Adverse effects in broiler chickens fed a high lycopene concentration supplemented diet

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Pozzo, L., Tarantola, M., Biasibetti, E., Capucchio, M. T., Pagella, M., Mellia, E., Bergagna, S., Gennero, M. S., Strazzullo, G. and Schiavone, A. 2013. **Adverse effects in broiler chickens fed a high lycopene concentration supplemented diet**. Can. J. Anim. Sci. **93**: 231–241. The present investigation was designed to assess the effects of the administration of a high dietary lycopene dose to broiler chickens on growth and slaughter performances, haemato–biochemical parameters, antioxidant enzymes, histological lesions and lycopene accumulation in the tissues. Thirty-six 1-d-old male Hubbard broiler chicks were examined and divided randomly into two groups (three replicates for each dietary treatment): the Control group, which received a basal diet, and the Lycopene group, which received the basal diet supplemented with 500 mg lycopene kg^{-1} diet. The experimental period lasted 35 d and growth performance was recorded on a weekly basis. At the end of the experiment (day 35), blood samples ($n=12$) were collected from the femoral vein. Erythrocyte and leukocyte numbers were assessed and the heterophil-to-lymphocyte ratio was determined. The alpha-1-acid glycoprotein, lysozyme, total protein and the electrophoretic patterns were evaluated in serum samples. Nine chickens per group were slaughtered to measure the carcass yields and the weight of the liver, spleen, bursa of Fabricius and thymus. Spleen, liver, and bursa of Fabricius samples were collected for histological examination and fixed in 10% neutral buffered formalin. The lycopene concentration, thiobarbituric acid reactive substances (TBARS) and chemical composition were assayed in breast meat and thigh samples. The TBARS, glutathione content, catalase and superoxide dismutase activity were measured in liver and kidney samples. The high lycopene concentration supplemented diet did not affect the birds' growth, slaughter performance or antioxidant enzymes in the breast meat, thigh meat, liver and kidney samples. The concentrations of lycopene in the breast and thigh of the lycopene-supplemented group were 0.10 ± 0.05 mg kg^{-1} and 0.42 ± 0.35 mg kg^{-1}, respectively, while no lycopene was found in the Control group. Spleen and bursa of Fabricius of birds fed a diet supplemented with a high level of lycopene showed weight decrease ($P<0.05$) and degenerative lesions ($P<0.05$). Moreover, lycopene supplementation reduced the serum protein concentration, albumin, alpha and the gamma globulin serum concentration ($P<0.05$).

Key words: Broiler chicken, lycopene, hemato–biochemical parameters, histological lesions

Pozzo, L., Tarantola, M., Biasibetti, E., Capucchio, M. T., Pagella, M., Mellia, E., Bergagna, S., Gennero, M. S., Strazzullo, G. et Schiavone, A. 2013. **Effets secondaires d'un supplément riche en lycopène chez le poulet de chair**. Can. J. Anim. Sci. **93**: 231–241. Cette étude devait évaluer les conséquences d'une dose élevée de lycopène dans la ration des poulets de chair sur la croissance et le rendement à l'abattage, sur les paramètres hémato-biochimiques, sur les enzymes antioxydants, sur les lésions histologiques et sur l'accumulation de lycopène dans les tissus. Les chercheurs ont examiné 36 poussins mâles Hubbard d'un jour qu'ils ont répartis au hasard en deux groupes (trois répétitions de chaque traitement): un groupe témoin, nourri avec la ration de base, et un groupe Lycopène, nourri avec la ration de base enrichie de 500 mg de lycopène par kg d'aliment. L'expérience a duré 35 jours et la croissance a été enregistrée chaque semaine. Au terme de l'expérience (jour 35), on a prélevé des échantillons de sang ($n=12$) de la veine fémorale, puis établi le nombre d'érythrocytes et de leucocytes ainsi que le rapport entre les hétérophiles et les lymphocytes (H:L). L'alpha-1 glycoprotéine acide (AGP), les lysozymes, la concentration totale de protéines et les fractions protéiques mesurées par électrophorèse ont été mesurés dans le sérum sanguin. Neuf poulets de chaque groupe ont été sacrifiés pour mesurer le rendement de carcasse ainsi que le poids du foie, de la rate, de la bourse de Fabricius et du thymus. Des échantillons de la rate, du foie et de la bourse de Fabricius ont été prélevés en vue d'un examen histologique, puis fixés dans une solution neutre tamponnée à 10 % de formaline. Les auteurs ont dosé la concentration de lycopène et des substances réagissant à l'acide

Abbreviations: **AGP**, alpha-1-acid glycoprotein; **A:G**, albumin to globulin ratio; **ALT**, alanino aminotransferase; **AST**, aspartate aminotransferase; **CAT**, catalase; **FCR**, feed conversion ratio; **GSH**, glutathione; **H:L**, heterophils to lymphocytes; **MDA**, malonaldehyde; **SOD**, superoxide dismutase; **TBARS**, thiobarbituric acid reactive substances

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thiobarbiturique (TBARS) puis établi la composition chimique des échantillons de viande de poitrine et de cuisse. Ils ont quantifié les TBARS, la concentration de glutathion (GSH) ainsi que l'activité de la catalase (CAT) et de la superoxyde dismutase (SOD) dans les échantillons du foie et des reins. La ration enrichie avec une forte dose de lycopène n'a eu aucune incidence sur la croissance des oiseaux, le rendement à l'abattage ni les enzymes antioxydants dans les échantillons de viande de poitrine, de viande de cuisse, de foie et de rein. La concentration de lycopène dans la viande de poitrine et de cuisse des poulets du groupe lycopène s'établissait respectivement à $0{,}10 \pm 0{,}05$ mg par kg et à $0{,}42 \pm 0{,}35$ mg par kg, aucun lycopène n'a été détecté chez les oiseaux du groupe témoin. La rate et la bourse de Fabricius des oiseaux qui avaient reçu la ration enrichie de lycopène étaient moins lourdes ($P < 0{,}05$) et présentaient des lésions dégénératives ($P < 0{,}05$). Par ailleurs, le supplément de lycopène a réduit la concentration sérique de protéines, d'albumine ainsi que de globuline alpha et gamma ($P < 0{,}05$).

Mots clés: Poulet de chair, lycopène, paramètres hémato-biochimiques, lésions histologiques

Lycopene is synthesized by plants and microorganisms but not by animals. Lycopene is known to exert an effective free radical scavenging activity and this action could be beneficial to poultry because free radicals are formed under stress, fast growth, high reproduction rates and intensive metabolic conditions of poultry (Englmaierová et al. 2011). Moreover, lycopene may play an important role in the antioxidant defense system (Sahin et al. 2008; Ševčíková et al. 2008). Lycopene and others carotenoids have been shown to act as pro-oxidants or antioxidants, depending on the dose at which they are administered. Many studies have reported the antioxidant effect of lycopene both in vivo (Sahin et al. 2006) and in vitro (Shi et al. 2007; Müller et al. 2011), although the dose-dependent pro-oxidant effect has only been shown in vitro (Lowe et al. 1999; Yeh and Hu 2000). It has been demonstrated that supplemented beta-carotene and other carotenoids are oxidatively degraded under heavy oxidative stress, which leads to the formation of high amounts of breakdown products with pro-oxidant properties (Siems et al. 2005).

Conflicting effects of lycopene on poultry meat oxidative stability were found. No effect of lipid stability of broiler chicken liver or meat was found by Leal et al. (1999) for chicken fed 25 mg lycopene kg^{-1} body weight d^{-1}, or by Ševčíková et al. (2008) in birds fed 50–100 mg lycopene kg^{-1} feed. An antioxidant effect of lycopene was demonstrated for raw meat of Japanese quail fed 50–100 mg lycopene kg^{-1} feed (Sahin et al. 2006). In Japanese quail, Botsoglou et al. (2004) found an antioxidant effect in raw and cooked meat when birds were fed 50 g dried tomato pulp kg^{-1} feed, while a pro-oxidant effect was found when the dried tomato pulp inclusion was 100 g kg^{-1} feed.

Lycopene-enriched eggs were obtained both in quail and laying hens. A dose-dependent increase was found in egg yolk with increasing dietary lycopene. The lycopene dietary supplementation varied as 100–200 mg lycopene kg^{-1} feed (Sahin et al. 2008), 65–840 mg lycopene kg^{-1} feed (Olson et al. 2008), 250–500 μg kg^{-1} feed (Gregosits et al. 2009), 13 μg lycopene g^{-1} feed (Rotolo et al. 2010), 75 g tomato seed or peel kg^{-1} feed (Knoblich et al. 2005). Furthermore, Mangiagalli et al. (2010) found that semen production, semen viability, fertility and immunity, measured as bactericidal activity, were increased in broiler breeders receiving drinking water supplemented with lycopene (0.5 g L^{-1}).

The purpose of the present study was to assess the effects of the administration of a high dietary lycopene dose to broiler chickens on growth and slaughter performance, haemato–biochemical parameters and antioxidant enzymes, histological lesions and lycopene accumulated in tissues.

MATERIAL AND METHODS

The experimental plan was designed according to the Italian legal guidelines for the care and use of experimental animals (Ministero della Salute 1992). Thirty-six 1-d-old male Hubbard broiler chickens were distributed over six pens and randomly assigned to two dietary groups (six birds per pen; three pens per dietary treatment) and reared from day 1 to day 35 of age. Each pen (0.9 m wide × 1.50 m long) was equipped with a feeder, a drinker and rice hulls as litter. Heating was provided in the first 2 wk to maintain the room temperature according to standard breeding practices (Hubbard Broiler Management Guide). The temperature and relative humidity were recorded 24 h a day by means of a thermo-hygrometer. A commercial basal diet, based on barley, wheat, and soybean meal (Table 1) and formulated to meet or exceed National Research Council requirements (NRC 1994), was adopted. The two groups, the Control group and the Lycopene group, were fed the basal diet or the same basal diet supplemented with 1% of a lycopene dry-extract (LycoBreads® 5% VBAF, LycoRed Ltd, Beer-Sheva, Israel), respectively. The lycopene concentration (500 mg kg^{-1} of basal diet) used in the present study was higher than those used in previous experiments conducted on growing poultry (Leal et al. 1999; Botsoglou et al. 2004; Sahin et al. 2006; Ševčíková et al. 2008). Mortality was recorded daily. Body weight and feed consumption per pen were recorded weekly ($n = 3$). The daily gain and feed conversion ratio (FCR) per pen were calculated weekly ($n = 3$).

At the end of the experiment (day 35), blood samples ($n = 12$) were collected (5 mL) from the femoral vein (four birds randomly chosen from each pen) and centrifuged for 15 min at $3000 \times g$ to separate the serum, which was stored at $-20^{\circ}C$ until analysis. A blood

Table 1. Ingredients and chemical composition of the basal diet

	Starter/grower	Finisher
Ingredients (g kg^{-1})		
Wheat	274.0	308.0
Corn	253.5	252.4
Soybean meal	376.7	332.4
Animal fat	56.0	68.0
Dicalcium phosphate	13.0	12.4
Calcium carbonate	11.5	11.2
Sodium chloride	2.3	2.2
Sodium bicarbonate	1.3	1.5
DL-methionine	3.9	3.8
L-lysine HCl	2.0	2.0
L-threonine	0.8	1.1
Vitamin-mineral premix[z]	5.0[1]	5.0[y]
Analyzed composition (g kg^{-1})		
Dry matter	904.9	907.4
Crude protein	230.5	195.0
Ether extract	96.6	95.7
Crude fiber	30.4	27.5
Ash	52.9	50.4
Metabolizable energy (MJ kg^{-1})[y]	13.1	13.5

[z]Supplied per kilogram of diet: vitamin A, 12 500 IU; cholecalciferol, 2000 IU; vitamin B_1, 1.5 mg; riboflavin, 3.0 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 15 μg; dl-α-tocopheryl acetate, 75 IU; niacin, 25.0 mg; d-pantothenic acid, 8.0 mg; cobalt, 0.2 mg; iron, 30.0 mg; iodine, 1.4 mg; manganese, 80 mg; copper, 1.5 mg; zinc, 30.0 mg.
[y]Calculated based on NRC (1994) ingredient composition.

smear was prepared from a droplet without anticoagulant. The total red and white cell counts were determined in an improved Neubauer haemocytometer, after mixing with a Natt–Herrick solution in a 1 to 200 ratio (Natt and Herrick 1952). The blood smears were stained with May–Grünwald and Giemsa–Romanowski stains (Campbell 1995). One hundred white blood cells were evaluated per smear in order to determine the heterophil to lymphocyte (H:L) ratio, and the blood cell type number was determined according to Campbell (1995). The serum alpha-1-acid glycoprotein (AGP) concentration (μg mL^{-1}) was assayed using a commercially available radial immunodiffusion tray (Cardiotech Services, Inc., Louisville, KY). The serum lysozyme assay employed *Micrococcus lysodeikticus* cells as the substrate for the lysozyme, using the Osserman and Lawlor method (Osserman and Lawlor 1996). The total proteins were quantified by means of the biuret method (Hospitex Diagnostics, Sesto Fiorentino, FI, Italy); the serum electrophoretic patterns were obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys, Evry, France). The alanine aminotransferase (ALT), aspartate aminotransferase (AST) and uric acid serum concentrations were measured with enzymatic methods on a clinical chemistry analyzer (Screen Master Touch, Hospitex diagnostics, Sesto Fiorentino, FI, Italy).

At 35 d of age, nine chickens per dietary treatment (three birds randomly chosen from each pen) were humanely euthanized, bled and dissected to measure the live weight and chilled carcass, breast, thigh, liver, spleen, bursa of Fabricius and thymus weights. The dressing percentages were then calculated. Thigh and breast meat samples were kept at −20°C for chemical composition analysis, thiobarbituric acid reactive substances (TBARS) evaluation and lycopene quantification. Liver and kidney aliquots were homogenized in five volumes of a 0.1 M potassium phosphate buffer (pH 7.4) and stored at −80°C until the assays for TBARS, glutathione (GSH) contents and catalase (CAT) and superoxide dismutase (SOD) activity.

Dry matter, crude protein, ether extract and ash quantification were performed for the breast and thigh meat according to methods of the Association of Official Analytical Chemists (2000). Susceptibility to lipid oxidation of the breast and thigh muscle was performed by means of a TBARS evaluation, according to the procedure described by Sárraga et al. (2006). The iron-induced TBARS assay was performed at 0, 15, 30, 60 min of incubation with $FeSO_4 \cdot 7H_2O$ (final concentration 1 mmol L^{-1} Fe^{+3}) as the oxidative agent and absorbance was read at 532 nm. Liquid malonaldehyde (MDA) (malonaldehyde bis diethyl acetal, Sigma Aldrich Co., St. Louis, MO) was used as the standard to determine the linear standard response and recovery. The TBARS values were expressed as nmol MDA kg^{-1} fresh meat. The Ohkawa et al. (1978) method was used for the TBARS evaluation in liver and kidney. The fluorimetric absorbance was assessed at 515 nm for λ_{ex} and at 553 nm for λ_{em} and compared with those obtained from an MDA standard. The results were expressed as nmol MDA mg^{-1} of protein. The total GSH content in the kidney and liver samples was determined according to the Mitchell et al. (1973) method. The absorbance was recorded at 412 nm. The results were expressed as nmol GSH mg^{-1} of protein. Catalase activity was determined according to the method described by Goth (1991). The yellow molybdate complex and the absorbance of H_2O_2 were measured at 405 nm in the spectrophotometer against a blank. CAT activity was expressed as U mg^{-1} protein. In the SOD activity assay, superoxide ions were generated from the conversion of xanthine and O_2 to uric acid and H_2O_2 by xanthine oxidase. The superoxide anion converts the nitroblue tetrazolium into a formazan dye. The addition of SOD to this reaction reduces the superoxide ion levels, thereby lowering the formazan dye formation rate. SOD activity is measured in an experimental sample as the percent inhibition of the formazan dye formation rate (Sun et al. 1988). The production of formazan was determined at 560 nm. The percent inhibition of the rate of increase in absorbance was calculated as follows: % inhibition = (slope of activity control − slope of activity sample) × 100. One unit of SOD is defined as the amount of protein that inhibited the NBT reduction rate by 50%. The total protein in the tissue homogenates of the kidney and liver samples was determined by means of the Bradford's standard protein assay procedure using bovine serum albumin as the standard (Bradford 1976).

Spleen, liver, and bursa of Fabricius samples were collected for histological examination and fixed in 10% neutral buffered formalin ($n = 9$). The tissues were routinely embedded in paraffin wax blocks, sectioned at a thickness of 5 μm and stained with haematoxylin and eosin. Each of the slide sections was examined under a light microscope by three pathologists. In the spleen and bursa of Fabricius samples the mean value of the apoptotic and mitotic cells was counted in 10 follicles at 40 × magnification. The severity of hepatic degeneration was classified using the following semiquantitative scoring system: no lesions (0), slight vacuolar degeneration with small vacuoles (1), moderate vacuolar degeneration (2), widespread vacuolar degeneration (3), severe widespread vacuolar degeneration (4), hydropic degeneration with necrotic foci (5).

Lyophilized thigh and breast samples (5 g) and feed samples were extracted with a 30 mL of chloroform-methanol (60:40 vol/vol) solution by means of sonication (SONICA® Ultrasonic Cleaner, SOLTEC Srl., Milano, Italy) for 90 min (Ametaj et al. 2003). The upper non-polar layer containing lycopene was filtered, concentrated in a Speed Vac Concentrator (Savant Speed Vac® Concentrator, Thermo Fisher Scientific, Waltham, MA) ($T < 35°C$) and stored at $-20°C$ until HPLC analysis, which was performed on the stored specimens reconstituted with 4 mL of dichloromethane with 1% of butylated hydroxytoluene. The HPLC apparatus consisted of a Dionex P680 pump (Dionex, Sunnyvale, CA) equipped with a Rheodyne injection valve (Rheodyne Model 7725i, Rheodyne, Rohnert Park, CA), a Dionex UVD-170/U UV-vis detector ($\lambda = 470$ nm), a Dionex thermostatted column compartment TCC-100, and a Chromeleon® 6 data handling system (Chromeleon® 6, Dionex, Sunnyvale, CA). An AscentisTM C18 column (25 cm × 4.6 mm, 5 μm particles) (AscentisTM C18, Supelco, Bellefonte, PA) was used for the analysis. Twenty micriliters of sample was injected into the chromatographic system using a full loop injection system. The system was run isocratically with a mobile phase containing acetonitrile-isopropanol (90:10 vol/vol) for 25 min at a flow rate of 2.5 mL min^{-1}. The column temperature was kept at 30°C for all of the chromatographic runs. The identification and quantification of lycopene were obtained through the combined use of the retention time, and co-chromatography with a commercial lycopene standard (Sigma-Aldrich, St. Louis, MO).

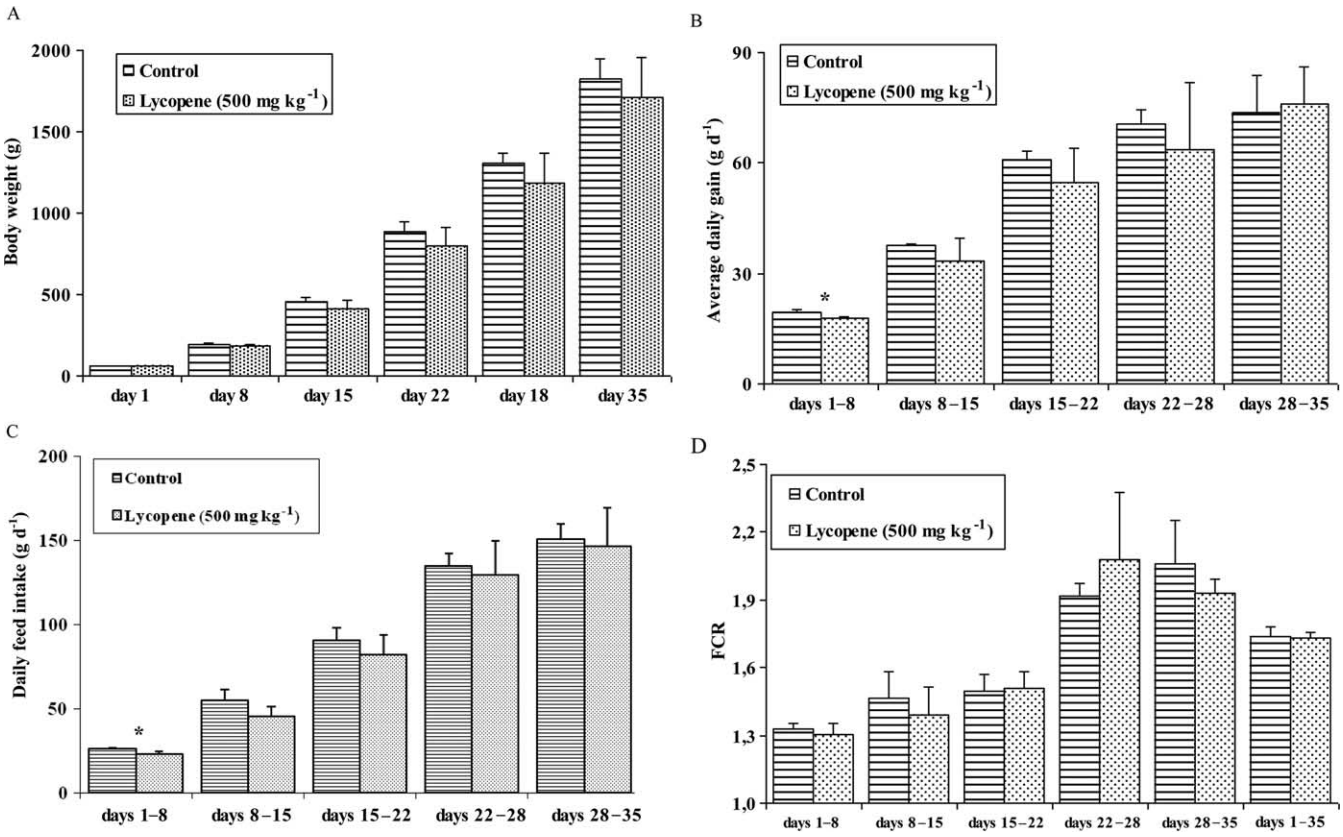

Fig. 1. A. Effects of lycopene-supplemented diet on body weight (g) of broiler chickens ($n = 3$ pens per treatment). B. Effects of lycopene-supplemented diet on average daily gain (g d^{-1}) of broiler chickens ($n = 3$ pens per treatment) (*$P < 0.05$). C. Effects of lycopene-supplemented diet on daily feed intake of broiler chickens (g d^{-1}) ($n = 3$) pens per treatment (*$P < 0.05$). D. Effects of lycopene-supplemented diet on feed conversion ratio (FCR) of broiler chickens ($n = 3$ pens per treatment).

Data were analyzed with a statistical package (SPSS 12.0 2003). Before testing for group differences, normality of the distribution of data was assessed in the two groups (Control group and Lycopene group) using the Shapiro–Wilk test. The results are presented as the mean value ± SD. Data relating to body weight, average daily gain, feed intake and FCR, pen ($n = 3$) was considered the unit of replication for analysis. For hemato–biochemical parameters, slaughter performance, meat chemical composition and antioxidant enzymes assessment, bird was considered the unit of replication for analysis ($n = 12$ or 9). The homogeneity of variance assumption was assessed by means of Levene's test. All the obtained data were statistically analyzed using an independent sample t-test (Control group vs. Lycopene group). The results were considered statistically significant when associated with a probability lower than 5%, differences with a probability lower than 1% were considered highly significant.

RESULTS

No bird mortality was observed during the whole experimental period. The mean body weight of the broiler chickens did not show any statistical difference between groups, although the body weight of the chickens in the Lycopene group tended to be lower than those in the Control group ($P < 0.10$) (Fig. 1A). The birds belonging to the Lycopene group showed a significantly lower average daily gain (g d^{-1}) than the Control group during the first week (days 1–8) ($P < 0.05$) (Fig. 1B). The daily feed intake of the Lycopene group was lower than the Control group during the 1–8 d period ($P < 0.05$) (Fig. 1C). No statistically significant differences were found for FCR for any time period (Fig. 1D).

The total number of the erythrocytes and leukocytes, the H:L ratio, AGP and lysozyme serum concentration were not influenced by the dietary treatment (Table 2). The birds fed the lycopene supplemented diet showed a lower concentration than the Control group for total protein (4.05 ± 0.69 g dL^{-1} and 3.20 ± 0.78 g dL^{-1}, respectively) ($P < 0.05$), albumin (1.49 ± 0.27 g dL^{-1} and 1.13 ± 0.37 g dL^{-1}, respectively) ($P < 0.05$), alpha (1.04 ± 0.19 g dL^{-1} and 0.80 ± 0.23 g dL^{-1}, respectively) ($P < 0.05$) and gamma (0.84 ± 0.14 g dL^{-1} 0.54 ± 0.16 g dL^{-1}, respectively) ($P < 0.001$) globulin serum concentrations. The beta globulin serum concentration and the albumin-to-globulin ratio (A:G) were not influenced by the diet (Table 2). The hepatic enzymes (ALT and AST) and the uric acid concentrations were not affected by the dietary treatment (Table 2).

The slaughter performance of the broiler chickens was not influenced by the diet (Table 3), except for the absolute ($P < 0.05$) and relative ($P < 0.01$) weight of the spleen and the bursa of Fabricius absolute weight ($P < 0.05$), which were lower for the birds fed the lycopene supplemented diet.

Traces of lycopene were found in the breast and thigh samples of the Lycopene group, but not in the samples from the Control group. The lycopene concentrations were 0.10 ± 0.05 mg lycopene kg^{-1} fresh weight in the breast and 0.42 ± 0.35 mg lycopene kg^{-1} fresh weight in the thigh (Table 3).

Table 4 shows the chemical composition and the TBARS content (nmol MDA kg^{-1} fresh meat) of the

Table 2. Effects of lycopene supplemented diet on haemato-biochemical parameters of broiler chickens (mean ± SD) ($n = 12$ birds per treatment)

	Control	Lycopene[z]	P
Erythrocyte (10^6 cells μL^{-1})	2.40 ± 0.33	2.31 ± 0.46	NS[t]
Leukocyte (10^3 cells μL^{-1})	14.1 ± 4.59	11.3 ± 5.16	NS
H:L[y] ratio	0.42 ± 0.12	0.51 ± 0.22	NS
Lysozyme (mg mL^{-1})	3.50 ± 0.88	2.98 ± 0.66	NS
AGP[x] (mg mL^{-1})	0.38 ± 0.12	0.38 ± 0.12	NS
Total protein (g dL^{-1})	4.05 ± 0.69	3.20 ± 0.78	<0.05
Albumin (g dL^{-1})	1.49 ± 0.27	1.13 ± 0.37	<0.05
Alpha globulin (g dL^{-1})	1.04 ± 0.19	0.80 ± 0.23	<0.05
Beta globulin (g dL^{-1})	0.64 ± 0.15	0.56 ± 0.21	NS
Gamma globulin (g dL^{-1})	0.84 ± 0.14	0.54 ± 0.16	<0.001
A:G[w]	0.59 ± 0.04	0.60 ± 0.07	NS
AST[v] (UI L^{-1})	181.34 ± 29.54	176.24 ± 25.08	NS
ALT[u] (UI L^{-1})	4.64 ± 1.97	4.69 ± 1.74	NS
Uric acid (mg dL^{-1})	9.22 ± 2.18	9.02 ± 1.98	NS

[z]500 mg lycopene kg^{-1} basal diet.
[y]Heterophil/lymphocyte.
[x]Alpha-1-acid glycoprotein.
[w]Albumin to globulin ratio.
[v]Aspartate aminotransferase.
[u]Alanino aminotransferase.
[t]NS, non-significant.

Table 3. Effects of lycopene-supplemented diet on slaughter performance traits and lycopene content in breast and thigh (mean ± SD) ($n = 9$ birds per treatment)

	Control	Lycopene[z]	P
Live weight (g)	1950.0 ± 352.7	1750.0 ± 398.7	NS[u]
Chilled carcass (g)	1421.0 ± 248.1	1237.4 ± 291.8	NS
Chilled carcass (%)[y]	73.0 ± 2.26	72.2 ± 2.07	NS
Breasts (g)	374.2 ± 96.2	297.8 ± 81.8	NS
Breasts (%)[x]	26.3 ± 4.73	23.9 ± 1.83	NS
Thighs (g)	366.2 ± 69.4	343.8 ± 71.7	NS
Thighs (%)[x]	25.9 ± 2.69	27.9 ± 2.01	NS
Liver (g)	51.8 ± 7.33	46.4 ± 12.6	NS
Liver (%)[w]	2.70 ± 0.40	2.66 ± 0.29	NS
Spleen (g)	3.04 ± 0.89	1.92 ± 0.73	<0.05
Spleen (%)[w]	0.16 ± 0.04	0.10 ± 0.04	<0.01
Bursa of Fabricius (g)	3.19 ± 1.07	2.08 ± 0.81	<0.05
Bursa of Fabricius (%)[w]	0.16 ± 0.05	0.12 ± 0.04	NS
Thymus (g)	10.3 ± 1.86	9.42 ± 2.60	NS
Thymus (%)[w]	0.50 ± 0.09	0.49 ± 0.12	NS
Breast (mg lycopene kg^{-1} fresh weight)	ND[v]	0.10 ± 0.05	–
Thigh (mg lycopene kg^{-1} fresh weight)	ND[v]	0.42 ± 0.35	–

[z]500 mg lycopene kg^{-1} basal diet.
[y]Chilled carcass:live weight.
[x]Organ weight:chilled carcass.
[w]Organ weight:live weight.
[v]Not detected.
[u]NS, non-significant.

Table 4. Effects of lycopene supplemented diet on chemical composition and TBARS[z] of breast and thigh (mean ± SD) ($n = 9$ birds per treatment)

	Breast			Thigh		
	Control	Lycopene[y]	*P*	Control	Lycopene[y]	*P*
Chemical composition						
Dry matter (%)	26.06 ± 3.45	25.96 ± 1.37	NS[w]	25.98 ± 1.21	26.33 ± 1.32	NS
Crude protein (%)	23.23 ± 3.28	22.59 ± 1.62	NS	18.41 ± 0.49	18.35 ± 0.26	NS
Ether extract (%)	0.37 ± 0.14	0.39 ± 0.15	NS	1.54 ± 0.37	1.68 ± 0.43	NS
Ash (%)	0.34 ± 0.11	0.32 ± 0.05	NS	1.28 ± 0.03	1.28 ± 0.04	NS
Iron-induced TBARS (nmol MDA[x] kg^{-1} fresh meat)						
0 min	0.52 ± 0.15	0.58 ± 0.08	NS	1.28 ± 0.31	1.14 ± 0.15	NS
15 min	0.66 ± 0.14	0.63 ± 0.24	NS	1.74 ± 0.58	1.17 ± 0.28	NS
30 min	1.05 ± 0.50	0.89 ± 0.27	NS	2.16 ± 0.71	1.75 ± 0.72	NS
60 min	0.73 ± 0.17	0.78 ± 0.08	NS	2.19 ± 0.80	1.49 ± 0.31	NS

[z]Thiobarbituric acid reactive substances.
[y]500 mg lycopene kg^{-1} basal diet.
[x]Malonaldehyde.
[w]NS, non-significant.

breast and thigh. Neither the chemical composition nor TBARS was influenced by lycopene supplementation. Similarly, neither the TBARS and GSH content nor the CAT and SOD activity in the liver and kidney samples was affected by the dietary treatment (Table 5).

The spleen samples of the chickens fed the lycopene-supplemented diet showed an increase in apoptosis in the follicles (Fig. 2). The number of apoptotic cells was significantly higher in the Lycopene group samples than the Control group birds ($P = 0.000$), with a mean value of 7.4 ± 1.8 and 1.7 ± 0.6 apoptotic cells/follicle, respectively. Bursae of Fabricius of Lycopene group birds showed an increase in apoptotic and mitotic cells, and lymphoid depletion (Fig. 4B) compared with the bursa of Fabricius of the Control group. However the result was only significant for the apoptosis ($P < 0.05$), with a mean value of 19.9 ± 10.6 (Lycopene group) and 5.9 ± 2.7 (Control group) apoptotic cells/follicle. Intra- or interfollicular multifocal cysts of different sizes were also found in the bursa of Fabricius samples of the lycopene-treated birds (Fig. 4C, D). All the liver samples of the Lycopene group showed degenerative lesions, which were classified in different grades of severity (3 cases: grade 4; 2 cases: grade 3; 1 case: grade 1) (Fig. 3). Only one control bird showed degenerative lesions in the liver.

DISCUSSION

Growth performance was not affected by lycopene dietary supplementation, except for the first week of age. In broiler chickens, no effects of lycopene on growth performance were observed by Leal et al. (1999) (lycopene intake: 25 mg lycopene kg^{-1} body weight d^{-1}) or Englmaierová et al. (2011) (lycopene supplementation 75 mg kg^{-1} feed). Similarly Sahin et al. (2006) did not find any effect in Japanese quail reared at

Table 5. Effects of lycopene supplemented diet on oxidative stress in liver and kidney (mean ± SD) ($n = 9$ birds per treatment)

	Liver			Kidney		
	Control	Lycopene[z]	*P*	Control	Lycopene[z]	*P*
Oxidative stress						
TBARS[y] (nmol MDA[x] mg^{-1} protein)	0.15 ± 0.04	0.13 ± 0.05	NS[t]	0.91 ± 0.25	1.03 ± 0.35	NS
GSH[w] (nmol mg^{-1} protein)	2.23 ± 0.70	2.01 ± 0.57	NS	0.54 ± 0.13	0.54 ± 0.05	NS
CAT[v] (U mg^{-1} protein)	539.6 ± 118.2	518.2 ± 53.8	NS	545.4 ± 60.7	569.7 ± 152.1	NS
SOD[u] (U mg^{-1} protein)	21.1 ± 2.02	22.6 ± 6.62	NS	22.6 ± 3.45	23.7 ± 6.32	NS

[z]500 mg lycopene kg^{-1} basal diet.
[y]Thiobarbituric acid reactive substances.
[x]Malonaldehyde.
[w]Glutathione.
[v]Catalase.
[u]Superoxide dismutase.
[t]NS, non-significant.

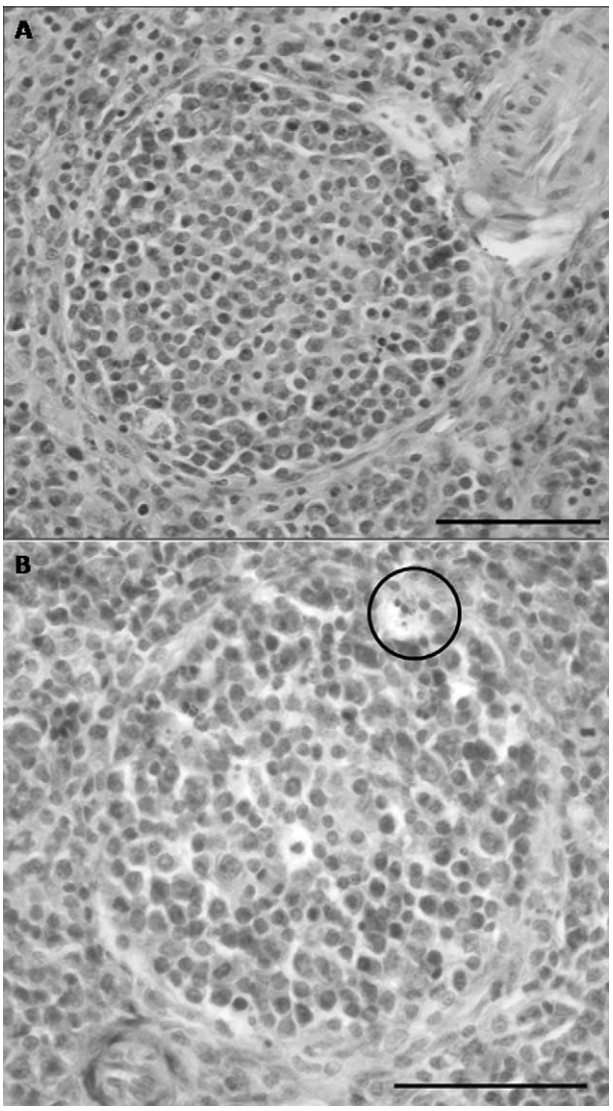

Fig. 2. Spleen: A: control group with a normal follicle; B: several apoptotic cells in chickens fed Lycopene (500 mg kg^{-1}) supplemented diet (circle) (HE, A: 40 ×, B: 60 ×).

thermoneutral temperature (lycopene supplementation 50, 100 and 200 mg kg^{-1} feed).

In our study, an increase in the number of apoptotic cells was detected in the spleen and bursa of Fabricius samples of the chickens fed with lycopene. In all the bursa of Fabricius samples in the Lycopene group, the increase in apoptotic cells could be related to the lymphoid depletion that was detected in all the samples. Some studies have suggested that lycopene induces apoptosis of cancer cells (Nara et al. 2001; Hantz et al. 2005). In our trial all of the liver samples of the chickens in the Lycopene group showed degenerative lesions. Lycopene has been found to have a low order of acute toxicity in rats, but some authors did not find any histopathological changes in the hepatocytes and spleen of rats treated with lycopene beadlet formulations in the diet at doses of up to 500 mg kg^{-1} body $weight^{-1}$ d^{-1} for 14 wk (Buser and Urwyler 1996) or 1000 mg kg^{-1} body $weight^{-1}$ d^{-1} for 4 wk (Niederhauser et al. 1996). No teratogenic effect was noted in rats treated with 1000 mg kg^{-1} body $weight^{-1}$ d^{-1} lycopene as beadlet formulations (Niederhauser et al. 1996).

Lycopene-treated groups showed decreased values of serum total protein, associated with hypoalbuminemia and decreased value of alpha globulin which may be due to liver damage. This is corroborated by the degenerative lesions observed in the liver sections, although the hepatic enzymes, ALT and AST, and uric acid were not affected by lycopene administration. Most serum proteins (albumin, globulins and total proteins) are synthesized in the liver and their total level in the blood reflects liver activity (Miller et al. 1951). The levels of these proteins may change in the presence of disease or certain types of tissue damage. A decreased level of albumin is common in many diseases, including liver disease, malnutrition, malabsorption, protein-losing nephropathy and enteropathy (Lumeij 2008). The decrement of gamma globulin seems rather to be related to the regressive changes of bursa and spleen (apoptosis and lymphoid depletion). Hypogammaglobulinemia could be due to lymphoid disorders, associated with toxic disorders or drug therapy (Alm et al. 1969; Nowak et al. 1982).

Several studies have reported that H:L and serum lysozyme are affected by stressors and can be used as sensitive hematological indicators of the stress response among chicken populations (Gross and Siegel 1983; Kowalski et al. 2006; Salamano et al. 2010). AGP and albumin are two other stress indicators in chickens (Salamano et al. 2010). The A:G ratio variations is an indicator inflammatory conditions (Lumeij 2008). In our study, no acute or chronic inflammation was detectable in the Lycopene treated group as suggested by the lack of modification of leukocyte count, H:L ratio, lysozyme, AGP and A:G ratio.

We found an accumulation of lycopene in breast (0.1 mg kg^{-1}) and thigh (0.4 mg kg^{-1}) meat. Lycopene is a lipophilic compounds and thus accumulates more in leg meat, which is characterized by higher ether extract amount than breast meat (Xiong et al. 1993; Quentin et al. 2003). Lycopene accumulation was mainly studied in laying hens to produce enriched eggs (Olson et al. 2008; Gregosits et al. 2009; Rotolo et al. 2010), while few studies report data on lycopene accumulation in avian tissues or biological fluids. Englmaierová et al. (2011) found that 1.66–2.82 mg lycopene kg^{-1} liver (as dry matter) in broiler chicken fed 75 mg kg^{-1} feed in combination with different dietary level of vitamin E (0, 50, 100 mg kg^{-1} feed). Sahin et al. (2006) found 4.3–8.2 μg lycopene dL^{-1} serum in Japanese quail fed different dietary level of lycopene (50, 100 and 200 mg kg^{-1} feed).

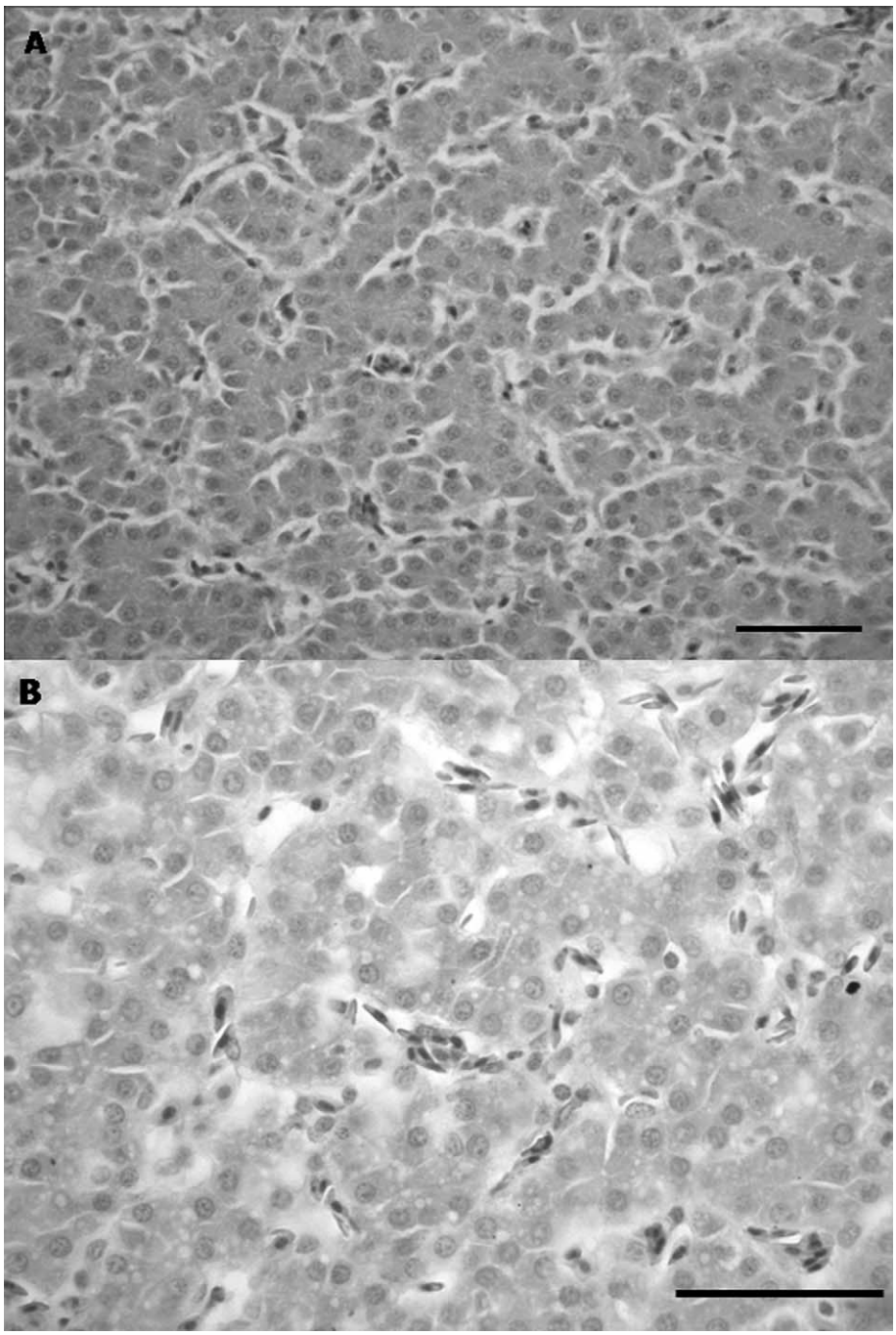

Fig. 3. Liver: A: control tissue; B: severe degenerative changes (grade 4) in chickens fed Lycopene (500 mg kg^{-1}) supplemented diet (HE, A: 40 ×, B: 60 ×).

In our study, lycopene administration did not affect the lipid peroxidation in either breast or thigh meat. Similarly, the antioxidant response in the liver and kidney samples was not influenced by lycopene supplementation. Few papers exist that deal with the antioxidant effect of lycopene in avian tissues with conflicting effects. Leal et al. (1999) found that lycopene supplementation in broiler chickens (25 mg lycopene kg^{-1} body weight d^{-1}) did not exert any antioxidant effect in liver. Englmaierová et al. (2011) found a significant reduction of the MDA values in fresh and 3-d stored broiler chicken leg meat, when birds were fed 75 mg lycopene kg^{-1} feed. Ševčíková et al. (2008) reported that a dietary supplement of 50 or 100 mg lycopene kg^{-1} did not affect the susceptibility to lipid oxidation (TBARS) of raw breast muscle in broiler chickens.

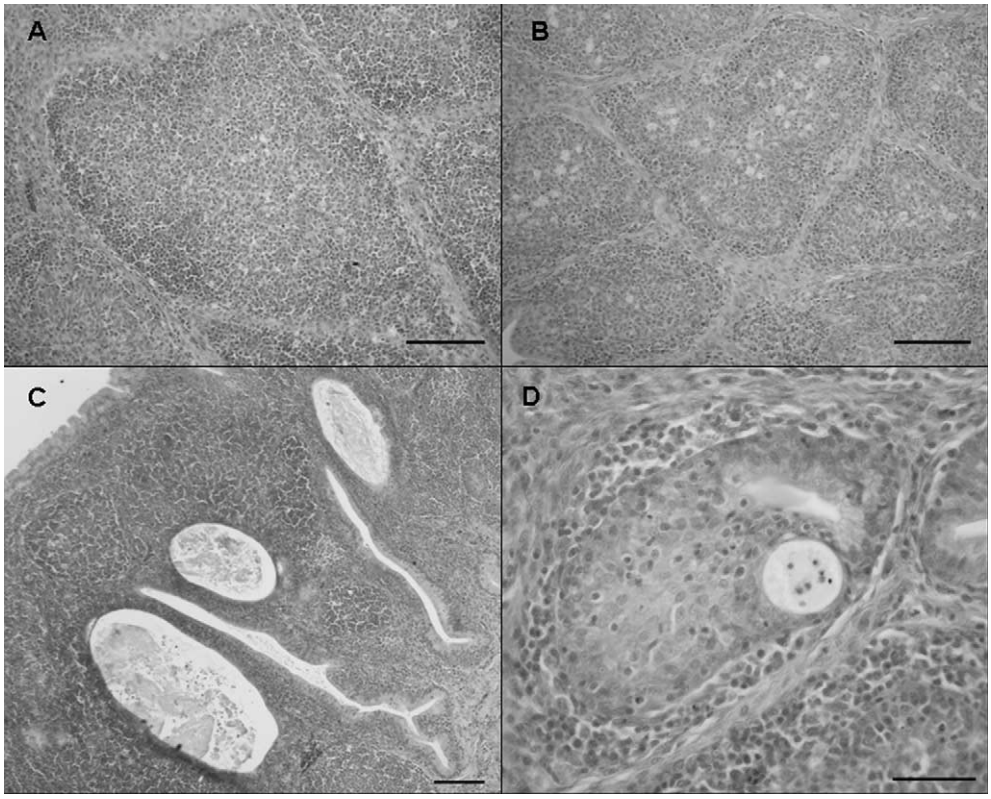

Fig. 4. Bursa of Fabricius: A: control tissue; B, C, D: chickens fed Lycopene (500 mg kg^{-1}) supplemented diet. B: lymphoid depletion in the follicles; C: inter follicular cysts; D: intra follicular cyst. (HE, A: 20 ×, B: 20 ×, C: 10 ×, D: 40 ×).

Sahin et al. (2006) reported that dietary lycopene (50, 100 and 200 mg kg^{-1} of diet) decreased the liver TBARS concentration in both thermoneutral and heat-stressed Japanese quail. Botsoglou et al. (2004) reported the MDA values in raw Japanese quail meat stored for 6 or 9 d 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 pro-oxidant effect. These studies suggest that carotenoids, although capable of trapping peroxyl radicals acting as antioxidants, can also form peroxyl radicals acting as pro-oxidants. It has been reported that carotenoids may work as pro-oxidants at high supplementation levels and as antioxidants at low levels, the balance between pro-oxidant and antioxidant behavior being very delicate and most pronounced at low oxygen partial pressure (Conn et al. 1991).

CONCLUSION

Increasing interest in natural bio-active molecules and plant extracts can currently be observed. Studies on these substances and products have shown that lycopene has positive effects on the performance of broiler chickens and laying hens. However, its dosage plays a very important role. In the present study, spleen and bursa of Fabricius of birds fed a diet supplemented with high level of lycopene showed weight decrease and degenerative lesions. Moreover, lycopene supplementation reduced the serum protein concentration, albumin, alpha and the gamma globulin serum concentrations, which seem rather to be associated to the degenerative lesions observed in liver, bursa of Fabricius and spleen. The observed negative effect in the Lycopene group mainly on spleen and bursa of Fabricius could predispose birds to infectious diseases. Our results have highlighted the general need for periodic monitoring of supplemented feedstuffs for animals when new natural products or new dosages are used to supplement animal diets.

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