The effects of turmeric supplementation on antioxidant status, blood gas indices and mortality in broiler chickens with T\textsubscript{3}-induced ascites

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The effects of turmeric supplementation on antioxidant status, blood gas indices and mortality in broiler chickens with T₃-induced ascites

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Abstract
1. A total of 320 one-day-old Ross male broiler chickens were used to investigate the effects of 0, 2.5, 5.0 and 7.5 g/kg turmeric rhizome powder (TRP) in the diet, on antioxidant status, biochemical gas indices and mortality in broiler chickens with triiodothyronine (T₃) induced ascites.
2. The TRP supplementation had no effect on blood pH, pO₂ or pCO₂ during the whole period of study. Moreover, supplementation of TRP did not influence the heart weight, right ventricle, left ventricle, or total ventricle weights, all relative to total live weight; RV/TV (right ventricle to total ventricle) ratio; or serum GPX (glutathione peroxidase) or SOD (superoxide dismutase) activities at week 6.
3. TRP supplementation influenced the blood HCO₃⁻ and O₂ saturation during the whole period of study, total mortality due to ascites, and serum total tocopherol and malondialdehyde (MDA) contents. Blood HCO₃⁻ and serum total tocopherol increased linearly as dietary TRP level increased. Blood O₂ saturation increased quadratically as dietary TRP increased.
4. Total ascites mortality and serum MDA content decreased linearly with increasing TRP level to 5 mg/kg and then reached a plateau.
5. The results of the study indicate that the addition of 5.0 g/kg TRP is sufficient to increase the blood O₂ saturation and bicarbonate (HCO₃⁻) concentration, and reduce the mortality due to ascites and serum MDA content.

INTRODUCTION

Pulmonary hypertension syndrome (PHS), commonly known as ascites, is a costly metabolic disease that occurs in the poultry industry worldwide (Huchzermeyer and DeRuyck, 1986; Huchzermeyer et al., 1988). It causes severe losses in the broiler industry in many countries, not only due to the high rate of mortality, but also due to reduced weight gain and increased condemnations at slaughter (Julian, 1993). It accounts for over one quarter of overall broiler mortality across the world (Guo et al., 2007).

The involvement of oxidative stress in PHS in broilers has been clearly demonstrated on ascites development (Enkvetchakul et al., 1993; Bottje and Wideman, 1995; Bottje et al., 1995, 1997). A major cellular source of oxidative stress in cells occurs within mitochondria due to incomplete reduction of oxygen to reactive oxygen species (ROS) (e.g., superoxide) (Chance et al., 1979). Increased mitochondrial ROS production has been observed in many studies (Maxwell et al., 1996; Cawthon et al., 2001; Iqbal et al., 2001).

Antioxidants play a major role in protecting cells from the actions of ROS by reducing chemical radicals and disrupting the process of lipid peroxidation (Yu, 1994). The low levels of antioxidants in birds with PHS could therefore lead to an inability to control lipid peroxidation (Bottje et al., 1995). Bottje et al. (1998) observed...
an increase in the GSGG to total glutathione ratio, an indicator of oxidative stress in birds exposed to high levels of dust and ammonia. Cawthon et al. (2001) observed lower levels of primary antioxidants, α and β-tocopherol, and glutathione (GSH) in the mitochondria in the liver of birds with PHS. Supplementation of tocopherol (vitamin E) in the diet (Bottje et al., 1997; Villar-Patino et al., 2002; Nain et al., 2008), tocopherol as an implant (Bottje et al., 1995), and vitamin C in the diet (Hassanzadeh et al., 1997; Nain et al., 2008) have been used to improve body antioxidant status and to prevent ascites to date, but there is no information about the antioxidant effect of Curcuma longa rhizome powder (turmeric rhizome powder) on ascites incidence in broiler chickens.

Dried turmeric rhizome powder (TRP) (used as a spice, food preservative, and a colouring agent) is a rich source of beneficial phenolic compounds: the curcuminoids (Srinivasan, 1953). Three main curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin (Balasubramanyam et al., 2003), have been isolated from turmeric. They have strong antioxidant activity (Asai et al., 1999).

Many studies have shown the capacity of curcumin, the most active component of turmeric, to prevent lipid peroxidation, a key process in the onset and progression of many diseases. For example; a reduction in lipid peroxidation (Venkatesan, 1998; Miquel et al., 2006) and lower susceptibility of low density lipoprotein (LDL) cholesterol to oxidation (Ramírez-Tortosa et al., 1999) have been observed in response to turmeric. Moreover, antioxidant properties of turmeric include protection of haemoglobin from oxidation; inhibition of the generation of ROS, H₂O₂ and nitrite radicals by activated macrophages; the reduction of ROS production in vivo; the inhibition of H₂O₂-induced damage in human keratinocytes and fibroblasts; the reduction of oxidised proteins in amyloid pathology in Alzheimer transgenic mice; decreased lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates; and the prevention of oxidative damage during indomethacin-induced gastric lesion by blocking the inactivation of gastric peroxidase and by direct scavenging of H₂O₂ and *OH; as reviewed by Chattopadhyay et al. (2004).

Since ROS have been implicated in the development of ascites, and the potential antioxidant ability of curcumin and its derivatives has been indicated in many studies, the effects of oral administration of different levels of TRP on the antioxidant capacity and incidences of ascites in broiler chickens were investigated.

### Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(g/kg)</th>
<th>Starter (0-21 d)</th>
<th>Grower (21-42 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>513-7</td>
<td>580-3</td>
<td></td>
</tr>
<tr>
<td>Soyabean meal (440 g/kg protein)</td>
<td>299-4</td>
<td>297-2</td>
<td></td>
</tr>
<tr>
<td>Maize gluten meal</td>
<td>88-7</td>
<td>30-1</td>
<td></td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>50-0</td>
<td>50-0</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>12-0</td>
<td>14-3</td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>17-4</td>
<td>11-6</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5-0</td>
<td>5-0</td>
<td></td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>4-7</td>
<td>3-4</td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0-6</td>
<td>0-1</td>
<td></td>
</tr>
<tr>
<td>Wheat bran²</td>
<td>7-5</td>
<td>7-5</td>
<td></td>
</tr>
<tr>
<td>T₃ hormone (mg/kg)</td>
<td>1-5</td>
<td>1-5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

1Free vitamin E vitamin-mineral premix was used and supplied per kg of diet: retinol, 10 000 IU; cholecalciferol, 9790 IU; menadione, 2 mg; cyanocobalamin, 20 μg; riboflavin, 4-4 mg; pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamine, 4 mg; riboflavin, 6-6 mg; pyridoxine, 3 mg; folic acid, 1 mg; zinc (zinc sulphate), 60 mg; manganese (manganese oxide), 60 mg; selenium (sodium selenite), 0-14; iron (ferrous sulphate), 20 mg; magnesium (magnesium oxide), 12 mg; copper (cupric sulphate), 0-76 mg; iodine (calcium iodate), 0-75 mg.

²0, 2, 5, 5-0 and 7-5 g turmeric rhizome powder per kg diet replaced wheat bran in starter and grower diets to provide 4 dietary treatments for each period.

### MATERIAL AND METHODS

In a completely randomised design with 4 treatments, 320 day-old male broiler chickens (Ross 308) were provided by a local hatchery and housed in 16 pens (2 m²) of 20 birds each. Of these, 4 pens were randomly assigned to each dietary treatment. Feed and water were provided for ad libitum intake. All 4 starter and/or grower diets were in mash form (Table 1). All diets were supplemented with 1-5 mg/kg T₃ (Levothyroxine Sodium, Sandoz Inc., Austria) to induce ascites (Hassanzadeh et al., 2000), with different levels of TRP at 0-0 (control diet), 2-5, 5-0 and 7-5 g/kg, and fed from d 1 to d 42 of age. The levels of 2-5, 5-0 and 7-5 g/kg TRP replaced wheat bran in the control diet. Fresh turmeric rhizome (imported from India) was purchased from the local market, ground and used in the diets. Determination of total phenolic compounds in the TRP was according to the standard extraction method of Seeviers and Daly (1970). Furthermore, gallic acid was used as the standard. For this purpose, one g turmeric rhizome was crushed in 10 ml of...
methanol in a pestle and mortar. Then the produced extract was filtered and centrifuged at 1000×g for 5 min. The collected supernatant was used for determination of total phenolic compounds by the colorimetric method, measuring absorbance at 720 nm using 20% sodium carbonate (Na2CO3) in Folin-Ciocalteau reagent (Merck Co., Darmstadt, Germany). A total amount of 48 mg/g phenolic compounds was found for the TRP.

Birds were exposed to continuous light. The temperature was 32°C during the first 2 d, then reduced by 2°C weekly until week 5, when the temperature was kept constant at 22°C until the end of the experiment (Luger et al., 2001). Two chickens from each replicate pen were randomly selected and wing vein blood samples were obtained every other week at 1100 h every Monday, after 3 hours of feed removal at 0800 h. Blood in heparinised syringes (1 ml, 29G, 0.33×12 mm) was kept for less than two hours on ice and used for blood gas measurements using a pH/blood gas analyser (ABL50, ABL 995, France). Means of all blood gases variables for samples taken at d 14, 28 and 42 of age were calculated and used for statistical analyses.

At week 6, blood samples from one bird per replicate in non heparinised syringes (2.5 ml, G23, 32 ml) were allowed to clot for 2 h at 37°C. The serum was then decanted and stored at −20°C for later analyses (Tankson et al., 2002). Serum samples were thawed and serum malondialdehyde (MDA) and total tocopherol content were determined colorimetrically (chemically), and serum glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities were determined colorimetrically (enzymatically), using an ELISA microplate reader (Tecan Co., Grodingen, Austria), MDA assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), total tocopherol assay kit (Jaica Co., Shizuoka, Japan), GPX assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) and SOD assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). All assays were carried out according to the manufacturer’s instructions without modifications. At the end of the experiment (week 6), 4 chickens from each replicate (pen) of treatments were randomly selected and sacrificed by decapitation. The heart was removed and the right ventricle (RV) was dissected from the left ventricle and septum. The right and left ventricles were weighed separately (Daneshyar et al., 2007, 2009). Mortality was recorded daily and all dead birds were examined for gross symptoms of ascites, such as amber-coloured fluid in the abdominal cavity and pericardium; and an enlarged heart and right ventricle.

The univariate test in SAS (SAS Institute Inc., Cary, NC, USA) was used to assess the normality of all data. The weekly ascites mortality of each pen was divided by the number of live birds at the beginning of the week, and the total ascites mortality was divided by the number of chickens in each pen at the beginning of the experiment and multiplied by 100. Percentage data for heart part weights and mortality were transformed to arcsine √% for analysis (Corzo et al., 2007). Orthogonal polynomial contrasts (linear and quadratic) were used to determine the optimal dietary TRP level on blood gas indices over the whole experimental period; proportional cardiac part weights at week 6; total mortality from ascites; and serum antioxidant indices (MDA and total tocopherol content, and GPX and SOD activities) at week 6. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad.

### RESULTS

#### Blood gas indices

Blood pH, pO2 and pCO2 were not affected (P>0.05) during the whole experimental period (Table 2). However, TRP did influence the blood HCO3⁻ (P<0.05). Blood HCO3⁻ increased linearly

### Table 2. Blood pH, HCO3⁻ pO2, pCO2 and O2 saturation of T3 administered birds fed 0.0, 2.5, 5.0 and 7.5 g/kg turmeric rhizome powder supplemented diets

<table>
<thead>
<tr>
<th>TRP (g/kg)</th>
<th>pH</th>
<th>HCO3⁻ (nmol/l)</th>
<th>pO2 (mmHg)</th>
<th>pCO2 (mmHg)</th>
<th>O2 saturation (m/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.29</td>
<td>22.41</td>
<td>37.86</td>
<td>48.30</td>
<td>524.1</td>
</tr>
<tr>
<td>2.5</td>
<td>7.31</td>
<td>22.99</td>
<td>36.51</td>
<td>42.82</td>
<td>718.3</td>
</tr>
<tr>
<td>5.0</td>
<td>7.36</td>
<td>24.60</td>
<td>40.92</td>
<td>41.51</td>
<td>773.8</td>
</tr>
<tr>
<td>7.5</td>
<td>7.30</td>
<td>25.95</td>
<td>40.87</td>
<td>51.08</td>
<td>718.3</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.01</td>
<td>0.34</td>
<td>1.64</td>
<td>1.93</td>
<td>32.8</td>
</tr>
</tbody>
</table>

*Orthogonal polynomials*<br>*Contrasts*<br>Linear 0.62 0.03 0.39 0.70 0.02<br>Quadratic 0.19 0.07 0.68 0.14 0.005

1Blood samples of two birds per pen were used for measurements on d 14, 28 and 42 of age and their mean values were calculated and used for statistical analyses.
as dietary TRP level increased (y = 52.41 + 6.38x; R² = 0.32).

Dietary TRP supplementation significantly affected O₂ saturation during the whole period of study (P < 0.05). When the diet was supplemented with 7.5 g/kg TRP, O₂ saturation reached a plateau (y = 21.2 + 37.6x - 6.2x²; R² = 0.56) and no additional response was observed at this level (Table 2).

Mortality from ascites

Dietary TRP supplementation’s effect on weekly mortality from ascites is indicated in Table 3. Increasing the dietary TRP to 2.5 g/kg reduced the mortality from ascites linearly in week one (P = 0.002) and then reached a plateau (P = 0.002). Addition of graded levels of TRP did not change the ascites mortality afterwards (in weeks 2, 3, 4, 5 and 6). TRP supplementation linearly decreased the mortality of birds due to ascites in week 1 (Figure, P = 0.003), whereas no significant reduction in total mortality was observed after this point (P > 0.05). Total ascites mortality decreased quadratically (y = 16.7 - 5.9x + 0.94x²; P < 0.05; R² = 0.52) as dietary TRP level increased.

Proportional heart part weights and RV/TV ratio

No effect of dietary TRP supplementation was observed for proportional heart weight, proportional right ventricle, left ventricle, total ventricle weights and RV/TV ratio at week 6 (P > 0.05) (data not presented).

Serum antioxidant indices

Dietary TRP supplementation had no effect on serum GPX and SOD activities at week 6 (P > 0.05) (Table 4), but serum total tocopherol

---

Table 3. Weekly and total ascites mortality¹ of T3 administered birds fed 0.0, 2.5, 5.0 and 7.5 g/kg turmeric rhizome powder supplemented diets

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
<th>Week5</th>
<th>Week6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>9.0</td>
<td>4.0</td>
<td>7.0</td>
<td>7.0</td>
<td>11.0</td>
<td>8.0</td>
<td>46.0</td>
</tr>
<tr>
<td>L-TRP</td>
<td>1.0</td>
<td>7.0</td>
<td>3.0</td>
<td>7.0</td>
<td>12.0</td>
<td>6.0</td>
<td>36.0</td>
</tr>
<tr>
<td>M-TRP</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
<td>8.0</td>
<td>5.0</td>
<td>8.0</td>
<td>27.0</td>
</tr>
<tr>
<td>H-TRP</td>
<td>0.0</td>
<td>5.0</td>
<td>4.0</td>
<td>9.0</td>
<td>9.0</td>
<td>5.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Orthogonal polynomials

Contras

²Linear 0.002 0.51 0.34 0.85 0.96 0.32 0.01

²Quadratic 0.002 0.73 0.48 0.95 0.99 0.60 0.01

¹The number of birds dying from ascites in each treatment.
²Dead birds of each pen divided by the number of live birds at the beginning of the week, and total dead birds divided by the number of chickens in each pen at the beginning of the experiment, and then multiplied by 100. These percentage data transformed to arcsine V% for linear and quadratic regression analyses.

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Figure. The relationship between the amount (g/kg) of turmeric rhizome powder (TRP) supplementation and total ascites mortality (y = 16.7 - 5.9x + 0.94x²; P < 0.05; R² = 0.52) of T3 treated birds over the whole study period.
Table 4. Serum total tocopherol, malondialdehyde (MDA) content, glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity* at week 6 of T3 treated birds fed with 0-0, 2-5, 5-0 and 7-5 g/kg turmeric rhizome powder supplemented diets

<table>
<thead>
<tr>
<th>TRP g/kg</th>
<th>Total tocopherol (μg/ml)</th>
<th>MDA (μM)</th>
<th>GPX (nmol/min/ml)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0</td>
<td>8.97</td>
<td>5.70</td>
<td>166.3</td>
<td>147.3</td>
</tr>
<tr>
<td>2-5</td>
<td>8.50</td>
<td>5.00</td>
<td>168.3</td>
<td>114.7</td>
</tr>
<tr>
<td>5-0</td>
<td>8.03</td>
<td>5.10</td>
<td>133.5</td>
<td>131.8</td>
</tr>
<tr>
<td>7-5</td>
<td>7.88</td>
<td>6.40</td>
<td>130.0</td>
<td>124.0</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.18</td>
<td>0.19</td>
<td>8.62</td>
<td>5.18</td>
</tr>
</tbody>
</table>

Orthogonal polynomials

Contrasts

Linear 0.02 0.22 0.06 0.30
Quadratic 0.18 0.002 0.18 0.05

*All measurements were performed on 4 chickens per treatment.

was increased linearly (y = 9.19 - 0.35x; P < 0.05) as dietary TRP increased. Furthermore, serum MDA content decreased quadratically at week 6 as dietary TRP level increased (y = 7.46 - 2.25x + 0.49x^2; P < 0.05; R^2 = 0.74).

DISCUSSION

Decuypere et al. (1994) and Hassanzadeh et al. (2000) found that dietary T3 supplementation (1 to 2 mg/kg) enhanced the incidence of ascites in broiler chickens. Thus 1.5 mg/kg T3 was added to diets to induce ascites in the present experiment.

The high mortality and altered RV/TV ratio of control birds in this experiment proved that T3 induces ascites and concurs with Decuypere et al. (1994). It seems that TRP supplementation was effective at enhancing the antioxidant ability of birds, which appeared to lower mortality during the first week. Even though the mortality of birds decreased linearly in week 1, it was decreased quadratically during the whole experimental period. Moreover, the serum MDA content was decreased quadratically during week 6; and an increase was observed in the birds fed the 7.5 g/kg TRP-supplemented diet compared with those fed the 2.5 and 5.0 g/kg TRP diets.

The lower serum MDA content and mortality of 5.0 g/kg TRP-fed birds in this experiment could be related to the antioxidant effect of TRP. There is no other report on the effect of TRP on ascites incidence or the antioxidant status of broilers, but several investigators have reported different results on the effect of TRP on MDA content. Reddy and Lokesh (1994) found that curcumin supplementation inhibited lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates. Doses of 2.4 to 9.6 μmol/l curcumin inhibited the oxidation of human LDL in vitro (Ramirez-Tortosa et al., 1998). Venkatesan (1998) observed a protective effect of curcumin against the cardio-toxicity produced by adriamycin in rats, indicating a reduction in the factors influencing lipid peroxidation. Unnikrishnan and Rao (1995) demonstrated that curcumin and its derivatives (demethoxy curcumin, bisdemethoxy curcumin) protect haemoglobin from oxidation at a concentration as low as 0.08 mM. The addition of 5 and 1 g/kg Curcuma longa during the laying period significantly decreased MDA formation in the egg yolk (Nadia et al., 2008). The administration of an aqueous extract of turmeric to streptozotocin induced diabetic rats decreased the MDA content (Halim Eshrat, 2002).

The results of this study regarding the higher serum MDA content in 7.5 g/kg fed birds conflict with above reports, which may be related to the relatively intense oxidative stress in this experiment, as reflected by the high mortality in the control birds. Inclusion of turmeric in the diets decreased the mortality in week 1 (unpublished data) and was possibly effective at reducing MDA and improving the antioxidant status only in this week, but increased feed intake and consequently high T3 intake may have caused more oxidative stress (but not in any other weeks, especially week 6). A higher serum MDA content was observed in the birds fed the 7.5 g/kg TRP-supplemented diet compared with those fed the 2.5 and 5.0 g/kg TRP-supplemented diets, and a quadratic response existed for MDA content vs TRP supplementation level. Furthermore, a linear reduction in total tocopherol was observed by increasing the level of TRP supplementation. This phenomenon might be related to the pro-oxidant activity of curcumin and appears to be mediated through the generation of the phenoxy radical of curcumin by the peroxidase–H2O2 system, which co-oxidises cellular glutathione or NADH, accompanied by O2 uptake to form ROS (Galati et al., 2002). So higher levels of TRP inclusion may have increased the formation of phenoxy radicals and caused a higher MDA content in 7.5 g/kg TRP-fed chickens. Kelly et al. (2001) reported that curcumin not only failed to prevent single-strand DNA breaks by H2O2, but also caused DNA damage. As this damage was prevented by antioxidant α-tocopherol, it proved the pro-oxidant role of curcumin. Curcumin also caused oxidative damage in rat hepatocytes by oxidising glutathione, and in human erythrocytes by oxidising oxyhaemoglobin, thereby causing haemolysis (Galati et al., 2002). The presence of several co-antioxidants might also be required for effective scavenging of free radicals, because some antioxidant molecules, per se, could...
paradoxically act as pro-oxidants once activated in the lipid moiety (Quiles et al., 2002).

Besides the antioxidant effects, the observed reduced mortality following the inclusion of 5.0 g/kg TRP may be related to its effect on other physiological aspects. Recently, it was demonstrated that TRP lowers the arterial blood pressure and heart rate in rats, probably due to the blockage of extracellular Ca$^{2+}$ influx and the inhibition of intracellular Ca$^{2+}$ release from inositol-1,4,5-triphosphate sensitive stores (Adaramoye and Medeiros, 2008). Hence the low RV/TV ratio of less than 0.27 observed in 5.0 g/kg TRP fed birds may indicate lower arterial blood pressure and possibly a lower heart rate which has resulted in lower ascites mortality in these birds.

**Conclusions**

The experiment revealed that the inclusion of 5.0 g/kg TRP is sufficient to increase the blood O$_2$ saturation and bicarbonate concentration, and reduce the mortality due to ascites and serum MDA content; but a higher dose of TRP (7.5 g/kg) was ineffective, which may be due to an increase in the formation of phenoxy radicals and consequently lipid peroxidation.

Moreover, the presence of several co-antioxidants may be required along with TRP to effectively scavenge free radicals, because some antioxidant molecules such as curcumin, *per se*, could paradoxically act as pro-oxidants once activated in the lipid moiety.

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