

The effect of lycopene and vitamin E on growth performance, quality and oxidative stability of chicken leg meat

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ABSTRACT: A 2 × 3 factorial design experiment was conducted to evaluate the effect of adding lycopene (0 and 75 mg/kg) and vitamin E (0.50 and 100 mg/kg) to the diet of chickens. Moreover, the study investigated growth traits, oxidative stability and chemical composition of leg meat and the vitamin content of meat and liver. The study was conducted using five hundred and forty Ross 308 male broilers that were assigned to one of the six dietary treatments. Significant interactions between lycopene and vitamin E additions affected the body weight of 21-days-old chickens ($P = 0.005$), the malondialdehyde content in fresh leg meat ($P < 0.001$) and leg meat stored for 3 days at temperatures of 2.5 to 4°C ($P = 0.032$), the cholesterol content in leg meat ($P < 0.001$) and the lycopene content in liver ($P = 0.006$). The chickens with the highest body weight were fed 75 mg/kg of lycopene and 50 mg/kg of vitamin E. The vitamin E supplement increased the oxidative stability of fresh and stored leg muscle ($P < 0.001$). The lowest mean cholesterol value (3.49 g/kg of dry matter) was found out in the meat from broilers that were fed 75 mg/kg of lycopene in contrast to broilers fed the control treatment without lycopene (3.93 g/kg of dry matter). Dietary vitamin E significantly reduced the fat content ($P = 0.033$) and increased the ash content of leg meat. The highest lycopene concentration in liver (2.82 mg/kg of dry matter) was in chickens that were fed the highest levels of vitamin E and lycopene in contrast with the control group (0.28 mg/kg of dry matter).

Keywords: antioxidants; lycopene; α -tocopherol; broiler; growth traits; meat quality; malondialdehyde

Although more than 700 carotenoids have been identified, only 6 carotenoid forms are present in observable amounts in Western diets and in human blood and tissues. These carotenoid forms are α - and β -carotene, lycopene, β -cryptoxanthin, lutein and zeaxanthin (Borel et al., 2007). Lycopene is most commonly found in vegetables and in some fruit species as a red pigment (e.g. pineapple, orange, grapefruit, tomato, sweet pepper and strawberry). Tomatoes and related tomato products are the major source of lycopene in the human diet. The amount of lycopene in fresh tomato fruits depends on the tomato variety and maturity as well as on

the environmental conditions under which the fruit matured (Shi and LeMaguer, 2000; Thompson et al., 2000; Marković et al., 2006).

Lycopene is an acyclic carotenoid and contains 11 conjugated double bonds. In nature, most carotenoids originally occur in the all-trans forms (Stahl and Sies, 1996; Gerster, 1997; Clinton, 1998; Marković et al., 2006). Lycopene, with its acyclic structure, large array of conjugated double bonds, and extreme hydrophobicity, exhibits many unique and distinct biological properties. With the use of an *in vitro* system, in which lycopene and other carotenoids were bound to the surface of human

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lymphoid cells, it was recently demonstrated that lycopene provided strong protection against singlet oxygen-induced cell damage (Sies and Stahl, 1995; Stahl and Sies, 1996; Gerster, 1997; Rao and Agarwal, 1999; Marković et al., 2006).

The discovery that carotenoids deactivate singlet molecular oxygen was an important advance in understanding the biological effects of carotenoids (Foote and Denny, 1968). The role of dietary antioxidants such as carotenoids in health and diseases is attracting increasing attention and numerous trials are in progress to ascertain the benefits of these compounds in the diet (Nierenberg et al., 1997; Leal et al., 1999). Lycopene is extraordinarily efficient in the control of degenerative diseases; it is a preventive against cardiovascular diseases and cancer of the prostate gland, digestive tract, skin, pancreas and uterine uvula. Lycopene also blocks the biosynthesis of cholesterol. Many studies have investigated the importance of lycopene in human health and diseases (Ševčíková et al., 2008). Blum et al. (2006) reported that a tomato-rich diet increased HDL-cholesterol levels. Napolitano et al. (2007) investigated the effects of lycopene on the induction of foam cell formation by modified LDL. Their findings suggest that lycopene may reduce the macrophage foam cell formation induced by modified LDL by decreasing lipid synthesis and downregulating the activity and expression of scavenger receptor activity. Few papers have reported the effects of lycopene in poultry diets. The effects of lycopene on the performance and quality of the meat and eggs of Japanese quail were studied by Botsoglou et al. (2004) and Sahin et al. (2006a,b), of laying hens by Yannakopoulos et al. (1992) and Dotas et al. (1999), and of broiler chicks by Leal et al. (1999). Lycopene is a potent antioxidant that provides protection against cellular damage caused by reactive oxygen species. Lycopene has an effective free radical scavenging activity, and this action could be beneficial to poultry because harmful free radicals are formed under the stress, fast growth, high reproduction rates and intensive metabolism conditions of poultry farming. Lycopene may play an important role in the antioxidant defence system (Ševčíková et al., 2008).

α -Tocopherol is the most active natural antioxidant used in animal feeds; it exhibits an antioxidant activity at low concentrations and a prooxidant activity at high concentrations (Chen et al., 1998; Franchini et al., 2002). Dietary supplementation of antioxidants is an effective way to maintain the lipid

stability of meat (Grau et al., 2001). Vitamin E is a major lipid-soluble antioxidant that is able to protect membrane-bound lipids from metmyoglobin/hydrogen peroxide-initiated oxidation (Buckley et al., 1989). Vitamin E is thought to reduce fatty acyl hydroxyperoxy radicals (ROO) to yield less reactive hydroperoxides (ROOH) (Perez et al., 2010). Tocopherols may also provide health benefits by preventing cancer and coronary diseases (Diplock, 1991; Knekt et al., 1991).

Vitamin E supplementation has improved the growth and feed utilisation of birds and has substantially improved the stability of meat quality against oxidative deterioration (Guo et al., 2001; Skřivan et al., 2010). Attia et al. (2001) reported that vitamin E supplementation significantly increased body weight at six weeks of age ($P < 0.05$) and that body weight gain, feed consumption and feed conversion were not influenced by dietary treatments.

This experiment was performed to determine the combined effects of antioxidants in poultry diets and to examine the possibility of affecting product quality. The aim of this study was to evaluate the effects of two dietary doses of vitamins E, alone or in combination with lycopene, on the health and growth performance of broiler chickens and on the oxidative stability of leg meat.

MATERIAL AND METHODS

Five-hundred and forty Ross 308 cockerels (0-day-old) were randomly assigned to 1 of the 6 dietary treatments (with 3 replicate pens of 30 chickens per pen) with supplementation of lycopene (0 and 75 mg/kg) and vitamin E (0, 50 and 100 mg/kg). A lycopene powder (10% lycopene, 60% lactose and 30% D-glucose; Alchimica, Prague, Czech Republic) was used as the lycopene source, and vitamin E was added in the form of Rovimix E-50 Adsorbate (50% DL- α -tocopheryl acetate and 50% silicon dioxide; Biofaktory, s.r.o., Prague, Czech Republic). The ingredients and chemical composition of the basal diet are presented in Table 1. Feed and water were provided *ad libitum*. The light cycle consisted of 16 h of light and 8 h of darkness. The chickens were housed in pens (2 m \times 1.1 m) on wood shavings with gas heating and ventilation with a temperature-controlled fan. Each pen was equipped with 7 nipple drinkers, 3 pan feeders and a feed hopper. The feed consumption, body weight and mortality were examined. Broilers were weighed at 0, 21, and 36 days of age. The ex-

Table 1. Composition of the basal diet^a

Ingredient	(g/kg)
Wheat	298.8
Maize	290
Soybean meal	320
Fish meal	20
Rapeseed oil	40
Dicalcium phosphate	8
Sodium chloride	2
Limestone	12.5
L-Lysine hydrochloride	1.7
DL-Methionine	2
Vitamin-mineral premix ^b	5
Analysed nutrient composition	
Dry matter	888
Crude protein	227
Crude fat	62
Crude fibre	28
Ash	51
Calcium	9
Phosphorus	6
Lycopene (mg/kg)	0.4
Vitamin E (mg/kg)	16.43
AME _N by calculation (MJ/kg)	12.92

^a0 and 75 mg/kg of lycopene and 0, 50 and 100 mg/kg of vitamin E were supplemented to experimental diets

^bthe vitamin/mineral premix provided per kg of diet: retinyl acetate 3.6 mg, vitamin D₃ 0.125 mg, vitamin K₃ 3 mg, niacin 50 mg, Ca pantothenate 20 mg, thiamine 3 mg, riboflavin 6 mg, pyridoxine 4 mg, folic acid 1.75 mg, biotin 0.2 mg, cobalamin 0.015 mg, choline Cl 250 mg, betain 100 mg, butylated hydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, butylhydroxyanisol 1 mg, Mn 100 mg, Zn 80 mg, Fe 50 mg, Cu 17.5 g, I 1 mg, Co 0.4 mg, Se 0.3 mg, Mo 0.5 mg, and salinomycin sodium 70 mg

periment was terminated when the broilers were 36 days of age. Ten broiler chickens representing the average live weight in each treatment were selected and sacrificed at a slaughtering plant. Ten samples of leg meat from each treatment were stored in plastic bags at –20°C before analyses for dry matter, ash, fat and crude protein, and at –70°C before analyses

for cholesterol, vitamin A, vitamin E and thiobarbituric acid-reactive substances (TBA). The livers ($n = 10$) were stored at –70°C for lycopene and lutein content assessment. The maximum freezer storage time was 2 months.

Laboratory analyses

The feed, leg meat and liver dry matter were determined by oven drying at 105°C to constant weight. The ash content of feed and meat were determined by ashing at 550°C in a muffle furnace (AOAC, 1997). The fat contents of feed and meat were analysed by extraction with petroleum ether in a Soxtec 1045 apparatus (Tecator Co., Hoganas, Sweden). The crude protein content of feed and meat were measured using a Kjeltac Auto 1030 Analyzer (Tecator Co., Hoganas, Sweden). The calcium content of feed was analysed in ashed samples by atomic absorption spectrometry (Solaar M-6, JTA Solutions, UK), and the phosphorous content was analysed colorimetrically using a molybdate reagent (Huxtable and Bressler, 1973). For determination of the cholesterol content in meat, lipids were saponified, and the unsaponified matter was extracted with diethyl ether in accordance with ISO 3596 (1988). Silyl derivatives were prepared using TMCS and HMDS silylation reagents (Sigma) and quantified on a gas chromatograph equipped with a SAC-5 capillary column (Supelco, Bellefonte, USA) that was operated isothermally at 285°C. The lipid oxidation in leg meat samples was measured using the method of Piette and Raymond (1999), and the results were expressed as TBA in mg of malondialdehyde (MDA)/kg muscle. Before the analysis, the leg meat was thawed and stored in a refrigerator at a temperature range of 2.5 to 4°C for 0 or 3 days. The α -tocopherol and retinol contents of the leg meat were determined in accordance with the EN 12822 European standard (2000) by Shimadzu HPLC system (VP series) equipped with a diode-array detector. The contents of lycopene and lutein in liver were detected by HPLC according to the methods of Leal et al. (1999) and Leeson et al. (2007), respectively.

Statistical analysis

The data from our experiment with a 2 × 3 full factorial design were analysed by a two-way analysis of variance (ANOVA) using the General Linear

Table 2. Growth traits

Characteristic	Levels of vitamin E (E) and lycopene (L) of the diet (in mg/kg)						SEM	Probability		
	0 E		50 E		100 E			L	E	L × E
	0 L	75 L	0 L	75 L	0 L	75 L				
BW (0 day; g)	44.5	45.0	44.6	44.7	44.7	45.0	0.17	n.s.	n.s.	n.s.
BW (21 st day; g)	989.6 ^b	994.2 ^b	975.0 ^b	1 030.4 ^a	988.9 ^b	965.9 ^b	5.00	n.s.	n.s.	0.005
BW (35 th day; g)	2 577.8 ^a	2 559.0 ^{ab}	2 539.9 ^{ab}	2 602.4 ^a	2 499.3 ^b	2 495.0 ^b	10.26	n.s.	0.004	n.s.
F:G (35 th day; g/g)	1.51	1.55	1.57	1.50	1.67	1.51	0.021	n.s.	n.s.	n.s.

BW = body weight; F:G = feed:gain

^{a,b}means with different superscripts differ significantly; n.s. = not significant

Models (GLM) procedure of the SAS software (SAS, 2001). The main effects were the supplementation with lycopene (L), the addition of vitamin E (E) and the interaction between these two factors (L × E). Differences were considered statistically significant at $P < 0.05$. The results in the tables are presented as the means and the standard errors of the mean (SEM).

RESULTS

Table 2 summarises the growth performance results. A significant interaction ($P = 0.005$) between lycopene and vitamin E supplementation was observed for the body weight of 21-days-old broilers. The highest mean body weight (1030.4 kg) was for broilers treated with 75 mg/kg of lycopene and 50 mg/kg of vitamin E. High doses of vitamin E (100 mg/kg) significantly decreased body weight ($P = 0.004$). Feed conversion (F:G) was not affected by the addition of either lycopene or vitamin E.

The oxidative stability of meat is presented in Table 3. The combination of vitamin E with lycopene significantly influenced the MDA value of

fresh leg meat ($P < 0.001$) and meat that had been stored for 3 days ($P = 0.032$). The MDA production was significantly decreased ($P < 0.001$) by the addition of 50 mg/kg of vitamin E without lycopene (0.271 mg/kg) and 100 mg/kg of vitamin E with lycopene (0.276 mg/kg) compared with the fresh meat from chickens treated with 75 mg/kg of lycopene alone (0.511 mg/kg). The lowest mean MDA values in meat that had been stored for three days at 2.5–4°C were from chickens treated with 50 mg/kg of vitamin E (0.474 mg/kg) and 100 mg/kg of vitamin E (0.427 mg/kg) compared with the control group (0.801 mg/kg). These differences were statistically significant ($P < 0.001$).

The analysis of chemical indicators (Table 4) of leg muscle quality revealed an interaction between lycopene and vitamin E on cholesterol content ($P < 0.001$). The addition of lycopene without vitamin E reduced the amount of cholesterol (3.49 g/kg DM) compared with the control (3.93 g/kg DM). Vitamin E decreased the fat content ($P = 0.033$) and increased the ash content ($P = 0.020$) of the leg meat. The content of fat, crude protein and ash was not significantly influenced by lycopene supplementation of the diet.

Table 3. The malondialdehyde value of fresh leg meat (MDA 0) and meat that had been stored for 3 days (MDA 3)

Characteristic	Levels of vitamin E (E) and lycopene (L) of the diet (in mg/kg)						SEM	Probability		
	0 E		50 E		100 E			L	E	L × E
	0 L	75 L	0 L	75 L	0 L	75 L				
MDA 0 (mg/kg)	0.387 ^b	0.511 ^a	0.271 ^d	0.367 ^{bc}	0.313 ^{cd}	0.276 ^d	0.0146	0.001	< 0.001	< 0.001
MDA 3 (mg/kg)	0.801 ^a	0.620 ^b	0.474 ^c	0.488 ^{bc}	0.427 ^c	0.487 ^{bc}	0.0258	n.s.	< 0.001	0.032

^{a-d}means with different superscripts differ significantly; n.s. = not significant

Table 4. Chemical analysis of leg meat (g/kg DM)

Characteristic	Levels of vitamin E (E) and lycopene (L) of the diet (in mg/kg)						SEM	Probability		
	0 E		50 E		100 E			L	E	L × E
	0 L	75 L	0 L	75 L	0 L	75 L				
Fat content	205.6 ^a	217.3 ^a	179.4 ^b	198.4 ^{ab}	195.7 ^{ab}	201.2 ^{ab}	3.50	n.s.	0.033	n.s.
Crude protein content	745.1	732.7	759.4	751.8	754.3	742.0	3.37	n.s.	n.s.	n.s.
Ash content	40.5 ^{ab}	39.5 ^b	41.7 ^a	41.2 ^a	41.0 ^a	41.2 ^a	0.22	n.s.	0.020	n.s.
Cholesterol content	3.93 ^a	3.49 ^d	3.65 ^c	3.78 ^b	3.59 ^{cd}	3.85 ^{ab}	0.025	n.s.	n.s.	< 0.001

DM = dry matter

^{a-d}means with different superscripts differ significantly; n.s. = not significant

Table 5. The vitamin E and vitamin A contents of leg meat and the lycopene and lutein contents of liver (mg/kg DM)

Characteristic	Levels of vitamin E (E) and lycopene (L) of the diet (in mg/kg)						SEM	Probability		
	0 E		50 E		100 E			L	E	L × E
	0 L	75 L	0 L	75 L	0 L	75 L				
Vitamin E	22.15 ^d	20.31 ^d	47.83 ^c	42.74 ^c	70.35 ^a	58.89 ^b	2.596	0.002	< 0.001	n.s.
Vitamin A	0.701 ^c	0.939 ^b	0.666 ^c	1.079 ^a	0.683 ^c	0.970 ^{ab}	0.0284	< 0.001	n.s.	n.s.
Lycopene	0.28 ^d	1.66 ^c	0.29 ^d	2.20 ^b	0.27 ^d	2.82 ^a	0.151	< 0.001	0.009	0.006
Lutein	4.73	4.57	4.40	4.39	4.69	4.31	0.068	n.s.	n.s.	n.s.

DM = dry matter

^{a-d}means with different superscripts differ significantly; n.s. = not significant

Lycopene supplementation significantly decreased ($P = 0.002$) the levels of vitamin E and significantly increased ($P < 0.001$) the levels of vitamin A in the leg muscles (Table 5). A higher concentration of vitamin E was found in leg muscles after supplementation of vitamin E alone to the diet, but this increase was not statistically significant. The simultaneous addition of lycopene and vitamin E into the basal diet significantly increased ($P = 0.006$) the levels of lycopene in the liver. Neither lycopene nor vitamin E addition had a statistically significant effect on the accumulation of lutein in the liver.

DISCUSSION

An interaction between the antioxidants lycopene and vitamin E was found in the body weight of 21-days-old chickens. Lycopene supplementation without vitamin E had no statistically significant effect on the performance characteristics of the chickens. Similar findings have been reported

for mammals. Jain et al. (1999) reported that the live weight and feed intake of rats were not affected by lycopene supplementation. In rabbits, treatment with lycopene and green tea extract did not negatively affect the performance characteristics of feed intake, body weight and feed conversion ratio (Tedesco et al., 2005). In contrast, an increased feed consumption as a result of lycopene supplementation was observed in Japanese quails reared at a high ambient temperature (Sahin et al., 2006a). In broiler chickens, Lira et al. (2010) reported that dietary supplementation of tomato waste that was high in lycopene increased feed intake in the periods from 1 to 7, 8 to 14 and 29 to 36 days of age and worsened weight gain and feed conversion up to 29 days of age. The authors also reported that vitamin E addition significantly reduced the live weight of chickens at the end of fattening. This finding may be related to those reported by Chen et al. (1998), who showed that vitamin E acted as a prooxidant in laying hens when supplementation levels were above 120 mg/kg. In contrast, Attia et al. (2001) reported an increase in the body weight of 6-weeks-old broilers that was

correlated with increasing doses of vitamin E dietary supplementation (0–1500 mg/kg). This finding corresponds to the results of our study. However, Attia et al. (2001) did not report on the effects of vitamin E level on feed intake and conversion. Brenes et al. (2008) showed an increase in fat digestibility in broiler chicks fed a diet enriched with vitamin E (200 mg/kg).

The uptake of vitamin E and carotenoids by the intestinal cell is poorly understood. It has long been assumed that these fat-soluble molecules are absorbed by a passive diffusion mechanism. However, recent results established that the scavenger receptor class B type I (SR-BI) is involved in the intestinal uptake of these micronutrients. Both vitamin E and the carotenoids are distributed to body tissues through plasma lipoproteins (Borel et al., 2007). Differences in the absorption of vitamin E and the carotenoids, including the lycopene in our study, as a result of their different concentrations and combinations in the diet may be the basic cause of increasing or decreasing the amounts deposited in tissues. Wang et al. (2010) reported that a higher dietary amount of one of the carotenoids they tested reduced the concentrations of the other carotenoids in the blood plasma and tissues of chickens. High doses of lutein decreased the level of zeaxanthin and *vice versa*. High β -carotene supplementation reduced the contents of both lutein and zeaxanthin.

The combination of lycopene and vitamin E significantly reduced the MDA values in fresh leg meat and in meat that had been stored for 3 days. The effects of the interaction between lycopene and vitamin E supplementation were not described previously. However, the ability of lycopene to quench singlet oxygen (more so than that of β -carotene), to trap peroxy radicals, and to inhibit lipid peroxidation was reported previously by Rao and Agarwal (1998). Leal et al. (1999) also described a decrease in the MDA production in broilers fed a mixture enriched with lycopene. The positive effect of a combination of lycopene and selenium on a reduction of the MDA value of breast meat after 5 days of storage was described by Ševčíková et al. (2008). Botsoglou et al. (2004) reported the MDA values in raw Japanese quail meat stored for 6 or 9 days in the refrigerator and suggested that the inclusion of dried tomato pulp in feed at a level of 5% exerted an antioxidant effect, whereas the addition at a level of 10% exerted a prooxidant effect. Tedesco et al. (2005) reported the interaction between the antioxidants lycopene and green tea extract on the improvement in the

lipid oxidation status of mammal meat stored for 7 days. DeWinne and Dirinck (1996) and Brenes et al. (2008) reported a decrease in the lipid oxidation of chicken meat due to vitamin E. Dietary supplementation of vitamin E increased the lipid stability in muscles and improved the meat quality and fatty acid composition, likely by influencing the expression of genes related to lipid metabolism (Li et al., 2009).

A reduction in the cholesterol content of leg meat was reported in chickens that were fed a diet enriched with lycopene, alone and in combination with vitamin E. Rao and Shen (2002) reported a decrease in blood plasma cholesterol as a result of dietary lycopene supplementation. Sahin et al. (2006a) described an increase in the blood plasma HDL concentration of Japanese quail and a significant decrease in the LDL concentration because of lycopene addition.

Lycopene supplementation influenced the vitamin content of leg meat. A positive effect of vitamin E and lycopene was found in the lycopene content of liver. Olson et al. (2008) described a significant increase in egg yolk lycopene and vitamin E with increasing dietary concentrations of these antioxidants, whereas lutein and zeaxanthin contents remained constant. The addition of lycopene or vitamin E had no effect on the lutein content of liver. The concentration of lutein in chicken liver was half the value reported by Wang et al. (2010), in whose study no lutein was supplemented to the diet.

CONCLUSION

Our results indicated a positive effect of dietary supplementation of lycopene and vitamin E on growth performance and meat quality. The interaction of these antioxidants was shown in the body weight of 21-days-old chickens, in the oxidative stability and cholesterol content of leg meat and in the liver lycopene content. Dietary vitamin E supplementation reduced the fat content and increased the oxidative stability of meat. The cholesterol content of leg meat was decreased by lycopene supplementation.

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Growth performance, pH and meat proximate composition in both species were not affected by dietary treatments. The TBARS value of chicken leg meat from the unsupplemented group was 3.86, while on average in CK-E200, CK-L200 and CK-O200 it was by 60, 55 and 63% lower ($P < 0.05$), whereas in CK-T200 it was by 25% higher ($P < 0.05$). Dietary treatments did not exert any antioxidant effects on chicken breast meat. @inproceedings{Marzoni2014EffectsOD, title={Effects of dietary natural antioxidant supplementation on broiler chicken and Muscovy duck meat quality}, author={M. Marzoni and R. Chiarini and A. Castillo and I. Romboli and M. D. Marco and A. Schiavone}, year={2014} }. M. Marzoni, R. Chiarini, +3 authors A. Schiavone. meat quality. Supplementation of vitamin E significantly improved the meat stability against oxidative deterioration in beef (Mitsumoto et al., 1993), pork (Phillips et al. Chickens performance. Vitamin E tissue accumulation has been shown to be correlated with the dietary level, the duration of the supplementing period, the animal race and sex as well as with the tissue type (Shehhy et al., 1991). In fact, it was shown that vitamin E saturation of the chicken thigh was reached after 3 weeks of dietary supplementation with 200 mg vitamin E/kg of food (Brandon et al., 1993). The effect of endogenous vitamin E on colour quality is more evident in species having higher levels of myo-globin and positive relationship between dietary vitamin E. Zouari et al. 2283. Many stud- effect of lycopene on performance and quality of ies dealing with the importance of lycopene for meat and eggs in Japanese quail was studied by. Supported by Research Plan of the Ministry of Agriculture of the Czech Republic (No. MZe 0002701403). The highest concentrations of Se were measured in The effect of lycopene on live weight of animals groups that received 100 mg dietary lycopene, both tends to be insignificant but lycopene has posi- in the inorganic and organic form of selenium. In tive effects on egg quality from the aspect of their many experiments the Warner and Bratzler method weight and yolk colour. Leal et al.