Effects of vitamin C or vitamin E supplementation on Cadmium induced oxidative stress and anaemia in broilers

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SUMMARY

The aim of this study was to investigate the possible protective effects of vitamins C and E on lipid peroxidation (LPO), antioxidant systems and erythrocyte parameters in broilers chronically exposed to cadmium (Cd). One day old Ross broiler chickens were assigned to 4 equal groups (n = 12) according to the diet regimen; in the control group, birds received basal standard starter and grower diets, in Cd group they were supplemented with Cd (60 mg/kg) and in the 2 other groups, they were supplemented with Cd and with vitamin C (400 mg/kg) or with vitamin E (250 mg/kg) during the 42 days long experimental period. The Cd exposition induced an oxidative stress characterised by the significant increase in plasma MDA concentrations coupled to significant decreases in enzyme antioxidant SOD (superoxide dismutase), CAT (catalase) and GSH-Px (glutathione peroxidase) activities and in plasma vitamin E, β-carotene and uric acid concentrations. A moderate regenerative anaemia (low haemoglobinemia, erythrocyte number and haematocrit associated with increase of the mean corpuscular volume) was also observed in the Cd group. Treatments with the vitamins C or E significantly reduced the lipid peroxidation but the antioxidant enzyme CAT and GSH-Px activities and vitamin E concentrations were significantly improved only with the vitamin E supplementation. Furthermore, the both types of vitamin supplementation, but particularly with ascorbate, have significantly increased the haemoglobinemia and the erythrocyte counts. These results clearly showed that vitamins C and E alleviate the oxidative effects of Cd at least partially and that the vitamin E exhibits more powerful antioxidant effects whereas vitamin C corrects more efficiently the anaemia.

Keywords: Cadmium, broiler, lipid peroxidation, antioxidants, anaemia, vitamin C, vitamin E.

Introduction

Cadmium (Cd) is a non-essential trace element which is widely used as a colour pigment in paints, in electroplating and galvanizing, and in batteries. It is also a by-product of zinc and lead mining and smelting [17]. Animals may accede to a cadmium excess from water, soil and plant contaminated with industrial and automobile emissions, and the cadmium toxicity has been reported in animals reared around different polluted areas [48]. Cadmium accumulates in many organs, such as liver, kidneys, testis, brain, bone, blood system and causes toxicity in these organs [51].

The detrimental Cd effects have been shown to result from oxidative damage throughout enhancement of the membrane lipid peroxidation, which may be a direct consequence of membrane damage [40] or of reduction of the antioxidant capacities [21]. Various enzyme activities are influenced by Cd [22, 36]. To counteract the damaging effect of lipid peroxidation, aerobic cells are normally protected by the antioxidant defence systems such as vitamins E and C and

RÉSUMÉ

Effets d’une supplémentation en vitamine C ou en vitamine E sur le stress oxydatif et l’anémie survenant chez des poulets intoxiqués par le Cadmium

L’objectif de cette étude a été d’étudier les effets éventuellement protecteurs des vitamines C et E sur la péroxydation lipidique (LPO), les systèmes antioxydants et les paramètres érythrocytaires chez des poulets exposés de façon chronique au Cadmium (Cd). Les poussins Ross de 1 jour ont été répartis en 4 groupes égaux en fonction du régime alimentaire ; les oiseaux du groupe contrôle ont reçu des aliments standards de démarrage et de croissance, ceux du groupe Cd ont été supplémentés en Cd (60 mg/kg) et ceux des 2 autres groupes ont non seulement été supplémentés en Cd mais aussi en vitamine C (400 mg/kg) ou en vitamine e (250 mg/kg) durant toute la période expérimentale de 42 jours. L’exposition au Cadmium a entrainé un stress oxydatif chez les poulets caractérisé par une augmentation significative des concentrations plasmatiques de MDA couplée à des diminutions significatives des activités des enzymes anti-oxydantes de type SOD (superoxyde dismutase), CAT (catalase) et GSH-Px (glutathion péroxydase) ainsi que des concentrations plasmaïques de vitamine E, β-carotène et acide urique. Une anémie régénérative modérée (hémoglobinémie, numération érythrocytaire et hématócrit faibles associés à une augmentation du volume globulaire moyen) a également été observée dans le groupe Cd. Les traitements par la vitamine C ou la vitamine E ont entraîné une diminution significative de la péroxydation lipidique mais les systèmes anti-oxydants (activités CAT et GSH-Px, concentrations en vitamine E) n’ont été significativement restaurés par rapport aux animaux intoxiqués que lors d’une supplémentation en vitamine E. En outre, avec les 2 types de supplémentation, mais surtout avec l’ascorbate, l’hémoglobinémie et la numération érythrocytaire ont significativement augmenté. Ces résultats montrent clairement que les vitamines C et E réduisent au moins partiellement les effets oxydants, et que la vitamine E exerce des effets anti-oxydants plus puissants alors que la vitamine C corrige plus efficacement l’anémie induite.

Mots clés : Cadmium, poulet, péroxydation lipidique, antioxydants, anémie, vitamine C, vitamine E.
glutathione (GSH) [41]. Vitamin C is a potent water-soluble antioxidant [10] capable of scavenging various types of reactive oxygen and nitrogen species [14]. It is known as a chelating and reducing agent that can interact with inorganic elements and may therefore inhibit their absorption [8]. Vitamin E is an efficient chain breaking agent acting as antioxidant in biological systems [1]. It is specifically incorporated into biological membranes which are critical targets of lipid peroxidation [38].

Many reports indicate the Cd toxicity in different animal species [2, 6, 7, 9, 12, 22, 32] as well as the protective effects of vitamin C [7, 9, 12] and of vitamin E [2, 6, 12, 32, 38] but there are few studies on oxidative stress in broilers exposed to Cd [7]. Therefore, the first objective of the present study was to determine the effects of a dietary Cd supplementation on lipid peroxidation and some antioxidant defence systems as well as on haematological parameters. The second was to evaluate the eventual protective effects of vitamins C and E towards the Cd supplementation.

**Materials and Methods**

**ANIMALS AND EXPERIMENTAL DESIGN**

Forty eight, one day old, broiler chicks were individually weighed then randomly allotted into four equal experimental groups (n = 12 in each group) according to the diet regimen. The experimental design consisted of four dietary treatments. First group was the control group in which birds were fed with commercial broiler rations (Table I); birds of the three other groups received the basal diets supplemented with Cd (60 mg/kg of food) but those of the 3rd and 4th groups were also supplemented with the vitamin C (ascorbic acid, 400 mg/kg of food) and the vitamin E (dl-α-tocopherol, 250 mg/kg of food), respectively. The chicks were fed with a basal starter diet until the age of 15 days and then they received a basal grower-finisher diet until the day 42. Chickens were housed in electrically heated batteries under fluorescent lighting and allowed *ad libitum* access to feed and water. This study was conducted in University of Kirikkale, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease. The animal care and use protocol was reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Kirikkale University (03. 02. 2005-05/01).

**BIOCHEMICAL AND HAEMATOLOGICAL ANALYSES**

At the end of the 42 days long experimental period, blood samples were collected into heparinised sterile test tubes by the *vena cephalicus* puncture to determine lipid peroxidation, some antioxidant systems and haematological parameters. Erythrocyte count and packed cell volume (PCV) values were measured by standard haematological techniques [25] and the haemoglobin concentration was measured by the cyan-met-haemoglobin method [5].

After centrifugation (1 600g, 4°C, 10 minutes), plasmas were carefully harvested and stored at -30°C until analysis.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter diet</th>
<th>Grower diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolisable Energy (kcal/kg)</td>
<td>3110</td>
<td>3215</td>
</tr>
<tr>
<td>Crude protein</td>
<td>23.40</td>
<td>20.30</td>
</tr>
<tr>
<td>Ether extract</td>
<td>8.70</td>
<td>10.10</td>
</tr>
</tbody>
</table>

1th the vitamin premix (Rovimix 124-F) supplemented to 2.5 kg of food had the following amounts of vitamins: vitamin A: 15 000 000 U; vitamin D3: 1 500 000 U; vitamin E: 50 000 mg; vitamin K3: 5 000 mg; vitamin B1: 3 000 mg; vitamin B2: 6 000 mg; Niacin: 25 000 mg, calcium D Pantothenate: 120 000 mg.

2the mineral premix: Mn: 80 000 mg; Fe: 30 000 mg; Zn: 60 000 mg; Ca: 5000 mg; Co: 500 mg; I: 2000 mg; CaCO3: 235 680 mg.

**STATISTICAL ANALYSIS**

Statistical analysis of data was performed using the by SPSS 10.0 version for Windows. One-way analysis of
variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan’s Multiple Range Test was performed. Differences were considered as significant when P value was less than 0.05. All data were expressed as means ± standard error of the mean (SEM).

Results

As shown in Table II, plasma MDA concentrations were markedly increased in animals exposed to Cd (group Cd) compared to the not supplemented controls (P < 0.05). In parallel, the erythrocyte enzyme SOD, CAT and GSH-Px activities as well as the plasma concentrations of anti-oxidants except those of the vitamins A and C were remarkably depressed in the Cd intoxicated birds (P < 0.001 to P < 0.05). When a dietary supplementation with vitamin C or vitamin E was performed, the plasma MDA concentrations significantly decreased compared to Cd exposed and not treated broilers (P < 0.05) but they remained significantly elevated compared to values measured in controls (receiving basal diets). In birds exposed to Cd, the enzyme CAT and GSH-Px activities were significantly restored by the vitamin E treatment (P < 0.05) but not by the vitamin C supplementation. In the same way, the SOD activity remained significantly depressed in the vitamin C supplemented birds compared to the controls (P < 0.05) whereas the difference in this parameter was not significant between the controls and the vitamin E treated birds. Surprisingly, the plasma vitamin A concentrations were significantly diminished in the 2 treated groups (with vitamin C or with vitamin E) compared to the healthy controls (P < 0.05) whereas they were not significantly depressed in Cd intoxicated birds and the β-carotene concentrations remained significantly decreased in the 3 groups exposed to cadmium (P < 0.001). As expected, the plasma vitamin C concentrations were maximal in the group treated with this vitamin compared to the 3 other groups (P < 0.01). However, in the vitamin E supplemented group, the plasma vitamin E concentrations were lower (not significantly) than in the not supplemented and not intoxicated controls but were markedly higher than in birds exposed to Cd and not treated (P < 0.001). In the vitamin C treated group, this parameter tended to increase but not significantly compared to the Cd group and remained significantly depressed compared to the control (P < 0.001).

On the other hand, plasma albumin and ceruloplasmin concentrations have not significantly differed between groups (Table III); nevertheless, the highest ceruloplasmin concentrations were observed in birds exposed to cadmium whereas the lowest values were found in healthy controls and birds treated with vitamin C or vitamin E have shown intermediate values. Compared to the controls, the uric acid concentrations were significantly decreased in the 3 groups exposed to cadmium (P < 0.05), particularly in birds treated with vitamin C or with vitamin E (group Cd vs. group Cd + C or group Cd + E: P < 0.05).

The variations of the erythrocyte characteristics were summarized in Table III. While the red blood cell counts (P < 0.001), haemoglobin concentrations (not significantly) and haematocrit values (not significantly) were depressed after Cd exposition compared to healthy controls, treatments with vitamin C or with vitamin E at a lesser extend induced marked increases of these parameters compared to the Cd intoxicated birds (P < 0.001 for erythrocyte numeration, P < 0.01 for haemoglobinemia and P < 0.05 for haematocrit) and to healthy controls (P < 0.05 to P < 0.001 only with vitamin C supplementation). Consequently, in birds exposed to Cd, erythrocytes exhibited a significantly greater mean corpuscular volume (given by the formula: MCV = PCV / (100 x RBC)) than in healthy controls (P < 0.05) and when the vitamin supplementation was performed, the cell size was significantly decreased (P < 0.05) but remained higher compared to controls in the case of vitamin C treatment. The mean haemoglobin load in erythrocytes (MHCH given by the for-

Table II: Lipid peroxidation intensity and changes in antioxidant systems in broilers dietary supplemented with Cadmium (60 mg/kg of food) (group Cd) and with vitamin C (400 mg/kg of food) (group Cd + C) or with vitamin E (250 mg/kg of food) (group Cd + E). Results are expressed as means ± standard error of the mean (SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cd</th>
<th>Cd + C</th>
<th>Cd + E</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/L)</td>
<td>5.66 ± 0.49a</td>
<td>8.04 ± 0.74c</td>
<td>7.31 ± 0.89b</td>
<td>6.75 ± 0.97b</td>
<td>&lt; 0.05</td>
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<tr>
<td>SOD (U/mg Hb)</td>
<td>0.28 ± 0.16a</td>
<td>0.17 ± 0.05b</td>
<td>0.20 ± 0.01b</td>
<td>0.23 ± 0.02ab</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CAT (katal/ mg Hb)</td>
<td>0.37 ± 0.10a</td>
<td>0.21 ± 0.04c</td>
<td>0.25 ± 0.06bc</td>
<td>0.30 ± 0.09ab</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>GSH-Px (U/mg Hb)</td>
<td>0.99 ± 0.20a</td>
<td>0.61 ± 0.14c</td>
<td>0.74 ± 0.11bc</td>
<td>0.84 ± 0.11b</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Vitamin A (mg/L)</td>
<td>1.01 ± 0.09a</td>
<td>0.79 ± 0.09ab</td>
<td>0.72 ± 0.07b</td>
<td>0.63 ± 0.08b</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>β-carotene (mg/L)</td>
<td>1.13 ± 0.09a</td>
<td>0.34 ± 0.02b</td>
<td>0.37 ± 0.03b</td>
<td>0.31 ± 0.06b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>6.01 ± 0.70b</td>
<td>6.78 ± 0.98b</td>
<td>9.21 ± 0.52a</td>
<td>5.54 ± 0.53b</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>8.32 ± 0.41a</td>
<td>4.63 ± 0.50c</td>
<td>5.25 ± 0.51bc</td>
<td>6.82 ± 0.81ab</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/L)</td>
<td>23.90 ± 3.11</td>
<td>28.04 ± 2.82</td>
<td>25.92 ± 1.24</td>
<td>24.44 ± 2.21</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>13.54 ± 0.52</td>
<td>12.16 ± 1.01</td>
<td>13.14 ± 0.61</td>
<td>11.86 ± 0.85</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid (mg/L)</td>
<td>65.82 ± 4.60a</td>
<td>54.36 ± 4.03b</td>
<td>41.18 ± 4.24c</td>
<td>42.06 ± 3.20c</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Hb: Haemoglobin.
Different superscripts a,b,c in the same row indicate significant difference between groups.
Discussion

The present study has clearly demonstrated that the Cd exposition induced oxidative stress in broilers as evidenced by, in one hand, increased plasma MDA concentrations and on the other hand, decreased SOD, CAT and GSH-Px activities in erythrocytes as well as decreased plasma α-carotene and vitamin E concentrations after 42 days of Cd treatment. These findings are in agreement with many previous studies performed on different species, indicating that Cd induces increase of the lipid peroxidation [2, 6, 7, 20, 23, 28, 32, 35, 50] and decreases in SOD [7, 42], in CAT [42] and in GSH-Px activities [23]. Although the mechanism of the Cd-induced oxidative stress is still not completely elucidated, some hypotheses have been proposed. As Cd inhibits the mitochondrial electron-transfer chain reaction leading to the accumulation of semi-ubiquinones [50], the unstable semi-ubiquinones would transfer one electron to molecular oxygen to form superoxide, providing a possible mechanism for Cd-induced generation of ROS in mitochondria [16]. It has also been suggested that cadmium could directly affect and weakened some antioxidant systems [21] such as the SOD, CAT and GSH-Px enzymes and the reactive scavengers of free radicals leading to an oxidative stress [49]. As SOD contains Zn and Cu in its active site, the interaction between Cd and Zn in the active site may induce the enzyme inhibition [31]. The decrease in the GSH-Px activity may result from a direct depletion of selenium by Cd [55] or from insufficient incorporation of sulphur amino-acids into GSH-Px because of competition with the Cd-metallothionein synthesis [33]. JURCZUK et al. [22] reported that the decreased CAT activity in the liver and kidneys of rats exposed to Cd may result from Fe deficiency. Indeed, Cd impaired the intestinal absorption of iron which is required for the CAT activity. In addition, SHUKLA and CHANDRA [44], HIJOVA et al. [18] and HIJOVA and NISTIAR [19] demonstrated that the vitamin E concentrations were significantly reduced in rats exposed to Cd. Because of vitamin E depletion, membrane lipids would be greatly oxidized by unscavenged ROS leading to the amplification of lipid peroxidation and severe cellular damage. Consequently, the vitamin E requirement would be increased during Cd intoxication. Nevertheless, plasma vitamin A concentrations were slightly and not significantly decreased in the Cd group whereas SUGAWARA et al. [45] has demonstrated that Cd interferes with the release of vitamin A especially in the storage form, from the liver. Various mechanisms have been evoked for limiting the vitamin A excretion into serum: Cd can induce the destruction of excretion canals of cell organelles or directly bind with specific proteins essential for the vitamin A excretion, transport and distribution such as retinol binding protein, prealbumin and the vitamin A hydrolase.

Different antioxidants such as the vitamins C and A, the β-carotene and quercetin have been investigated for alleviating the Cd-induced lipid peroxidation [6, 7, 9, 12, 20, 31, 43]. In the current study, the dietary supplementation with vitamin C significantly reduced the Cd-induced lipid peroxidation as evidenced by the significant decrease in plasma MDA concentrations and these results are in agreement with previous reports [7, 20]. The SOD, CAT and GSH-Px activities and the plasma β-carotene and vitamin E concentrations were slightly increased but not significantly compared to Cd intoxicated birds. However, GUPTA et al. [12] have reported significant increases of SOD and GSH-Px activities in rats exposed to Cd and treated with vitamin C. This discrepancy can be attributed to the dose used of vitamin C which could be insufficient for completely preventing the toxicity of 60 mg/kg of Cadmium. Vitamin E is an important antioxidant, mainly located in cell membranes. The oral vitamin E treatment appeared in the present study as very effective in the prevention of oxidative damage induced by Cd: plasma MDA concentrations were deeply depressed and the antioxidant enzyme activities (except SOD) and plasma vitamin E concentrations were markedly increased (P < 0.05) compared to the intoxicated birds although they remained lower than activities measured in healthy controls. Similarly, BEYTUT et al. [2], SARKAR et al. [41] and OGNJANOVIC et al. [32] also reported pro-

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>26.60 ± 1.21(^{b})</td>
<td>25.20 ± 1.31(^{b})</td>
<td>30.20 ± 0.61(^{a})</td>
<td>28.40 ± 0.75(^{a b})</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>58.01 ± 1.52(^{bc})</td>
<td>55.61 ± 1.10(^{c})</td>
<td>62.30 ± 1.38(^{a})</td>
<td>60.90 ± 1.32(^{a b})</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RBC (10(^12)/L)</td>
<td>2.83 ± 0.69(^{b})</td>
<td>2.34 ± 0.91(^{c})</td>
<td>3.10 ± 0.72(^{a})</td>
<td>2.97 ± 0.45(^{a b})</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>94.76 ± 5.52(^{a})</td>
<td>109.84 ± 8.71(^{c})</td>
<td>97.69 ± 3.67(^{b})</td>
<td>95.99 ± 2.99(^{a b})</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>223.17 ± 13.60(^{a})</td>
<td>226.18 ± 12.99(^{a})</td>
<td>208.29 ± 7.58(^{b})</td>
<td>217.34 ± 8.88(^{a})</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red Blood cells; MCV: Mean corpuscular volume given by the following formula: MCV = PCV / (100 x RBC); MCHC: Mean corpuscular haemoglobin concentration given by the following formula: MCHC = 100 x Hb / PCV; NS: Not significant. Different superscripts a,b,c in the same row indicate significant difference between groups.
ective antioxidant effects of vitamin E during Cd exposition in rabbits and rats respectively. Although the treatment of Cd exposed broilers with vitamin E or vitamin C at a lesser extend attenuated the induced oxidative stress by notably increasing the antioxidant systems, the plasma vitamin A and β-carotene concentrations were poorly affected and even the vitamin A concentrations were lowered compared to the healthy controls. By increasing in membranes ROS scavenging and their consequent reduction into hydroperoxides and by promoting anti-oxidant enzyme activities, it is possible that the regeneration into the reduced form of the incremented tocopherol pool throughout dietary supplementation requires indirectly an increased consumption of retinol and β-carotene (as provitamin A) leading to decreases of their plasma concentrations.

Compared to controls, significant decreases in plasma uric acid concentrations were observed in groups exposed to Cd treated or not with vitamins E or C (P < 0.05). Many researchers [3, 18, 19, 43] have previously shown that the Cd exposition induced significant reduction of plasma uric acid concentrations in rats and CASALINO et al. [3] and SHAIKH et al. [43] have reported that long term Cd administration induced inhibition of antioxidant defence systems in kidneys. The decrease in uric acid level can be caused by its consumption after formation of free radicals. Furthermore, uric acid concentrations were significantly lower in treated birds than in not treated and Cd exposed broilers (P < 0.05). These results suggested that the vitamins E and C did not exert any preventive effect on the uric acid utilisation.

In addition, the Cd exposition has induced anaemia which was evidenced by the reduction of haemoglobinemia, the erythrocyte number and the haematocrit in exposed birds. As previously shown in rats, mice and broilers [6, 24, 26, 32, 39, 54], the anaemia is due to haemolysis due to Cd-induced erythrocyte membrane damage. Besides, HAMADA et al. [15] and KTAPCINSKA et al. [27] have demonstrated that the erythrocyte ATP (adenosine triphosphate) and 2,3 DPG (2,3 diphosphoglycerate) contents were depressed in Cd exposed animals favouring cell deformation and shakiness that coupled to oxidative membrane alteration leads to haemolysis and anaemia. Indirectly the decrease of haematocrit values and the occurrence of haemolysis plasma would confirm the erythrocyte haemolysis [15, 37]. Additionally, some authors [9, 26] have also suggested that the impairment of iron absorption by Cd would lead to decrease the haemoglobin synthesis and would contribute to anaemia. In the present study, in broilers exposed to Cd and not treated, the erythrocytes exhibited in average a higher size than in healthy controls and were normally loaded in haemoglobin suggesting a regenerative anaemia consecutive to the intense haemolysis instead of an iron deficiency (which would lead to small erythrocytes poorly loaded with haemoglobin). Oral vitamin treatment, particularly with ascorbate has considerably improved the erythrocyte parameters (numeration, haematocrit and haemoglobinemia) even leading to values significantly more elevated than values observed in controls in the case of vitamin C treatment. The 2 antioxidant vitamins have successfully prevented anaemia in Cd exposed chickens and these findings are in agreement with previous data [32, 35, 43]. Furthermore, the higher efficiency of the vitamin C would be linked to its specific effect on iron absorption from the gastrointestinal tract [9, 11]. In this way, the occurrence of erythrocytes larger than in controls with low haemoglobin content is compatible with an intense erythropoiesis.

In conclusion, a chronic exposition to Cd (60 mg/kg) has induced an oxidative stress through lipid peroxidation and inhibition or consumption of some antioxidant systems, particularly in erythrocytes leading to anaemia. Dietary supplementation with vitamin C (400 mg/kg) or with vitamin E (250 mg/kg) succeeded in alleviating many of the harmful effects of Cd in broilers. Especially, the vitamin E treatment appeared more efficient on anti-oxidant systems (particularly CAT and GSH-Px) than treatment with vitamin C, whereas a best correction of anaemia was achieved with the ascorbate.

Acknowledgement

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