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Influence of dietary graded levels of lycopene on the growth performance, muscle cholesterol level and oxidative status of Japanese quail fed high-fat diet

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Abstract: This study investigated the impact of supplementing normal and high-fat diets with graded levels of lycopene on the growth performance, cholesterol level of the muscle, and antioxidant markers in Japanese quail. A total of 192, 14 day-old unsexed Japanese quail were part of a 2 x 4 factorial arrangement consisting of a control group; birds that were fed a normal fat diet (NFD), another control group; birds that were fed a high-fat diet (HFD) with four levels of lycopene for NFD and HFD (0, 100, 200 and 300mg lycopene/kg diet). Lycopene level of 300mg/kg gave the greatest body weight, body weight gain, and relative growth rate when added to the NFD, but this level showed non-significant improvement in growth performance when supplemented to an HFD. Superoxide dismutase in the muscle and liver was noted to be high in NFD+ 300mgL, HFD+ 200mgL, and HFD+ 300mgL groups, while malondialdehyde level in the muscle and liver and cholesterol level in the muscle was found to be low in the same groups. Lycopene slightly improved growth performance, but significantly improved the antioxidant status and lowered cholesterol concentration in the muscle. A diet supplemented with 300 mg lycopene/kg could be recommended for Japanese quail.

Key words: Antioxidant activity, cholesterol, growth performance, Japanese quail, lycopene.

INTRODUCTION

Fat is one of the key dietary components of both livestock and poultry feed. A high-fat diet (HFD) is linked to improving the productivity of meat production in the poultry industry. The interruption of oxidative balance following HED feeding, as well as the production of lipotoxic effects due to excess production of reactive oxygen species "ROS", is collectively referred to an oxidative stress (Furukawa et al. 2017). For a long time, antioxidant supplements have been used to minimize oxidative stress in animals (Tauler et al. 2006). Reactive oxygen species (ROS) are normally produced and are important to cell signalling, the immune system, and many other physiological functions. However, if ROS are produced in excess, the body's oxidative balance is altered, and this promotes inflammation, and cellular damage leading to degenerative diseases such as cardiovascular disease, cancer, aging, and metabolic disorders (Halliwell 2006). The use of carotenoids and antioxidants offers great potential to maintain the oxidative balance in a host body through several mechanisms, such as scavenging reactive oxygen species (ROS) or upregulating the production of antioxidant enzymes, such

as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (Blokhina et al. 2003, Martínez et al. 2008). That lycopene is a carotenoid pigment that possesses great health-promoting potentials, including antioxidant agents, has been known since the late 1950s (Lauretani et al. 2008). Because lycopene contains a high amount of conjugated dienes, it acts as a powerful scavenger of free oxygen radicals, with an ability twice to ten times higher than that of β-carotene and α-tocopherol, respectively (Palozza et al. 2012). The antioxidant potential of lycopene is reflected to be mainly involved to improve health.

Among the carotenoids, lycopene is considered the highest in antioxidant ability and acts as a powerful free radical scavenger (Omoni & Aluko 2005). Previously, several published studies showed the beneficial health effects of lycopene under conditions of physiological stress in both *in vitro* and *in vivo* models (Powell 2001, Ogundeji et al. 2012). As demonstrated by Bae & Bae (2011), lycopene down regulates lipoperoxidation and maintains the haematological and behavioural response of the human and animal bodies. Recently, it was found that lycopene directly modulates several molecular signalling pathways responsible for the activation of the antioxidant defence system, such as ROS producing enzymes, nuclear factor TNF-α, and κB (Palozza et al. 2012). Thus, several previous *in vitro* studies have been conducted on different models, including humans, laboratory animals, and other livestock species. Studies on lycopene and its effects on Japanese quail are, however, limited. Therefore, the aim of this study was to investigate the impact of supplementing normal and high-fat diets with graded levels of lycopene on growth performance, muscle cholesterol level, and the antioxidant status of the chest muscle and the livers of Japanese quail.

MATERIALS AND METHODS

Ethical statement

The experiment was performed according to the guidelines provided by Zagazig University, and all standard methods were officially approved by the institutional ethical committee of Zagazig University, Egypt. Throughout all procedures and experiments, all efforts were made to minimize animal suffering.

Lycopene extraction

Fresh tomatoes were purchased from a local vegetable market (Zagazig, Egypt), After being cleaned and dried, the tomatoes were ground with an equal weight of distilled water for 2 min with a Moulinex Super Blender (Type LM 207, France) at the maximum speed and lyophilized until a constant weight was obtained. A 5% (w/v) lyophilized sample in a solvent system of hexane, acetone, and alcohol in a 2:1:1 ratio containing 0.05% (w/v) butylated hydroxytoluene (BHT) was then dispersed. This was followed by the addition of 15 ml distilled water. The suspension was then agitated to achieve a homogenous mixture. Finally, the solution was allowed to stand for 15 min for separation of polar and non-polar layers. Lycopene was obtained from the polar layer of suspension and then lyophilized.

Lycopene determination

The spectrophotometric determination of lycopene from tomatoes was made according to Sadler et al. (1990), with a slight modification in the extraction with hexane/ethanol/acetone and absorbance measurement at 472 nm.

$$\text{Lycopene (mg/Kg fresh wt.)} = A472 \times 171.7 / W$$

In the above formula, W is the exact weight of tomato added, in grams.

According to the previous formula, the concentration of lycopene in the tested sample

was 80 mg/kg fresh weight (0.8mg lycopene/ gm dried tomato). These results are similar to those recorded in previous studies. Normally, tomatoes contain about 3 to 5 mg lycopene per 100g of raw material (Hart & Scott 1995). Higher amounts are found in some tomato varieties. Recently, Tonucci et al. (1995) reported that lycopene content in whole tomato fruit was more than 9.27 mg/100 g.

Experimental design and feeding management

A total of 192 14 day-old unsexed Japanese quail (*Coturnixcoturnix japonica*) were purchased from a private farm for the present study (initial body weight 35.71 ± 0.25g). At the time of arrival, the birds were randomly assigned into eight treatment groups (n = 24), and each consisted of six replicates (n = 4). The 2 x 4 factorial design of the experiment consisted of a control group of birds that were fed a normal fat diet (NFD); another control group was fed a high-fat diet (HFD) with 4 graded levels of lycopene for the NFD and HFD (0, 100, 200 and 300 mg lycopene/kg diet [air dry basis]) for 28 days. Feed and fresh drinking water were provided *ad libitum* to all birds during the entire experimental period. The compositions of the experimental diets are shown in Table I. The diets were calculated to meet the nutrient requirements of birds as recommended by the NRC (1994)1994.

Growth Performance

Body weight (BW) per replicate was recorded at the beginning and end of the trial, and total weight gain was estimated. Feed consumption was recorded weekly for each replicate throughout the experiment. Data recorded for weight gain and feed consumption were used to calculate the feed conversion ratio (FCR). Body weight gain (BWG), feed conversion ratio (FCR), relative growth rate (RGR), and protein efficiency ratio (PER) were calculated by using the following formulas (McDonald et al. 2011, Rehman et al. 2017, Wanger et al. 1983):

$$BWG = W2 - W1 \; [(W1 = \text{initial live weight (g)}, W2 \text{ final live weight (g)}]$$

$$FCR = \text{Amount of feed consumed (g)} / \text{Body weight gain (g)}$$

$$RGR = W2 - W1 / \; 0.5 \; (W1 + W2) \times 100$$

$$PER = \text{Live weight gain (g)} / \text{Protein intake (g)}$$

Sampling and laboratory analysis

At the end of the trial, six quail birds were randomly selected from each group and slaughtered as per the recommendation of the institutional committee. Chest muscle and liver samples were taken from all sacrificed birds from each group. After removing the visible fat, six grams of muscle and liver samples from each bird were homogenized and transferred into individual sterilized tubes containing 10% w/v homogenate of 0.05 M phosphate buffer (pH 7) and then centrifuged at 12,000 ×g for 60 min at 4°C. The supernatant was obtained and utilized for further analysis of cholesterol in chest muscle, lipid peroxidation, and malondialdehyde (MDA) in chest muscle and liver according to the methods described previously (Uchiyama & Mihara 1978), as well as for further analysis of SOD (Qwele et al. 2013).

Statistical analysis

The data was analysed by using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA) and expressed as the mean ± standard deviation (SD). The variation was assessed by a two-way (ANOVA) and the differences between experimental groups were calculated by Duncan's multiple-range test. The statistical significance of the results was calculated at ($P \leq 0.05$).

Table I. Ingredients and proximate composition of the experimental diets.

Ingredients	NFD[#]	HFD[##]
Ground yellow corn	558	504.5
Corn gluten	68	28
SBM	342.3	412.3
Oil	1.5	26
caco3	12	12
ca dibasic	9.2	9.2
Nacl	3.5	3.5
Premix*	3	3
Antimycotoxin	0.5	0.5
Methionine	1	1
Lysine	1	0
Chemical composition (g/kg)		
DM	900.5	900.5
ME(kcal/kg diet)	2906.99	2946.06
Crude protein	245.4	243.5
Lipid	27.0	48.6
Ca	8.0	8.2
Ap	3.2	3.3
CF	37.1	40.3
Lysine	13.0	13.7
Methionine	5.4	5.1

[#]NFD: Control group fed normal fat diet. [##]HFD: Control group fed high-fat diet.
***Premix per kg of diet: vitamin A, 1 500 IU; vitamin D3, 200 IU; vitamin E, 10 mg; vitamin K3, 0.5 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; pantothenic acid, 10 mg; folic acid, 0.55 mg; pyridoxine, 3.5 mg; niacin, 35 mg; cobalamin, 0.01 mg; biotin, 0.15 mg; Fe, 80 mg; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.35 mg; Se, 0.15 mg.**
Nutrient level of the diets was based on NRC (1994).

RESULTS

Growth performance

Effects of different dietary levels of lycopene supplementation, dietary fat levels, and their interactions on the growth performance of Japanese quail are shown in Tables II and III. Birds fed the NFD or HFD were not significantly different ($P > 0.05$) in the final body weight, body weight gain and RGR. Feed intake and FCR were significantly decreased ($P \leq 0.05$) in birds fed HFD than those fed NFD. PER was significantly increased ($P \leq 0.05$) in birds fed HFD than those fed NFD. Varying levels of lycopene supplementation did not significantly ($P > 0.05$) affect the final body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) or the protein efficiency ratio (PER), when compared to the control group. Birds fed the diet supplemented with 300 mg/kg lycopene significantly ($P \leq 0.05$) increased RGR compared to the other groups. Supplementing

a NFD with 300 mg/kg lycopene significantly *(P≤ 0.05*) increased BW, BWG and RGR compared to the control groups. However, these parameters were not significantly (*P >0.05*) different in the group of birds fed the HFD supplemented with 300 mg/kg lycopene compared to that of the control group (no lycopene supplementation). Birds fed HFD supplemented with 100 or 200 mg/kg lycopene showed significantly decreased BW, BWG and RGR than the control group. Feed intake and FCR were not significantly different (*P >0.05*) in lycopene supplemented groups in comparison with the control group. PER was not significantly different (*P >0.05*) in NFD supplemented with different levels of lycopene, while it was significantly increased (*P≤ 0.05*) in HFD supplemented with 100 mg lycopene/kg.

Antioxidant status

The effects of various graded levels of lycopene supplementation, fat levels, and their interactions on antioxidant enzymes in the liver and muscle, as well as the cholesterol level in

the chest muscle of Japanese quail, are shown in Tables IV and V. Supplementing a NFD with 300 mg/kg lycopene significantly (*P≤ 0.05*) decreased the MDA level in the liver and muscle compared to all other lycopene supplemented groups (0, 100, and 200 mg/kg diet). Supplementing HFD with 200 or 300 mg/

Table II. Effects of graded levels of lycopene supplementation to normal fat diet (NFD) and high-fat diet (HFD) on the final body weight and body weight gain of growing Japanese quail.

parameters		Initial weight (g)	Final BW.(g)	BWG(g)
Diet				
NFD		36.05±.1.85	165.94±11.76	129.89±11.65
HFD		35.36±1.62	166.96±13.37	131.59±13.02
Lycopene level mg/kg				
0		36.21±1.50	168.64±15.81^{ab}	132.42±15.56^{ab}
100		35.18±1.22	162.40±10.53^{b}	127.22±10.87^{b}
200		35.83±2.56	159.75±8.20^{b}	123.91±6.73^{b}
300		35.60±1.513	175.01±9.17^{a}	139.40±9.30^{a}
Interaction effects				
NFD	0	36.33±1.69.	159.87±13.18^{c}	123.54±13.20^{c}
	100	35.41±0.97	163.87±12.23^{abc}	128.45±11.93^{abc}
	200	36.92±2.43	163.87±9.42^{abc}	126.95±7.76^{bc}
	300	35.54±2.09	176.16±6.63^{a}	140.62±6.94^{a}
HFD	0	36.10±1.45.	177.42±13.83^{a}	141.32±13.01^{a}
	100	35.37±0.91.	160.94±9.45^{bc}	125.56±10.02^{bc}
	200	36.41±0.97	155.62±4.30^{c}	119.20±3.46^{c}
	300	35.66±0.80	173.86±11.75^{ab}	138.19±11.78^{ab}

BW: body weight. BWG: body weight gain.
^{abc}Means within the same column carrying different superscripts are significantly different at (*P*≤0.05).

kg also significantly (*P≤ 0.05*) decreased the MDA level in the liver. All graded levels of lycopene (100, 200, 300 mg/kg) combined with the HFD significantly (*P≤ 0.05*) reduced the MDA level in chest muscle of Japanese quail compared to the control group. Birds fed the NFD together with 300mg lycopene/kg had significantly higher SOD enzyme concentrations in the liver and muscle compared to all other lycopene supplemented groups (0, 100, and 200 mg/kg diet)., while the birds fed the HFD with 200 and 300 mg/kg added lycopene showed significantly (*P*≤ 0.05) higher SOD levels in the liver and muscle compared to the control group and the group fed the HFD together with 100 mg lycopene/kg.

Cholesterol level

The effects of various graded levels of lycopene supplementation, fat levels, and their interactions on the cholesterol level in the chest muscle of Japanese quail, are shown in (Table V). The group receiving the NFD together with the 300 mg/kg lycopene had a significantly (*P≤ 0.05*) decreased muscle cholesterol level compared to the control group and other lycopene supplemented groups. The group receiving the HFD, together with 200 and 300 mg/kg lycopene displayed significantly decreased muscle cholesterol levels compared to the control group and the 100mg/kg lycopene supplemented group.

Table III. Effects of graded levels of lycopene supplementation to normal fat diet (NFD) and high-fat diet (HFD) on FI, FCR, PER and RGR of growing Japanese quail.

Parameters		FI(g)	FCR	PER	RGR
Diet					
NFD		506.79±46.32^{a}	3.92±0.43^{a}	1.05±0.11^{b}	128.41±4.79
HFD		395.42±42.19^{b}	3.02±0.36^{b}	1.37±0.17^{a}	128.48±4.88
Lycopene level mg/kg					
0		459.72±62.05^{a}	3.53±0.71^{ab}	1.20±0.23^{ab}	128.94±5.59^{b}
100		411.41±81.37^{b}	3.25±0.72^{b}	1.31±0.28^{a}	127.27±4.33^{bc}
200		456.89±53.77^{a}	3.68±0.38^{a}	1.12±0.11^{b}	125.29±2.16^{c}
300		476.39±76.43^{a}	3.41±0.51^{ab}	1.22±0.18^{ab}	132.27±3.80^{a}
Interaction effects					
NFD	0	499.25±66.31^{ab}	4.06±0.57^{a}	1.02±0.14^{d}	125.68±5.54^{b}
	100	486.17±28.49^{b}	3.82±0.52^{ab}	1.09±0.15^{cd}	128.72±4.06^{ab}
	200	505.29±26.20^{ab}	3.99±0.30^{a}	1.03±0.07^{d}	126.45±2.54^{b}
	300	536.44±48.38^{a}	3.82±0.31^{ab}	1.08±0.09^{cd}	132.82±3.87^{a}
HFD	0	420.18±17.7^{b}	2.997±0.33^{cd}	1.38±0.15^{b}	132.21±3.54^{a}
	100	336.65±18.49^{c}	2.69±0.30^{d}	1.53±0.16^{a}	127.76±4.50^{b}
	200	408.49±7.2^{b}	3.42±0.12^{bc}	1.19±0.04^{c}	124.17±0.79^{b}
	300	416.34±43.13^{b}	3.021±.31^{cd}	1.37±.13^{b}	131.73±4.02^{a}

FI: feed intake. FCR: feed conversion ratio. PER: protein efficiency ratio. RGR: relative growth rate.
^{abcd}Means within the same column carrying different superscripts are significantly different at (*P*≤0.05).

Table IV. Effects of graded levels of lycopene supplementation to normal fat diet (NFD) and high-fat diet (HFD) on antioxidant enzymes in the liver of Japanese quail.

parameters		MDA(µmol/gm)	SOD(µmol/gm)
Diet			
NFD		220.00±5.64[a]	92.54±13.09[b]
HFD		204.66±9.88[b]	120.70±9.52[a]
Lycopene level mg/kg			
0		218.66±5.46[a]	101.91±16.56[b]
100		217.83±7.24[a]	95.91±14.11[b]
200		207.83±14.62[b]	106.50±21.01[b]
300		205.00±8.58[b]	122.16±9.27[a]
Interaction effects			
NFD	0	222.83±2.48[a]	86.16±1.16[d]
	100	223.66±3.98[a]	83.16±4.70[d]
	200	221.33±3.66[a]	87.00±4.60[d]
	300	212.16±3.06[b]	113.83±3.06[b]
HFD	0	214.50±4.23[b]	117.66±2.58[b]
	100	212.00±4.24[b]	108.66±5.12[c]
	200	194.33±4.41[c]	126.00±6.13[a]
	300	197.83±5.41[c]	130.50±3.61[a]

MDA:malondialdehyde. SOD:superoxide dismutase.
[abcd]Means within the same column carrying different superscripts are significantly different at (*P*≤0.05).

DISCUSSION

Antioxidant compounds are often used in poultry diets. Supplementation of these compounds is commonly practiced in poultry feeding systems to improve the productivity and maintain the health of animals, including poultry species. Lycopene is a carotenoid pigment, predominantly found in many kinds of ripe fruits and vegetables, and, among all commonly known carotenoids, has been shown to possess the greatest promise as a potent antioxidant agent (Sevcikova et al. 2008). The present results showed that lycopene supplementation at a dose of 300mg/kg diet to the NFD significantly improved the growth performance of Japanese quails, while this level of lycopene supplementation did not result in any significant improvements in growth performance when added to the HFD in comparison to that of the control group. Similarly, previous studies showed that green tea extract and lycopene fed to rabbits did not induce negative effects on growth performance, body weight gain (BWG), feed intake, and feed conversion ratio (FCR) (Tedesco et al. 2005).

Sahin et al. (2006), observed that the feed intake of Japanese quails, reared under a high ambient environmental temperature, was

Table V. Effects of graded levels of lycopene supplementation to normal fat diet (NFD) and high-fat diet (HFD) on antioxidant enzymes and cholesterol level in the chest muscle of Japanese quail.

parameters		MDA(µmol/gm)	SOD(µmol/gm)	Cholesterol(mg/dl)
Diet				
NFD		142.33±5.30^{a}	95.95±11.53^{b}	103.16±1.47^{a}
HFD		125.20±6.50^{b}	119.16±8.19^{a}	88.12±1.63^{b}
Lycopene level mg/kg				
0		139.00±6.72^{a}	103.50±8.44^{b}	98.33±6.84^{a}
100		134.83±9.69^{ab}	100.75±14.85^{b}	102.58±8.25^{a}
200		133.50±12.81^{ab}	104.91±19.32^{b}	96.50±10.50^{a}
300		127.75±9.64^{b}	121.08±8.08^{a}	85.16±9.16^{b}
Interaction effects				
NFD	0	144.83±3.18^{a}	95.83±1.72^{d}	104.50±2.16^{b}
	100	143.66±3.50^{a}	87.33±3.88^{e}	109.16±5.26^{a}
	200	144.83±5.34^{a}	86.83±3.48^{e}	105.50±6.15^{ab}
	300	136.00±3.63^{b}	113.83±3.06^{c}	93.50±2.42^{c}
HFD	0	133.16±2.78^{b}	111.16±3.60^{c}	92.16±2.63^{c}
	100	126.00±2.68^{c}	114.16±6.17^{c}	96.00±4.24^{c}
	200	122.16±4.95^{c}	123.00±4.93^{b}	87.50±3.27^{d}
	300	119.50±5.28^{d}	128.33±2.87^{a}	76.83±3.48^{e}

MDA: malondialdehyde. SOD: superoxide dismutase.
^{abcd}Means within the same column carrying different superscripts are significantly different at ($P \leq 0.05$).

improved in the treated groups fed dietary supplemented lycopene (50, 100 or 200 mg of lycopene/kg of diet). Supplementation of tomato waste containing a predominant amount of lycopene in broiler diets showed significantly increased FI in the starter and grower phases from 1 to 28 days of age, but feed intake and FCR were drastically decreased from 29 to 36 days of age (Lira et al. 2010). Lycopene is a well-known carotenoid pigment that possesses great health-promoting potentials, including as an antioxidant agent (Lauretani et al. 2008). Because lycopene contains a high amount of conjugated dienes, it acts as a powerful scavenger of free oxygen radicals, with an ability twice and ten times higher than of β-carotene and α-tocopherol, respectively (Palozza et al. 2012). Another important antioxidant effect of lycopene suggested by Rao & Agarwal (1998) is its potential to inhibit the lipid peroxidation of nutritional fat.

The results of the present study showed that the supplementation of 300 mg/kg lycopene to a NFD and 200 and 300 mg/kg lycopene supplementation to a HFD significantly enhanced the superoxide dismutase enzyme. On the other hand, a low concentration of malondialdehyde was noted in the liver and muscles of Japanese quail. Similarly, a reduction in the concentration of MDA was found in broilers treated with various levels of plant-derived mixture containing rich amounts of lycopene (Leal et al. 1999). The combined effects of lycopene and selenium were investigated to evaluate the status of breast meat stored for 5 days, and indicated that the combined application of lycopene with other antioxidants synergistically inhibit the formation of MDA (Sevcikova et al. 2008). Another study examined the MDA concentration in the meat of Japanese quail after storage for 6 to 9 days; these results indicated that the dietary inclusion of dried tomato pulp by 5% in the feed of Japanese quail exhibits antioxidant effects. However, the inclusion levels were increased up to 10% to produce pro-oxidant effects (Botsoglou et al. 2004). Similarly, Tedesco et al. (2005) evaluated the antioxidant interaction of lycopene- and green tea-derived extract on the improvement of the lipid oxidation status of stored mammal meat for 7 days; positive effects were reported.

Extra cholesterol accumulation in the body impairs the normal homeostasis of the living being. The current findings showed that levels of lycopene supplementation in NFDs and HFDs showed significantly lower cholesterol levels in the chest muscles of the Japanese quail. The results of the present study are in line with Englmaierova et al. (2011), who reported that the lycopene-enriched diet fed to Japanese quail significantly reduced the meat cholesterol level. Numerous other studies have also suggested that the dietary addition of lycopene produces cholesterol-lowering effects in circulating blood (Rao & Shen 2002), improves plasma concentration of HDL in Japanese quail, and effectively lowers the LDL concentration (Sahin et al. 2006).

CONCLUSION

From the aforementioned results and discussion, we can conclude that lycopene carotenoid (active component of tomato) could be used as a natural antioxidant, to reduce the oxidative stress. Our results showed that lycopene supplementation resulted in a slight improvement in growth performance of the quail, together with significant improvement meat quality. Muscle total cholesterol was significantly reduced in birds receiving the NDF supplemented with 300mg/kg and in those receiving either the 200, or 300 mg/kg lycopene together with the HFD. These findings will positively affect the production of healthy meat for consumers. The 300 mg lycopene/kg diet could be recommended in future to ensure ideal performance for Japanese quail.

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