Influence of Tomato Powder as Dietary Supplementation on Some Physiological, Biochemical and Histological Parameters in New Zealand White Growing Rabbits

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Abstract

Tomato and tomato-by products contain some phytochemicals that may have health benefits and are important sources of many nutrients. Therefore, this study aimed to investigate the effect of two different concentrations (1 and 2%) of tomato powder (TP) in diet of New Zealand white growing rabbits on some physiological, biochemical and histological parameters. Thirty unsexed New Zealand white growing rabbits (42 days old) were randomly assigned into 3 groups (10 rabbits each). The first group served as control (no additives). The second and the third groups were fed diets containing 1 and 2% tomato powder (TP), respectively, for 8 weeks. Results revealed that, no significant differences were observed in the absolute weights of some organs (kidney and thymus) and serum electrolyte concentrations (Ca, P, K, Na) between TP treated groups and control. Dietary 1 and 2% TP supplementation induced alterations in serum biochemical parameters. Serum albumin (g/dl), A/G ratio urea, uric acid and creatinine concentrations were significantly (p < 0.05) decreased by TP administration (1 and 2%), whereas globulin concentration (g/dl) was significantly (p < 10.05) increased compared to control group. Furthermore, no histopathological alterations were recorded in liver and kidney of TP treated groups. In conclusion, TP can be safely used in New Zealand white growing rabbits diet as natural growth enhancer.

Keywords: biochemical, histological, New Zealand white growing rabbit, physiological, tomato

Introduction

Tomatoes (*Solanum lycopersicum*) are one of the most broadly used vegetable crops. Tomato fruits is a healthful in the development of chronic degenerative disease which may be due to its containing antioxidant compounds (flavonoids and hydroxycinnamic acids) which decrease oxidative stress and scavenge free peroxyl radicals in the body (García-Valverde, *et al.*, 2013; Ray *et al.*, 2016). Detrimental effects of environmental stress could be partly consequent to induction of oxidative stress. Recent studies have been shown that, diets containing natural antioxidant substances could be used to attenuate the negative effects of environmental stress (Sahin and Kucuk, 2003; Sahin *et al.*, 2008).

Tomatoes is a vegetable rich in carotenoids such as lycopene, carotene, phytoene, phytofluene, lutein and L-ascorbic acid (Soma, 2013; Marković *et al.*, 2006). Lycopene is the major pigment (~ 87%) found in tomatoes and tomato products. It is an acyclic carotenoid compound contains 11 conjugated double bonds.

Due to its antioxidant activity, it has engaged attention with other carotenoids and recently showed that, it is produced potent protection versus oxygen-induced cell damage (Marković et al., 2006). Moreover, polyphenols extracted from peel, pulp and seeds of tomatoes, have an antiproliferative effect in several cancer cell lines (Li et al., 2013). Specific strategies, such as use of chemical preservatives (Giménez et al., 2015), antioxidants addition (Realini et al., 2015) and feed systems adoption (Rossi et al., 2014) have been used to reduce or prohibit damage by oxidation. One of the most important strategies which used to improve animal lambs productivity is inclusion of phytochemicals in feed of animal lamps (Salem et al., 2014; Valdes et al., 2015). In search for new safe and effective phytochemicals which can improve the growth performance in rabbits, this study aimed to investigate the effect of tomato powder supplementation on physiological, biochemical and histological parameters of New Zealand white growing rabbits.

Material and Methods Preparation of tomato Powder

Tomato was provided from a private commercial Farm at El-Minya Governorate Egypt. Then, oven dried on 50°C for 16 hrs until constant weight. The dry tomato powder (TP) was finally milled, sieved (1 mm mesh) and stored for several days in a well tight polyethylene bags at room temperature 25°C until use.

Experimental design

Thirty unsexed New Zealand white growing rabbits (42-day old, 1350 ± 150 g) were randomly as-

signed into three dietary groups (10 rabbits each) with supplementation of 0, 1 and 2 % tomato powder (TP) in diet, respectively, for 8 weeks. Animals were kept at the experimental rabbit farm site in the Department of Animal and Poultry Production, Faculty of Agriculture, Minia University. Then individually housed in cages (35x 40x 40cm) in a controlled house (14 L:10 D and 18-24°C) and fed a grower standard ration. The Experimental diets contents were as follows; protein 18%, fiber 14%, energy 2600 kcal/kg, 44 % ground yellow corn, 15.5% wheat bran, 25% sugar beet pulp, 13.5% soybean meal (44%) crude protein), 0.75% limestone, 0.5% sodium chloride and 0.75% premix (vitamins and minerals). Tomato powder was added and thoroughly hand mixed with other feed ingredients of each batch at 0, 1 and 2%. The basal experimental diets were formulated and pelleted to cover the nutrient requirements of rabbits according to National Research Council (NRC, 1977). Feed and water were provided ad libitum during the experimental period (8 weeks).

Physiological response

At the end of the experiment, ten rabbits from each group were fasted for 12 hrs before slaughtering, weighed and handily slaughtered and left complete bleeding. Slaughtered animals were de-skinned. Edible offal's (liver, kidneys, spleen and thymus) were separately weighed and recorded. All organs were determined to live body weights.

Blood constituents

Blood samples were collected by decapitation into test tubes without anticoagulant then centrifuged (K15 Ultra cooling centrifuge, ENVAIR Deutschland GmbH) at 3000 rpm for 10 min; serum was collected and kept at -20° C until analysis.

Determination of liver functions such as some liver enzymes and metabolites like; alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Babson, 1965), Gamma glutamyl transferase (GGT) (Rosalki et al. 1970), and lactate dehydrogenase (Horecker and Kornberg (LDH) 1948) were determined using diagnostic kits (Vitro, Germany). Also, total protein and albumin concentrations (Peters, 1968; Doumas et al., 1971) were determined using commercial kits (Bio-Med, Egypt). Serum globulin concentration and albumin /globulin (A/G) ratio were calculated using the following equations: -Globulin (g/dl) = Total proteins (g/dl)- Albumin (g/dl). All analytical testes were done using T80 UV Spectrophotometer UK

The serum was analyzed for levels of urea (mg/dl), uric acid (mg/dl), creatinine (mg/dl), creatinine kinase (U/l), using commercially Bio-Med reagent kits Egypt according to (Tietz, 1986, Tietz and Saunders 1990). While calcium (mg/dl), phosphate (mg/dl), potassium (mmol/L) and sodium (mmol/L) were determined using Vet scan VS2 Blood chemistry and electrolyte analyzer (ABAXIS Germany).

Histopathological Examination

Microscopic changes were observed through random selection of liver and kidney samples from each treatments and control animals. Tissues were preserved in 10% formalin followed by dehydration in ascending grades of alcohol. Clearing by xylene and embedding in paraffin wax. Paraffin sections (5µm thickness) were stained with hematoxylin and eosin (H & E) for histological examination. **Statistical analysis**

Data were statistically analyzed by one-way ANOVA and Duncan's multiple range test, using the SAS software (Statistical analysis system, version 9.1.3, 2003). (Duncan, 1955). Comparison between the control and the treatments were considered statistically significant with 95% confidence interval (P < 0.05).

Results and Discussion

Absolute and relative weights of some organs

The change in absolute and relative weights of liver, kidney, spleen and thymus were recorded for the rabbits of different groups and the results are presented in Table 1. Liver, kidney and thymus relative weights were significantly (p < 0.05) decreased by the administration of 1 and 2 % TP by 19.8, 17.3, 15.5, 22.4, 16.6 and 33.3% respectively compared to the control group. Nonsignificant decrease was observed in spleen relative weight among all groups.

	Groups		
	Control	1% TP	2% TP
Live body weight (g)	2000.3 ± 44.03^{b}	2667.3 ± 14.76^{a}	2710.4 ± 11.20^{a}
Carcass weight (g)	1152.0 ± 33.09^{b}	1328.1 ± 11.22^{a}	1343.3 ± 8.39^{a}
Liver weight (g)	47.5 ± 6.86^{b}	50.8 ± 0.75^{b}	53.2 ± 0.89^{a}
Liver (%)	2.3 ± 1.55^{a}	1.9 ± 0.03^{b}	1.9 ± 0.03^{b}
Kidney weight (g)	11.7 ± 3.68	13.1 ± 0.45	12.3 ± 0.39
Kidney (%)	0.5 ± 0.83^{a}	0.4 ± 0.01^{b}	0.4 ± 0.01^{b}
Spleen weight (g)	1.4 ± 1.12^{b}	1.8 ± 0.12^{a}	1.8 ± 0.05^{a}
Spleen (%)	0.07 ± 0.25	0.06 ± 0.004	0.06 ± 0.002
Thymus weight (g)	2.5 ± 1.52	2.8 ± 0.20	2.3 ± 0.09
Thymus (%)	0.1 ± 0.34^{a}	0.1 ± 0.007^{a}	0.08 ± 0.003^{b}

Table 1. Effect of tomato powder (TP) supplementation on live body weight, carcass and some organs absolute and relative weights of New Zealand white growing rabbits at 14 weeks of age.

1% TP, diet supplemented with 1% tomato powder; 2% TP, diet supplemented with 2% tomato powder. Values are presented as mean \pm SEM. One-way analysis of variance (ANOVA) and Duncan's multiple range test showed statistically significant differences in relation to the control at p < 0.05. Different letters in a row represent significant difference and the same letters or if no letters are shown in a row represent no significant difference.

These results in partially agreed with those obtained by Elkomy et al., (2016) who found that, there was a significant decrease in liver relative weight with increase dried tomato pomace (DTP) inclusion level. A similar study conducted by Sayed and Abdel-Azeem, (2009), revealed that, DTP can be safely used in rabbits diet up to 20%. Also, the present study shows that, when TP (1 and 2%) was fed to rabbits, live body weight and carcass weight were increased (Table 1). These results were in agreement with the study of Yitbarek, (2013), which showed that, DTP exposure by 5% in rhode island red grower chicks diet promoted increase in carcass vield values compared with other treatment groups. The effect of TP on body and carcass weight, as well as some organs in the present study may be due to containing TP a several types of carotenoids.

Liver Function Tests (LFTs)

As shown in Table 2, administration of TP (1 and 2%) significantly depleted serum AST, GGT and LDH activities within the normal range (AST: 14-113 U/L, GGT: 2-50 U/L, LDH: 34-129 U/L) compared to the control group. Also, TP administration showed non-significant differences in ALT and ALP activities. This could be attributed to the improvement in liver functions in rabbits does to the potent antioxidant activities of lycopene and other carotenoids present in TP which may have contribution to prevent against oxidative stress (Zeb and Haq, 2016). In line with the present findings, Elkomy et al., (2016), revealed that, 10, 15 and 20% of tomato pomace administration promoted a significant reduction in serum AST and ALT activities of growing rabbits.

Tabl	e 2. Alterations in serum hepatic markers enzyme ac	tivities of New Zealand
	white growing rabbits at 14 weeks of age fed on tomat	to powder supplementa-
	tion	

	GROUPS		
	Control	1% TP	2% TP
ALT (U/L)	41.7 ± 0.11	40.9 ± 0.60	40.9 ± 0.34
AST (U/L)	109.8 ± 0.25^{a}	107.9 ± 0.21^{b}	108.7 ± 0.22^{b}
ALP (U/L)	34.5 ± 1.32	34.0 ± 0.40	33.5 ± 0.20
GGT (U/L)	5.1 ± 0.02^{a}	4.1 ± 0.04^{b}	4.5 ± 0.08 ^b
LDH (U/L)	124.1 ± 0.73^{a}	100.2 ± 0.85^{b}	$101.8 \pm 1.73^{\rm b}$

1% TP, diet supplemented with 1% tomato powder; 2% TP, diet supplemented with 2% tomato powder. Values are presented as mean \pm SEM. One-way analysis of variance (ANOVA) and Duncan's multiple range test showed statistically significant differences in relation to the control at p < 0.05. Different letters in a row represent significant difference and the same letters or if no letters are shown in a row represent no significant difference.

Furthermore, the alterations of concentration of total proteins and their fractions in serum were recorded for the rabbits of different groups and the results are presented in Table 3. Tomato powder (1 and 2 %) exposure significantly (p < 0.05) decreased serum concentration of albumin (g/dl) and A/G ratio by 29.5, 39.2, 56.3 and

65.1 % respectively compared to the control group. Contrary, TP (1 and 2%) administration significantly raised globulin concentration (g/dl) by 36.4 and 41.8%, respectively. In addition, TP (1 and 2%) treatment maintained serum total protein level similar to the control group.

Table 3. Alterations in serum protein profiles of New Zealand white growing rab-
bits at 14 weeks of age fed on tomato powder supplementation.

	Groups		
	Control	1% TP	2% TP
Total protein (g/dl)	5.2 ± 0.08	5.6 ± 0.04	5.7 ± 0.17
Albumin (g/dl)	3.0 ± 0.08^{a}	2.1 ± 0.05^{b}	1.3 ± 0.04^{b}
Globulin (g/dl)	2.2 ± 0.11^{b}	$3.5\pm0.06^{\rm a}$	3.8 ± 0.20^{a}
Albumin/Globulin Ratio	1.3 ± 0.11^{a}	0.5 ± 0.02^{b}	0.4 ± 0.03^{b}

1% TP, diet supplemented with 1% tomato powder; 2% TP, diet supplemented with 2% tomato powder. Values are presented as mean \pm SEM. One-way analysis of variance (ANOVA) and Duncan's multiple range test showed statistically significant differences in relation to the control at p < 0.05. Different letters in a row represent significant difference and the same letters or if no letters are shown in a row represent no significant difference.

These findings conflict with the similar study, conducted by Elkomy *et al.*, (2016), which revealed that, administration of different concentration of dried tomato pomace (DTP), to male rabbits diet induced an increase in serum total protein and albumin concentrations. These observed differences in protein profile

may be due to various administrative concentrations of TP. Increase of serum globulin which play an important role in fighting infections within the normal range (1.5-3.8 g/dl) observed in the present study is a good indicator to improve the immune response by the administration of TP in rabbits diet. This seems to be due to preventive activity and antioxidant potential of β -carotene, lycopene and α tocopherol present in TP (Zeb and Haq, 2016).

Kidney Function Tests (LFTs)

Renal function analyses include creatine kinase (U/L), urea (mg/dl), uric acid (mg/dl) and creatinine (mg/dl) were determined in the present study to assess the safety of TP administration in rabbits diet on the kidney functions and the results are shown in Table 4. Tomato powder administration maintained serum creatine kinase (U/L) level close to control group, suggesting that TP has no deleterious effects on the kidneys. Whereas, TP (1 and 2%) administration promoted a significant (p < 0.05) decrease within the normal range in serum urea (36.6 - 84.2 mg/dl), uric acid (1 - 4.3 mg/dl) and creatinine (0.5 - 2.5 mg/dl) concentrations by 22.3, 22.8, 26.5, 37.3, 26,7 and 41.1%, respectively. Serum biochemical markers (urea, uric acid and creatinine) of renal functions are useful to evaluate the kidney health status and an increase in these markers may be associated with kidney disease (Mitchell and Kline, 2006). Decrease of serum urea, uric acid and creatinine within the normal range noted in the present study is a potent indicator to improve the kidney functions by TP administration in rabbits diet and could be due to the antioxidant defense of lycopene present in TP (Avdin et al., 2013).

 Table 4. Alterations in kidney function testes of New Zealand white growing rabbits at 14 weeks of age fed on tomato powder supplementation.

	GROUPS		
	Control	1% TP	2% TP
Creatine kinase (U/L)	118.0 ± 2.85	116.5 ± 1.02	115.2 ± 0.60
Urea (mg/dl)	55.4 ± 0.22^{a}	43.0 ± 1.08^{b}	42.7 ± 0.85^{b}
Uric Acid (mg/dl)	3.3 ± 0.06^{a}	2.4 ± 0.02^{b}	$2.1 \pm 0.04^{\circ}$
Creatinine (mg/dl)	1.4 ± 0.02^{a}	1.0 ± 0.04^{b}	$0.8 \pm 0.02^{\circ}$

1% TP, diet supplemented with 1% tomato powder; 2% TP, diet supplemented with 2% tomato powder. Values are presented as mean \pm SEM. One-way analysis of variance (ANOVA) and Duncan's multiple range test showed statistically significant differences in relation to the control at p < 0.05. No letters are shown in a row represent no significant difference.

Serum electrolytes

In order to confirm the results about biochemical markers of renal functions, analysis of serum electrolytes includes Ca (mg/dl), P (mg/dl), K (mmol/L and Na (mmol/L) were determined in the current study to assess renal functions and metabolic biochemistry profiles (Yousafzai *et al.*, 2012). As shown in Table 5, the serum electrolytes level of New Zealand white growing rabbits fed on tomato powder were similar to control group, suggesting that TP has no harmful effects on the serum electrolytes thus on the kidney functions.

14 weeks of age led on tomato powder supplementation.				
		Groups		
	Control	1% TP	2% TP	
Ca (mg/dl)	9.5 ± 0.11	9.9 ± 0.08	9.4 ± 0.13	
P (mg/dl)	7.4 ± 0.30	7.6 ± 0.08	7.8 ± 0.25	
K (mmol/L)	6.5 ± 0.12	6.1 ± 0.07	6.1 ± 0.06	
Na (mmol/L)	154.6 ± 1.03	153.3 ± 1.02	154.0 ± 2.27	

Table 5. Alterations in serum electrolytes of New Zealand white growing rabbits at14 weeks of age fed on tomato powder supplementation.

1% TP, diet supplemented with 1% tomato powder; 2% TP, diet supplemented with 2% tomato powder. Values are presented as mean \pm SEM. One-way analysis of variance (ANOVA) and Duncan's multiple range test showed statistically significant differences in relation to the control at p < 0.05. Different letters in a row represent significant difference and the same letters or if no letters are shown in a row represent no significant difference.

Histopathological alterations

In order to assess the safety of TP administration in rabbits diet on some organs, liver and kidney tissues were examined for histopathological alterations after 8 weeks of TP administration. As shown in Figure 1B and C, respectively TP (1 and 2 %) treated groups have no pathological alterations and didn't differ from the control group (Figure 1A), showed normal hepatic cells and numerous hepatic lobules, the central vein is located in the middle of the lobule and the hepatocytes are polygonal in shape with granulated, eosinophilic cytoplasm and centrally located nuclei with one or two nucleoli and delicate strands of chromatin.



Figure (1): Histopathological examination of New Zealand white growing rabbits liver following the exposure of two different concentrations of tomato powder (TP) in diet for 8 weeks. (H&E 40×). H&E = Hematoxylin and eosin stained. (A): Control rabbits, (B): 1% TP administrated group and (C): 2% TP administrated group. Thick arrows showing numerous hepatic lobules, Thin arrows showing central vein is located in the middle.

In addition, analysis of sections of kidney from the TP (1 and 2 %) treated groups revealed that, no pathological alterations were observed and didn't differ from the control group (Figure 2A), showed outer cortex contains the renal corpuscles which appear as large spherical struc-

ture and renal tubules. Each renal corpuscle is surrounded by the Bowman's capsule composed of simple squamous epithelial cells. It encloses the urinary space and the capillary tuft of the glomerulus which consists of blood capillaries.



Figure (2): Histopathological examination of New Zealand white growing rabbit's kidney following the exposure of two different concentrations of tomato powder (TP) in diet for 8 weeks. (H&E 40×). H&E = Hematoxylin and eosin stained. (A): Control rabbits, (B): 1% TP administrated group and (C): 2% TP administrated group. Thick arrows showing renal corpuscle is surrounded by the Bowman's capsule composed of simple squamous epithelial cells.

In conclusion, the current study was conducted to assess the safety of tomato powder (TP) administration by 2 different concentrations (1 and 2%) in rabbit's diet on some physiological, biochemical and histological parameters. It can be concluded from the results that, TP hasn't deleterious effects on the relative weights of some organs as well as liver and kidney functions. Furthermore, no pathological alterations were observed in rabbits' liver and kidney following the exposure of TP. Thus, TP can be considered safe to use in rabbit's diet as natural growth inducer. Further studies are needed to investigate the effect and mode of action of TP administration in animals.

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تأثير مسحوق الطماطم كمكمل غذائي على بعض القياسات الفسيولوجية والبيوكيميائية والهستولوجية في الأرانب النيوزيلندي البيضاء النامية حمادة عبد الحميد مهني علوان'، عبد الرحمن إبراهيم زنوني'، عاطف عبد المحسن عبد الرحمن' أقسم الإنتاج الحيواني والداجني – كليه الزراعة – جامعه المنيا – المنيا – مصر أقسم الكيمياء الزراعية – كليه الزراعة – جامعه المنيا – المنيا – مصر

الملخص

تحتوى الطماطم ومنتجاتها على العديد من المواد الكيميائية النباتية التى قد يكون لها فوائد صحية بالإضافة الى أنها مصدر هام للعديد من العناصر الغذائية. لذلك فقد هدفت هذه الدر اسة إلى در اسة تأثير إضافة تركيزين مختلفين (١، ٢٪) من مسحوق الطماطم الى علائق الأرانب النيوز يلندي البيضاء النامية على بعض القياسات الفسيولوجية والبيوكيميائية والهستولوجية. تم تقسيم ٣٠ أرنب (عمر ٤٢ يوم) من الارانب النيوزيلندي البيضاء النامية عشوائياً إلى ٣ مجموعات (١٠ أرانب لكل مجموعة). استخدمت المجموعة الأولى كمجموعة ضابطة. أما المجموعة الثانية وُ الثالثة فقد تم تغذيها على علائق تحتوى على ١، ٢٪ من مسحوق الطماطم لمدة ٨ أسابيع على التوالي. وقد أظهرت النتائج أنه لا توجد فروق معنوبة في الأوزان المطلّقة لبعض الأعضاء. (الكلر، الغدة الزعترية) بالإضافة الى تركيز ات بعض العناصر الموجودة في سيرم الدم (كالسيوم، فوسفور، بوتاسيوم، الصوديوم) بين المجموعة الضابطة والمجموعات المعاملة بمسحوق الطماطم. وقد أدت تغذية الارانب على علائق تحتوى على مسحوق الطماطم الى حدوث تغير إت في القياسات البيو كيميائية لسير م الدم حيث انخفض تركيز الألبيو مين ونسبة الألبيو مين الي الجلوبيولين، اليوريا، حمض اليوريك والكيرياتينين بشكل ملحوظ عند معاملة الأرانب بمسحوق الطماطم (١، ٢ %)، بينما ارتفع تركيز الجلوبيولين بشكل ملحوظ وذلك مقاربه بالمجموعة الضابطة. علاوة على ذلك لم تظهر أي تغيرات هستيولوجية مرضية في كل من انسجة الكبد والكلي في الار انب المعاملة بمسحوق الطماطم. وبالتالي فانة يمكن التوصية باستخدام مسحوق الطماطم بأمان في علائق الار انب النيوزيلندي البيضاء النامية كمحسن نمو طبيعي.