Effects of Rosemary (Rosmarinus officinalis) Extract on Performance, Antioxidant Ability and Blood Gas Indices of Broiler Chickens Treated with Sodium Nitrate in Drinking Water

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ABSTRACT

A total of 220 broiler chicks in five groups were used to reveal the effects of different levels of 0.0, 1.5, 3.0 and 6.0 mL/L rosemary extract along with sodium nitrate (27.4 mg/L) in drinking water as compared to the control (without any supplement in water) on performance and antioxidant potential of treated broiler chickens. Body weight gain and feed conversion ratio were negatively affected by nitrate during finisher period and was compensated by all the rosemary levels. Both the blood uric acid and total antioxidant capacity were diminished by nitrate while consumption of 3.0 mL/L rosemary extract returned it to the control level. Nitrate decreased venous blood pO2, and rosemary extract levels of 3.0 and 6.0 mL/L increased pO2 to the same level as the control. Venous blood pCO2 was affected in an opposite way to pO2 by nitrate and rosemary extract. It is concluded that rosemary extract supplementation in drinking water can improve antioxidant ability and performance functions of broiler chickens treated with sodium nitrate.

KEY WORDS: blood gas, body weight gain, creatinine, total antioxidant capacity, uric acid.

INTRODUCTION

Changes in the patterns of agricultural practice, food processing and industrialization have resulted in accumulation of nitrates and nitrites in the environment. Intensive farming practice increased the use of nitrogen-based fertilizers (Chow and Hong, 2002). However, the continuous use of nitrogenous fertilizers in agriculture is the major source of nitrates and nitrites which have been shown to be present in relatively high concentrations in a wide variety of food plants and drinking water (Walker, 1975). The main problem of nitrate consumption is methemoglobin production which cannot bind oxygen. Accumulation of methemoglobin (methemoglobinemia) occurs if this oxidation process overwhelms the protective reduction capacity of the cells (Jaffe, 1981). A further concern relating to the metabolism of dietary nitrate is the potential in vivo formation of N-nitroso compounds from nitrite. Carcinogenesis, hepatotoxicity, impairment of reproductive functions, endocrine disturbances, growth retardation, destruction of vitamin A, methaemoglobinemia and impairment of certain defense mechanisms linked to the inflammatory response and tissue injury are the toxicological effects of nitrates and nitrites in different mammalian species (Slepchenko and Rhnemitskh, 1988). Conversion of nitrate to nitrite potentiate the formation of N-nitroso compounds such as peroxynitrite that is the most damaging reactive nitrogen species (RNS). Peroxynitrite causes the oxidative stress, lipid peroxidation and damage to cellular components (Seven et al. 2010). Although the nitrates and nitrites effects have been investigated extensively in mammals, little is known about their possible negative effects in poultry. In an experiment on...
broiler chickens, 20 mg/L nitrate consumption in water decreased the growth rate (Barton et al. 1986). In the other experiment on slow growing native breed (Balady), dietary sodium nitrate (4.2 g/kgfeed) have retarded growth and caused methaemoglobinemia (Atef et al. 1991). Oxidative damage, which is a consequence of excessive oxidative stress and/or insufficient antioxidant potential is the other consequence of nitrate or nitrite treating in animals (Safari and Daneshyar, 2012). Antioxidants play a major role in protecting cells from the actions of reducing chemical radicals and disrupting the process of lipid peroxidation (Yu, 1994). Today, there is increasing interest in the use of natural antioxidants such as rosemary (Rosmarinus officinalis) extracts, flavonoid and tocopherol for food preservation (Hras et al. 2000; Williams et al. 2004) because these natural antioxidants avoid undesirable health problems that may arise from the use of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (Aruoma et al. 1992). Antioxidant effect of aromatic plants is due to the presence of hydroxyl groups in their phenolic compounds (Shahidi and Wanasundara, 1992). Rosemary, belonging to the Lamiaceae family, is well known for its antioxidant properties, is used for flavouring foods and beverages, and is also used in several pharmaceutical applications. These polyphenols also have important biological activities in vitro such as anti-tumor, chemo-preventive and anti-inflammatory activities (Shuang-Sheng and Rong-Liang, 2006; Cheung and Tai, 2007). Rosemary contains a variety of phenolic compounds, including carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial and caffeic acid, with substantial antioxidant activity (Ibanez et al. 2003). Some experiments have indicated the potential antioxidant effect of rosemary and its components. Polat et al. (2011) investigated the dietary supplementation effects of rosemary plant (57, 86 and 115 g/kg) and its volatile oil (100, 150 and 200 mg/kg) in broilers. They showed that supplementation of 100 mg/kg rosemary volatile oils or 8.6 g/kg rosemary plant increases the plasma superoxide dismutase (SOD) activity. In the other study, feeding 5, 10 and 15 mL/L rosemary essential oil increased the serum serum superoxide dismutase (SOD) activity in broilers under oxidative stress (Yasar et al. 2011). The objective of this study was to evaluate the effects of hydroalcoholic extract of rosemary on broiler performance and antioxidant status chickens treated sodium nitrate in drinking water.

MATERIALS AND METHODS

Birds, diets and management

Two hundred and twenty one-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery. All the birds were weighed on arrival and randomly divided among 25 pens (1 m², 11 birds each pen) and four pens were assigned to one of the five experimental treatment groups. The subgroups of 11 chicks (mean weight 39±0.5 g) were kept in a well-ventilated house with wood shavings litter. All the birds were fed the same starter (from day one to day 10 of age), grower (from day 11 to day 24 of age) and finisher (from day 25 to day 42 of age) diets in mash form (Table 1). Vitamin and mineral premixes did not include anticoagulants or antioxidants (except ethoxyquin in the vitamin premix). The birds of different groups received the different water treatments. Control birds consumed the natural (having 5.4 mg/L nitrate) water whereas the birds of other groups received the sodium nitrate (27.4 mg/L) in drinking water alone or along with the different levels of 1.5, 3 and 6 mL/L hydroalcoholic extract of rosemary (Rosmarinus officinalis) from day one to the end of the experiment. The rosemary extract was purchased from Exir Gol Sorkh Company of Mashhad City, Iran. One of the active component of the extract (1, 8-cineol) was determined using a GC chromatograph (PV 4500 Shimaozu GC Chromatograph). For this determination, the extract was dissolved in a polar solvent of dichloromethane to derive the essential oils. Then the essential oils were injected to GC Chromatograph. The amount of 1.5 mg/kg was obtained for 1, 8-cineol in rosemary extract. Feed and water were provided ad libitum consumption. Birds were exposed to 23 h light and 1 h darkness during the experiment. Birds were exposed to 23 h light and 1 h darkness during the experiment.

Collection of samples and measurements

Body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined for the starter, grower, finisher and whole the experimental periods. At the end of the experiment (week 6), four birds per treatment were randomly selected and slaughtered. Before slaughter, one series of venous blood samples were collected in heparinised (20 U/mL) syringes (1 mL, 29 G, 0.33X 12 mm) for determination of blood gas indices. These blood samples were placed on ice and moved to the laboratory in less than 2 h. The blood gas indices of pH, bicarbonate, pCO₂, pO₂ and O₂ saturation then were determined using a pH/blood gas analyzer (Nova biomedical model pHox plus, USA). At time of slaughter, another series of blood samples was collected in anticoagulant tubes (ethylenediaminetetraacetic acid (EDTA), 7.5%) and transferred immediately to the laboratory, then centrifuged at 2800 × g for 5 min, for plasma separation, and stored at -20 °C for the later analyses. Plasma total antioxidant capacity (TAC) was determined using Randox test kit (Randox Laboratories Ltd., Crumlin, UK).
Plasma malondialdehyde (MDA) concentration was determined by MDA reaction with thiobarbituric acid followed by extraction with butanol (Kolahi et al. 2011). Additionally, plasma creatinine, uric acid and urea were determined spectrophotometrically (Alcyon 300 USA) using enzymatic kits (Pars Azmon Co., Iran).

### Results and Discussion

**Performance**

The effects of treatments on FI, BWG and FCR during the starter, grower, finisher, and the whole experimental periods are shown in Tables 2, 3 and 4, respectively. No significant difference was observed among the treatments for FI during the starter, grower, finisher and whole the experimental periods (P>0.05). Moreover, there was no significant differences between the treatments for BWG during the starter, grower, and the whole experimental periods (P>0.05), but during finisher period, nitrate diminished the BWG.

### Statistical analyses

The data were analyzed based on a completely randomized experimental design using the GLM procedure of SAS (2002). Duncan’s multiple range test was used to separate the means when treatment means were significant (P<0.05).

### Table 1: Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Starter (0-10 d)</th>
<th>Grower (11-24 d)</th>
<th>Finisher (25-42 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>329.9</td>
<td>344.5</td>
<td>392.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>200.0</td>
<td>250.0</td>
<td>250.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>393.3</td>
<td>335.0</td>
<td>282.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>29.4</td>
<td>29.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>21.0</td>
<td>21.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>11.0</td>
<td>8.60</td>
<td>8.60</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>2.90</td>
<td>2.20</td>
<td>2.00</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>3.80</td>
<td>0.80</td>
<td>1.40</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.70</td>
<td>3.40</td>
<td>3.40</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Calculated analysis:

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter (0-10 d)</th>
<th>Grower (11-24 d)</th>
<th>Finisher (25-42 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (g/kg)</td>
<td>859.8</td>
<td>862.1</td>
<td>862.7</td>
</tr>
<tr>
<td>Metabolisable energy (kcal/g)</td>
<td>2.86</td>
<td>2.93</td>
<td>3.00</td>
</tr>
<tr>
<td>Crude protein (g/kg)</td>
<td>219.9</td>
<td>199.9</td>
<td>179.9</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>48.7</td>
<td>49.3</td>
<td>54.7</td>
</tr>
<tr>
<td>Fiber (g/kg)</td>
<td>39.6</td>
<td>37.0</td>
<td>34.4</td>
</tr>
<tr>
<td>Calcium (g/kg)</td>
<td>10.0</td>
<td>9.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Available phosphorus (g/kg)</td>
<td>4.5</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Calcium/phosphorus</td>
<td>2.22</td>
<td>2.00</td>
<td>2.66</td>
</tr>
<tr>
<td>Chloride (g/kg)</td>
<td>3.3</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Sodium (g/kg)</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Methionine (g/kg)</td>
<td>7.0</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Lysine (g/kg)</td>
<td>14.2</td>
<td>12.4</td>
<td>10.9</td>
</tr>
<tr>
<td>Arginine (g/kg)</td>
<td>15.3</td>
<td>13.7</td>
<td>12.2</td>
</tr>
<tr>
<td>Methionine + cysteine (g/kg)</td>
<td>10.7</td>
<td>7.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Tryptophan (g/kg)</td>
<td>2.9</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Tyrosine (g/kg)</td>
<td>9.8</td>
<td>8.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Threonine (g/kg)</td>
<td>8.5</td>
<td>7.7</td>
<td>6.9</td>
</tr>
</tbody>
</table>

1 Supplied per kilogram of diet: vitamin A: 9000 mg; vitamin D₃: 2000 mg; vitamin E: 18 mg; vitamin B₆: 0.15 mg; Riboflavin: 6.6 mg; Calciumpantothenate: 10 mg; Niacin: 30 mg; Choline: 500 mg; Biotin: 0.1 mg; Thiamine: 1.8 mg; Pindoxin: 3 mg; Folic acid: 1 mg; vitamin K₃: 2 mg; Antioxidant (ethoxyquin): 106 mg; Zinc: 50 mg; Manganese oxide: 100 mg; Copper: 10 mg; Fe: 50 mg; I: 1 mg and Se: 0.2 mg.
However, supplementation of all the rosemary levels returned BWG to the control level (P<0.05). In orthogonal comparisons, consumption of rosemary improved the BWG as compared to nitrate during both finisher and whole periods (P>0.05).

Meanwhile the FCR was affected by treatments during the all periods (P<0.05). In the starter period, consumption of low rosemary extract level (0.15%) lowered the FCR as compared to the other treatments (P<0.05). During the grower, finisher and whole the experimental periods, all the rosemary levels returned the FCR increment due to nitrate consumption to the normal level (P<0.05).

### Blood parameters

There was no significant difference among the treatments for blood urea and MDA concentrations at week 6 of age (P>0.05; Table 5). The blood creatinine was decreased by consumption of 1.5 and 3 mL/L rosemary extract of drinking water as compared to other treatments (P<0.05). Uric acid was decreased by nitrate consumption, and consumption of 1.5 and 3 mL/L rosemary extract returned uric acid to a level that was not different from the control. However, the consumption of rosemary extract at 6.0 mL/L caused plasma uric acid to remain at a level similar to consumption of nitrate alone.
Nitrate decreased the blood TAC and consumption of 3 mL/L rosemary returned it to the normal level (P<0.05).

**Blood pH and gases indices**

Venous blood pH and O₂ saturation and were not affected significantly by the treatments at week 6 of age (P>0.05; Table 6). Bicarbonate was decreased by nitrate in the drinking water, and consumption of all rosemary levels returned bicarbonate to the level of the control or slightly higher (P<0.05). The venous blood pCO₂ was decreased by nitrate in drinking water, and consumption of the rosemary extract at 3 and 6 mL/L increased pO₂ to a level similar to the control (P<0.05). In contrast, venous blood pCO₂ was elevated by nitrate in the drinking water, and supplementation of all rosemary extracts decreased pCO₂ to the level of the control (P<0.05).

The results of recent experiment showed that consumption of nitrate in drinking water negatively affected the growth performance, especially BWG and FCR, of broiler chickens. The decreased performance due to nitrate consumption has been reported in many animals especially broiler chickens. A study with commercial broilers showed that nitrate levels greater than 20 mg/L had a negative effect on weight, feed conversion, or performance (Lil, 2009). Reeder (1996) indicated that consumption of sodium nitrate at 2033 mg/kg diet reduced the growth performance and body weight of broilers. Grizzele et al. (1996) reported that a water nitrate levels of 3.55 and 5.19 mg/L negatively affected broiler growth performance (body weight and feed conversion ratio). Barton et al. (1986) found a negative correlation between nitrate consumption and feed conversion ratio in chickens. The compromised growth performance of nitrate-treated chickens could be related to their lower body antioxidant capacity as suggested by the nitrate-related decreased plasma TAC and uric acid. Safariy and Daneshyar (2012) reported the lower plasma TAC content in nitrite-fed laying hens and connected it to the potential for increased peroxide production.

Peroxide is a reactive oxygen specious (ROS), which has the potential to cause oxidative damage, a conclusion supported by the nitrate-associated decrease in plasma total antioxidant activity (TAC). Excessive levels of ROS or peroxynitrite-the most damaging of the reactive nitrogen species (RNS)- results in oxidative stress and causing severe damage to cellular components and lipid peroxidation (Seven et al. 2010). Reeder (1996) observed the decreased uric acid level in chicks consuming 50, 200 or 1000 mg/kg diet of calcium nitrate. The current research showed that addition of rosemary extract to the nitrated drinking water of broiler chickens ameliorated the nitrate-associated negative effects. It has been reported that growth performance parameters of broiler chickens can be enhanced by the addition of aromatic herbs and their extracts to poultry diets (Yesilbag et al. 2011). The current findings agree with those obtained by Radwan (2003) who reported improved body weight and feed conversion ratio of chickens fed rosemary leaves at 5 g/kg diet. Al Kassie (2008) indicated that addition of anise at 10 g/kg and rosemary 10 g/kg diet improved feed conversion ratios in broilers. Yesilbag et al. (2011) reported that addition of rosemary volatile oils at 100, 150 and 200 mg/kg diet improved both weight gain and feed conversion ratio in broilers. In Japanese quail, Cifci et al. (2013) found a decrease in feed conversion ratio by adding rosemary oil at 125 and 250 mg/kg diet when the birds were under a heat stress condition. In broiler chicken fed with poultry fat, adding 0.1 rosemary powder to diet improved the BWG and feed efficiency (Khazaee et al. 2016). It is highly likely that an improved body antioxidant system could be the reason for improved growth performance associated with rosemary treated broilers in this experiment. Both the blood TAC and uric acid were higher in rosemary extract treated broilers, which indicated improved antioxidant capacity of these birds.

Uric acid is the main nitrogenous waste product of birds (Wright, 1995), and it is also a potent antioxidant (Ames et al. 1981).
Uric acid inactivates the peroxynitrite and consequently decreases the ROS production in the body. It is likely that uric acid is degraded or metabolized when it scavenges peroxynitrite (Santos et al. 1999).

Allantoin, the oxidative product of uric acid, has been proposed as a potential biomarker for in vivo free radical reactions. Since birds do not possess the enzyme urate oxidase, the presence of allantoin in their plasma indicates a direct oxidation of uric acid possibly by peroxynitrite. Strong oxidizers such as hypochlorous acid and hydroxyl radicals rapidly oxidize uric acid into allantoin and other products (Kaur and Halliwell, 1990).

The improved body antioxidant capacity by rosemary extract consumption possibly have caused the lower methemoglobin production and better status of blood gaseous (lower pCO2 and higher pO2) in current experiment.

The antioxidant ability of rosemary extract or one or more of its components has been reported by some researchers. Krause and Ternes (2000) found the improvement of the oxidative stability of egg yolk when carnosic acid (an antioxidant constituent of rosemary) was used as a dietary supplement in laying hens. Galobart et al. (2001) reported that the dietary supplementation of a rosemary extract to laying hens had no effect on lipid oxidation of eggs. Yasar et al. (2011) detected the highest SOD activity by dietary consumption of 100 mg/kg rosemary oil in broilers. In quails, dietary supplementation of 250 and 500 ppm rosemary essential oils alleviated the heat stress induced testicular lipid peroxidation (Turk et al. 2016). The decreased blood creatinine was the other consequence of rosemary consumption in recent experiment. Creatinine is a chemical waste molecule that is generated from muscle metabolism. The kidneys maintain the blood creatinine in a normal range (Polat et al. 2011). It has been reported that nitrate consumption can impair the kidney functions (Zurovsky and Haber, 1995).

Current finding suggests that rosemary extract at levels of 1.5, 3.0 and 6.0 mL/L of drinking water may cause an amendatory effect on antioxidant ability and growth performance of broiler chickens. It can be concluded that the biochemical activities attributed to rosemary extract phenolic compounds and metabolites could be important to chicken and human health. Additional studies are necessary to ascertain plant and plant extracts that have the potential to provide benefits to the animal and reduce the harmful effects of nitrate exposure.

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### REFERENCES


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