomato powder/kg diet for 8 weeks.

Items	Experimental treatments			*p-value*
	Control	10 g GTP/kg diet	20 g GTP/kg diet	
Triglycerides (mg/dl)	178.5 ± 0.98 [a]	171.5 ± 0.26 [b]	166.1 ± 0.44 [c]	< 0.0001
Cholesterol (mg/dl)	213.6 ± 1.26 [a]	143.9 ± 3.09 [b]	134.9 ± 0.71 [c]	< 0.0001
HDL-c (mg/dl)	97.13 ± 0.85 [b]	121.5 ± 0.56 [a]	128.6 ± 1.82 [c]	< 0.0001
LDL-c (mg/dl)	147.3 ± 1.42 [a]	63.0 ± 3.02 [b]	46.0 ± 1.95 [c]	< 0.0001
VLDL-c (mg/dl)	35.38 ± 0.18 [c]	34.0 ± 0.15 [b]	32.88 ± 0.12 [a]	< 0.0001

Mean ± standard error of mean SEM, [a,b,c] different letters in a row differ significantly ($p < 0.05$).
Control = green tomato powder free-diet; GPT = green tomato powder.

The hypolipidemic action of GTP may be associated with its high tomatine content, which is the most abundant saponin in the green tomato and can reduce blood lipids by inducing permeabilization of the cell membrane (Medina *et al*., 2015), binding with sterols like cholesterol and formatting of tomatine cholesterol complex which excreted in feces (Friedman *et al*., 2000). Moreover, in the current study, it was noticed that GTP could elevate the serum HDL-c (good cholesterol) level (Table 3)., suggesting that GTP may assist as a precious supplement in the rabbit diet.

Oxidative stress and lipid peroxidation biomarkers

The addition of GTP has been investigated for its role in oxidative stress. For this purpose, serum oxidative stress enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) as well as serum malondialdehyde (MDA) concentration as a reliable marker of lipid peroxidation were determined and data illustrated in Figure 2. As evident from these data in Figure 2, there was a highly significant ($p < 0.05$) increase in oxidative stress enzyme activities like CAT, SOD and GST induced by the administration of two various levels of GTP (10 or 20 g/ kg diet) to growing rabbit diet compared to the control group. Contrarily, serum MDA concentration in rabbits fed on 10 or 20 g GTP/ kg diet has shown a significantly ($p < 0.05$) lower level compared to the control group. Furthermore, data clarified that the highest level of 20 g GTP/ kg diet has shown significantly ($p < 0.05$) elevated in oxidative stress enzyme (CAT, SOD, GST) activities compared to the lowest level of 10 g GTP/ kg diet. Also, MDA concentration did not significantly ($p < 0.05$) differ with 10 or 20 g GTP /kg diet administrated levels (Figure 2).

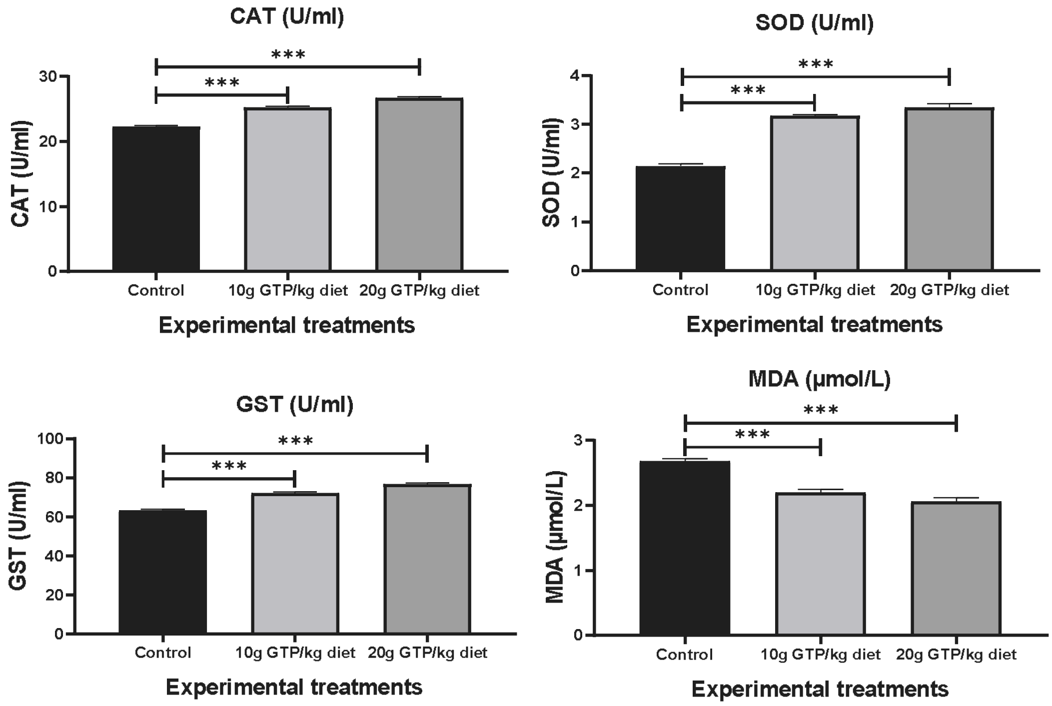

Fig (2): Oxidative stress and lipid peroxidation biomarkers (Mean ± SEM) of growing rabbits fed diets containing two various concentrations of green tomato powder for 8 weeks.

*$P < 0.05$ significant differences, ** $P < 0.01$ highly significant differences and *** $P < 0.001$ very high significant differences compared to normal group. CAT, catalase; SOD, superoxide dismutase; GST, glutathione S-transferees; MDA, malondialdehyde; Control = green tomato powder free-diet; GPT = green tomato powder.

The dramatic improvement in serum CAT, SOD, GST activities, and MDA level induced by GTP administration to rabbits diet may be connected to bioactive GTP components like chlorophyll, which well known for its potent antioxidant properties (Lanfer-Marquez *et al.*, 2005 and Hsu *et al.*, 2013). Interestingly, the current study confirmed that GTP could act as a scavenging agent and protect serum lipids against peroxidative damage. These findings clarified by the low level of MDA (lipid peroxidation end product) in the serum of feeding rabbits on 10 or 20 g GTP/ kg diet. (Figure 2).

Conclusion

This study was done to clarify the effect of dietary green tomato powder GTP supplementation on growing rabbits. The addition of GTP (10 or 20g /kg diet) to the diet of growing rabbits resulted in a significant improvement of some physiological, hematological, biochemical and oxidative stress parameters. These findings suggest that GTP can be used to improve the growth and fattening of New Zealand white growing rabbits.

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المؤشرات الفسيولوجية والهيماتولوجية والبيوكيميائية والاجهاد التأكسدي في الأرانب النامية المغذاه علي علائق مضاف اليها مسحوق الطماطم الخضراء

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[2]قسم الانتاج الحيواني والداجني - كلية الزراعة – جامعة المنيا

الملخص

تعتبر مضادات الأكسدة الغذائية التي تستخدم في نظم تغذية الحيوانات من أهم الوسائل في تحسين الثروة الحيوانية. وبحثا عن مكمل غذائي غير تقليدي لتحسين صحة وانتاجية الحيوانات فقد أجريت هذه الدراسة باضافة تركيزين من مسحوق الطماطم الخضراء (١٠ أو٢٠ جم /كجم عليقة) الي علائق الأرانب البيضاء النيوزيلاندية ودراسة تأثيرها علي بعض القياسات الفسيولوجية والهيماتولوجية والبيوكيميائية ومضادات الأكسدة. فتم استخدام ٢٤ أرنب غير محددة الجنس عمر ٤٥ يوم، وتم تقسيمهم عشوائيا الي ثلاث مجموعات كل مجموعة اشتملت علي ٨ أرانب. اعتبرت المجموعة الأولي هي المجموعة الضابطة (الكنترول) والتي تغذت علي عليقة لا تحتوي علي مسحوق الطماطم الخضراء، أما المجموعة الثانية والثالثة فتم تغذيتها علي علائق تحتوي علي مسحوق الطماطم الخضراء بتركيز ١٠ او ٢٠ جم /كجم عليقة علي التوالي لمدة ثمانية أسابيع. وقد أظهرت النتائج أن مسحوق الطماطم الخضراء بتركيز ١٠ او ٢٠ جم /كجم عليقة له تأثيرات ايجابية كبيرة علي الأرانب النامية من خلال زيادة عدد كرات الدم الحمراء ، ونسبة الهيموجلوبين ، وطول عمود الدم ، وتركيز بروتينات السيرم ، وانخفاض نسبة الدهون في السيرم ، وزيادة النشاط المضاد للأكسدة بالاضافة الي تقليل مستوي المانوالدهيد في السيرم والذي يعتبر العلامة المميزة لأكسدة الدهون. وعلي ذلك يتضح من هذه النتائج أن مسحوق الطماطم الخضراء له تأثير قوي وايجابي لتحسين صحة وانتاجية الأرانب البيضاء النيوزيلاندية النامية.