Effects of *Astragalus membranaceus* root processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens

G. G. Zhang,* Z. B. Yang,* Y. Wang,† and W. R. Yang*1

*Department of Animal Sciences and Technology, Shandong Agricultural University, Tai-an, Shandong, P. R. China, 271018; and †Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, AB, Canada T1J 4B1

ABSTRACT The objectives of this study were to assess the effects of supplementation of *Astragalus membranaceus* root powder (AMP) and AMP processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. The experiment was conducted with one hundred twenty 1-d-old Arbor Acres broilers in 5 groups of 4 cages and for both starter (0 to 21 d) and grower (22 to 42 d) phases. The treatments were basal diet only (control) and basal diet supplemented with 5 g/kg of diet of AMP processed to particle sizes of 300, 149, 75, or 37 μm. Average daily gain, ADFI, and feed conversion rate (FCR) were determined weekly, and carcass yield, serum antioxidant enzyme activity, and metabolites were determined at 21 and 42 d of the experiment. Supplementation of AMP increased \( P < 0.01 \) activities of total superoxide dismutase (TSOD) and glutathione peroxidase (GSHPx), but reduced \( P < 0.01 \) concentrations of malondialdehyde (MDA) and cholesterol in the serum of chickens at 21 and 42 d. Reducing AMP particle sizes from 300 to 37 μm linearly increased \( P < 0.01 \) TSOD and GSHPx activities at 21 and 42 d, but linearly decreased \( P < 0.01 \) MDA at 42 d. Concentrations of total protein, albumin, and globulin in the serum were also increased \( P < 0.05 \) or tended to be increased \( P = 0.05 \) to 0.10) by AMP and linearly increased \( P < 0.01 \) as the AMP particle sizes decreased. However, both treatments had no effect on ADG, ADFI, or FCR throughout the entire experiment period, although carcass yield increased \( P < 0.05 \) at 42 d. Dietary supplementation of AMP at the concentration of 5 g/kg of diet enhanced serum antioxidant status and its efficacy linearly increased as the AMP particle size decreased from 300 to 37 μm, but had no effect on growth performance of broilers.

Key words: *Astragalus membranaceus*, broiler, particle size, growth, antioxidant status

INTRODUCTION

The increasing restriction of synthetic compounds as growth-promoting agents (such as antibiotics) in livestock production has promoted the use of natural plant-derived compounds (phytogenic feed additives) as alternative feed additives (Jamroz et al., 2003; Hernandez et al., 2004; Naidoo et al., 2008). However, researchers have shown that efficacy of phytogenic feed additives in enhancing nutrients metabolism and productivity varies greatly depending on their origin and dosage, animal species, and the feeding management (Barreto et al., 2008; Willis et al., 2008; Tang et al., 2011). The dry *Astragalus membranaceus* root powder (AMP) is one of the popular additives used as a therapeutic agent in humans and livestock to treat various diseases such as diabetes, nephrotoxicity (Cho and Leung, 2007; El-Kenawy, 2010), nasopharyngeal carcinoma (Cho and Chen, 2009), foot-and-mouth disease (Zhang et al., 2010), and to improve immune functions (Yang et al., 2010; Wang et al., 2012). Recently, there has been increasing interest in the use of AMP as a feed additive. However, like other phytogenic feed additives, the observed effects of AMP on animal performance and feed efficiency varied among the published reports (Chen et al., 2003; Mao et al., 2005; Wang et al., 2010). Many factors such as those mentioned above could be attributable to the variation of the animal response to AMP supplementation. However, the processing property such as particle size of AMP may have also affected the efficacy of AMP supplementation in improving animal performance, but this has not been investigated. Zhao et al. (2010) showed that water holding capacity and polysaccharide solubility increased as the particle

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1Corresponding author: wryang@sdau.edu.cn

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sizes of AMP decreased from 300 to 7.56 μm (Zhao et al., 2010). Research conducted in our laboratory also showed that effects of ginger (Zingiber officinale) root powder as feed additive on growth performance, carcass quality, and blood antioxidant status of broilers were mediated by its particle size (Zhang et al., 2009a). The objectives of this study were to assess the effects of supplementation of AMP and AMP processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens.

MATERIALS AND METHODS

Preparation of Astragalus membranaceus Powder

Fresh, mature Astragalus membranaceus roots [Radix Astrali; Astragalus membranaceus (Fisch.) Bge. var. mongholicus (Bge.) Hsiao] were obtained from a local farm, cleaned with tap water, and then oven-dried at 40°C. The dried samples were first ground coarsely using a disc mill and screened through different-sized sieves to get the powders with particle size of 300 and 150 μm. Then the coarse particles with a particle size of 150 μm were ground in an HMB-701 type micronizer (Huanyatianyuan Machinery Company, Beijing, China) to obtain the superfine powders with the particle sizes of 74 and 37 μm (Zhao et al., 2010). The processed samples were stored in covered containers at ambient temperature (21 to 24°C) before being mixed into diets.

Experimental Design, Broilers, and Management

One hundred twenty 1-d-old vaccinated (against Marek’s disease and infectious bronchitis) Arbor Acres broiler chicks (mixed sex) were obtained from a local commercial hatchery. Broilers were randomly allocated into 20 wire cages of 5 treatments (6 birds per cage and 4 cages per treatments). The experiment was conducted with a completely randomized experimental design and the cages were considered replicate units. One group was fed the basal diet only (control) and the remaining 4 groups were fed a basal diet supplemented with 1 of 4 types of AMP (denoted as AMP300, AMP149, AMP74, and AMP37 containing AMP ground to particle sizes of 300, 149, 74, and 37 μm, respectively) at a dose of 5 g/kg of diet. Broilers were fed a starter diet from d 1 to 21 and a grower diet from d 22 to 42. Diets were formulated to meet nutrient requirements for starter and grower broiler chickens (Feeding Standard of Chicken of the People’s Republic of China; NY/T 33–2004) and were fed as mash. The diet compositions are shown in Table 1. All diets were formulated in a single batch. The AMP was first mixed with a premix that was subsequently mixed with other ingredients and then stored in covered containers.

The feeding experiments were carried out in an environmentally controlled house. The temperature was maintained at 32°C from d 1 to 7 and gradually decreased to 20°C at a rate of 3°C per week, and then maintained until the end of the trial. The lighting cycle was 24 h from d 1 to 3, 18 h from d 4 to 20, 21 h from d 21 to 35, and 23 h from d 36 to 42 of the experiment. The birds were fed for ad libitum intake and had free access to water through nipple drinkers for the entire duration of the experiment.

Average daily gain and ADFI were determined weekly by weighing the BW of the birds and their feed consumption per cage (sum of feed offered − feed leftover at the weighing time). The feed conversion rate (FCR) was calculated as ADFI dividing by corresponding ADG. The ADG, ADFI, and FCR were determined separately for starter (1 to 21 d), grower (22 to 42 d), and the entire feeding period (1 to 42 d). The animal care and use protocols were approved by the Animal Nutrition Research Institute of Shandong Agricultural University.

Sample Collection

Eight chicks (2 birds per cage) were randomly picked out from each treatment in the morning of d 21 and 42 of the experiment, after 12 h without feed, and BW of each bird was measured followed by blood samples (5.0 mL) taken from wing vein into nonheparinized tube. Blood sample was incubated in a water bath at 37°C for 2 h and subsequently centrifuged at 1,500 × g for 10 min at room temperature, and the serum was stored in 1.5-mL Eppendorf tubes at −20°C for further assay. The bird after bleeding was then slaughtered by cervical dislocation, and the carcass was obtained by removing abdominal fat pad, feathers, feet, and all the visceral organs (except the kidneys). The carcass yield

<table>
<thead>
<tr>
<th>Table 1. Diet formulation and compositions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Item</td>
</tr>
<tr>
<td>CP</td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Total phosphorus</td>
</tr>
<tr>
<td>Lys</td>
</tr>
<tr>
<td>Met</td>
</tr>
</tbody>
</table>

1Supplied per kilogram of diet: vitamin A, 8,050 IU; cholecalciferol 1,800 IU; vitamin E, 20 IU; vitamin K3, 5.1 mg; thiamine, 2.4 mg; riboflavin, 8.2 mg; pantothenic acid, 15.3 mg; pyridoxine, 3.1 mg; cobalamin, 0.02 mg; niacin, 32 mg; choline chloride, 1,000 mg; biotin, 0.20 mg; folic acid, 1.2 mg; Mn, 68 mg; Fe, 85 mg; Zn, 58 mg; Cu, 8.6 mg; I, 0.27 mg; and Se, 0.20 mg.
was calculated as percentage of carcass weights relative to fasted BW.

Assay of Antioxidant Enzymes and Metabolites in Serum

The activities of total superoxide dismutase (TSOD) and glutathione peroxidase (GSHPx), and concentrations of malondialdehyde (MDA) were all assayed according to the spectrophotometrical method described by Zhang et al. (2009a) with assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China; Zhang et al., 2009a). The activity of TSOD was determined by measuring the reduction of optical density (OD) at 550 nm of the reaction solution, GSHPx by measuring the OD of the reaction solution at 412 nm and MDA content by measuring OD of the reaction solution at 532 nm with corresponding substrates provided in the assay kits. Content of total protein (TP), albumin, globulin, and cholesterol in serum were determined using the methods described by Zhang et al. (2009a). These blood metabolites assays were analyzed by an automatic biochemical analyzer (Hitachi 7600-020, Beijing, China).

Data Calculations and Statistical Analyses

Data were statistically analyzed using 1-way ANOVA using the GLM procedure of SAS version 9.0 (Cole, 2001; SAS Institute Inc., 2002). The effect of AMP supplementation was determined by the contrast option of the GLM procedure. When this effect was significant (i.e., with \( P < 0.05 \)), polynomial contrasts were used to determine linear and quadratic responses of the parameters to particle sizes for the AMP treatment only. The significance of differences among treatments was tested using LSMEANS with the PDIF option in SAS v.9.0 (SAS Institute Inc., 2002).

RESULTS

Growth Performance and Carcass Characteristics of Broilers

The production performance of broilers is presented in Table 2. Throughout the duration of the experiment, all broilers appeared to be healthy and no mortality occurred (data not shown). All broilers had similar BW, ADG, ADFI, and FCR over the course of the 42-d experiment. Irrespective of particle size, the inclusion of AMP in broiler diets markedly increased the carcass yield (\( P = 0.006 \), control vs. AMP). Reducing particle sizes of AMP from 300 to 37 \( \mu \)m increased (linear, \( P = 0.006 \); quadratic, \( P = 0.012 \)) the carcass yield over the 42-d experiment. Broilers in AMP149, AMP74, and AMP37 had higher (\( P < 0.01 \)) carcass yield than that in control at 42 d of the experiment. In addition, carcass yield of broilers fed the AMP37 diet was higher (\( P < 0.05 \)) than that of broilers fed the AMP300 and control diets and was slightly higher (\( P = 0.054 \)) than broilers fed AMP149 and AMP74 diets at d 42 of the experiment.

Effect of Different Particle Sizes of AMP on Serum Antioxidant Status

In comparison with the control, the inclusion of AMP in broiler diets, irrespective of the AMP particle size, increased (\( P < 0.05 \)) the activities of TSOD and GSHPx, but reduced (\( P < 0.001 \)) the MDA content in the serum of chicks at the ages of 21 and 42 d (Table 3).

The serum antioxidant enzymatic activities of TSOD (\( P < 0.01 \)) and GSHPx (\( P < 0.05 \)) linearly increased as the particle sizes of AMP decreased from 300 to 37 \( \mu \)m, at both 21 and 42 d of age. On the contrary, the concentration of MDA in the serum of AMP-fed broilers linearly decreased (\( P < 0.01 \)) at d 42, but not at d 21 as AMP particle sizes decreasing from 300 to 37 \( \mu \)m. Birds supplemented with AMP had lower (\( P < 0.05 \)) MDA content in the serum than that of the control.

Effect of Different Particle Sizes of AMP on Serum Metabolites of Broilers

Concentrations of TP, albumin, globulin, and cholesterol in the serum of broilers at 21 and 42 d of age are presented in Table 4. Irrespective of particle size, the broilers fed AMP diets had higher (\( P = 0.034 \) at 21 d, and \( P = 0.053 \) at 42 d) TP concentration than that of control. In contrast, inclusion of AMP did not affect albumin concentration at 42 d, but tended to increase (\( P = 0.088 \)) at 21 d of age, and tended to increase the globulin concentration in the serum at 21 d (\( P = 0.067 \)) and 42 d (\( P = 0.078 \)) of age. The serum cholesterol concentration was decreased (\( P < 0.001 \)) by the addition of AMP in broiler diet compared with the control. Broilers fed AMP74 and AMP37 diets had higher (\( P < 0.05 \)) TP and albumin concentrations, and broilers fed AMP37 had higher (\( P < 0.05 \)) globulin concentration as well than that fed control diet.

Further analysis showed that serum concentrations of TP, albumin, globulin, and cholesterol responded linearly or quadratically (or both) to the particle sizes of AMP. Whereas concentrations of TP, albumin, and globulin linearly and quadratically (\( P \)-values ranging from <0.001 to 0.05) increased at 21 and 42 d of age, the cholesterol concentration linearly decreased (\( P < 0.05 \)) at 21 d and quadratically decreased at 42 d of age as the AMP particle sizes reducing from 300 to 37 \( \mu \)m.

DISCUSSION

Effect of AMP and Particle Sizes on Growth Performance of Broilers

The similar BW, ADG, ADFI, and FCR between the control and the AMP-supplemented broilers and among
different particle size treatments indicated that AMP supplemented at a dose of 5 g/kg of diet and processed to particle sizes ranging from 300 to 37 μm did not affect the growth performance of broilers under the feeding conditions of this study. This observation is consistent with Wang et al. (2010), who reported no effect of AMP supplementation at 5 g/kg of diet on growth performance of broilers. However, they observed increased ADG of broilers during the 42-d period feeding experiment by AMP supplemented at 10 g/kg of diet (Wang et al., 2010). This suggests that lack of growth response of broilers to AMP may be due to the low dosage of AMP in this study. The growth performance of broiler was also reported to be dose response to *Astragalus* polysaccharides, the main effective compound of AMP (Chen et al., 2003; Zhang et al., 2009b). This, together with the lack of response of broilers' growth performance to the particle sizes of AMP, suggests that dosage may be more important than the particle size for AMP to exert an effect on the growth of broilers. This is consistent with the observation of Zhang et al. (2009a) in ginger root processed to the same particle sizes. It needs to be pointed out that relatively small numbers (120) of broilers were used in this study due to the restriction of the research facility. Further study involving a higher dosage of AMP and larger number of broilers needs to be conducted to confirm the finding of this study.

### Table 2. Growth, feed intake, feed conversion rate, and carcass yield of broilers fed a diet with or without *Astragalus membranaceus* root powder (AMP) supplementation

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>AMP300</th>
<th>AMP149</th>
<th>AMP74</th>
<th>AMP37</th>
<th>SEM</th>
<th>Control vs. AMP (P)</th>
<th>Effect of particle size (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>683</td>
<td>685</td>
<td>691</td>
<td>691</td>
<td>700</td>
<td>14.6</td>
<td>0.593</td>
<td>0.548 0.823</td>
</tr>
<tr>
<td>42 d</td>
<td>1,911</td>
<td>1,901</td>
<td>1,953</td>
<td>1,947</td>
<td>1,973</td>
<td>47.8</td>
<td>0.534</td>
<td>0.319 0.614</td>
</tr>
<tr>
<td>ADG, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 21 d</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>32</td>
<td>0.4</td>
<td>0.100</td>
<td>0.359 0.477</td>
</tr>
<tr>
<td>22 to 42 d</td>
<td>59</td>
<td>58</td>
<td>61</td>
<td>60</td>
<td>61</td>
<td>2.2</td>
<td>0.624</td>
<td>0.392 0.688</td>
</tr>
<tr>
<td>0 to 42 d</td>
<td>45</td>
<td>45</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>1.1</td>
<td>0.447</td>
<td>0.32 0.620</td>
</tr>
<tr>
<td>ADFI, g/d</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0 to 21 d</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>46</td>
<td>0.4</td>
<td>0.900</td>
<td>0.194 0.437</td>
</tr>
<tr>
<td>22 to 42 d</td>
<td>129</td>
<td>130</td>
<td>133</td>
<td>133</td>
<td>134</td>
<td>3.4</td>
<td>0.384</td>
<td>0.376 0.684</td>
</tr>
<tr>
<td>0 to 42 d</td>
<td>87</td>
<td>87</td>
<td>89</td>
<td>89</td>
<td>90</td>
<td>1.7</td>
<td>0.369</td>
<td>0.287 0.578</td>
</tr>
<tr>
<td>Feed conversion rate (ADFI/ADG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 to 21 d</td>
<td>1.47</td>
<td>1.43</td>
<td>1.46</td>
<td>1.43</td>
<td>1.44</td>
<td>0.013</td>
<td>0.052</td>
<td>0.879 0.484</td>
</tr>
<tr>
<td>22 to 42 d</td>
<td>2.20</td>
<td>2.24</td>
<td>2.19</td>
<td>2.21</td>
<td>2.21</td>
<td>0.049</td>
<td>0.824</td>
<td>0.739 0.948</td>
</tr>
<tr>
<td>0 to 42 d</td>
<td>1.95</td>
<td>1.96</td>
<td>1.95</td>
<td>1.95</td>
<td>1.95</td>
<td>0.026</td>
<td>0.382</td>
<td>0.641 0.859</td>
</tr>
<tr>
<td>Carcass yield, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>70.6</td>
<td>69.7</td>
<td>69.5</td>
<td>70.6</td>
<td>70.7</td>
<td>0.58</td>
<td>0.436</td>
<td>0.171 0.232</td>
</tr>
<tr>
<td>42 d</td>
<td>71.2</td>
<td>72.6</td>
<td>73.5</td>
<td>73.7</td>
<td>75.3</td>
<td>0.65</td>
<td>0.006</td>
<td>0.006 0.012</td>
</tr>
</tbody>
</table>

a–c Means within a row with different letters differ (P < 0.05).

1AMP300, AMP149, AMP74, or AMP37 represents AMP processed to particle size of 300, 149, 74, or 37 μm, and all were supplemented at a dose of 5 g/kg of diet.

2Comparison of control with pooled AMP treatments.

3All data are means of 4 replicate cages of 6 chicks per cage otherwise mentioned.

4Data are means of 4 replicate cages of 2 chicks per cage.

### Table 3. Antioxidant enzymatic activity and concentration of malondialdehyde in the serum of broilers fed diets with or without *Astragalus membranaceus* root powder (AMP) supplementation

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>AMP300</th>
<th>AMP149</th>
<th>AMP74</th>
<th>AMP37</th>
<th>SEM</th>
<th>Control vs. AMP (P)</th>
<th>Effect of particle size (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSOD, U/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>111d</td>
<td>122c</td>
<td>125bc</td>
<td>127b</td>
<td>136a</td>
<td>1.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>42 d</td>
<td>106e</td>
<td>114b</td>
<td>117a</td>
<td>117a</td>
<td>118a</td>
<td>2.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001 &lt;0.001 0.004</td>
</tr>
<tr>
<td>GSHPx, U/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>1,156c</td>
<td>1,190bc</td>
<td>1,236b</td>
<td>1,520a</td>
<td>1,534a</td>
<td>24.6</td>
<td>0.024</td>
<td>&lt;0.001 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>42 d</td>
<td>961c</td>
<td>1,146b</td>
<td>1,198bc</td>
<td>1,205ab</td>
<td>1,319a</td>
<td>40.0</td>
<td>&lt;0.001</td>
<td>0.011 0.026</td>
</tr>
<tr>
<td>MDA, nmol/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>13.2a</td>
<td>10.4b</td>
<td>10.4b</td>
<td>9.8b</td>
<td>9.7b</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>0.190 0.338</td>
</tr>
<tr>
<td>42 d</td>
<td>11.0a</td>
<td>9.3b</td>
<td>9.3bc</td>
<td>9.0c</td>
<td>8.6d</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>0.003 0.001</td>
</tr>
</tbody>
</table>

a–d Means within a row with different letters differ (P < 0.05).

1Data are means of 4 replicate cages of 2 broilers per cage.

2TSOD, total superoxide dismutase; GSHPx, glutathione peroxidase; MDA, malondialdehyde.

3AMP300, AMP149, AMP74, or AMP37 represents AMP processed to particle sizes of 300, 149, 74, or 37 μm, respectively, and all were supplemented at a dose of 5 g/kg of diet.

4Comparison of control with pooled AMP treatments.
However, inclusion of AMP in the diet increased the carcass yield of broilers compared with the controls. Carcass yield was increased by AMP supplementation and linearly increased by reducing AMP particle sizes from 300 to 74 μm, indicating that both treatments affected carcass yield. The increased carcass yield by AMP supplementation and by reducing particle sizes observed in this study is likely due to the effect of the treatment improving muscle (breast and leg) yield, although this is not measured in this study. Wang et al. (2010) reported that supplementation of AMP at the same concentration of this study did not affect carcass yield. The increased carcass yield by AMP supplementation and by reducing particle sizes indicated both treatments increased the solubility and thereby increased the absorption of AMP bioactive compounds upon ingestion. The particle size was regarded as a vital factor that influences the release of active constituents contained in phytoenetic feed additives used as feed additives (Gião et al., 2009a) and reduction of particle sizes has been demonstrated as an effective method to increase poorly dissoluble drugs. Zhao et al. (2010) also found that solubility and utilization of bioactive compounds in AMP were improved by reducing AMP particle sizes from 300 to 37 μm. This is likely due to the increase of contact surface area of particles with digestion medium as the particle size decreased, which increased the solubility and thereby increased the absorption of AMP bioactive compounds upon ingestion.

**Effect of AMP Processed to Different Particle Sizes on Serum Antioxidant Status and Metabolites**

The higher activities of TSOD and GSHPx but lower MDA concentration in the serum of broilers supplemented with AMP compared with that of controls indicated that AMP supplemented at the dietary concentration of 5 g/kg of diet enhanced serum antioxidant enzymatic activity and improved antioxidant status of broilers. Other researches have also demonstrated the improvement of antioxidant status by supplementation of AMP to the diet of broilers (Wang et al., 1994; Toda and Shirataki, 1999; Lee et al., 2003; Hei et al., 2005). The enhanced antioxidant enzyme activity and antioxidant status of broilers by AMP is likely due to various bioactive compounds in AMP, including 2,4-dihydroxy-5,6-dimethoxyisoflavone, kumatakenin, beta-saponins, and flavones (Cho and Leung, 2007; El-Kenawy, 2010). These compounds have been reported to possess various biological and pharmaceutical activities such as antioxidant and free radical scavenging functions (Feng et al., 1997; Ding and Wu, 2003; Li et al., 2009; Wang et al., 2010). Further research is needed to assess the effects of these AMP bioactive compounds on antioxidant enzyme activity and antioxidant status of broilers.

This is the first study that showed a linear increase of antioxidant enzyme activity but linear decrease of MDA concentration in the serum with the reduction of AMP particle sizes from 300 to 37 μm. This is likely due to the increase of contact surface area of particles with ingestion. The particle size was regarded as a vital factor that influences the release of active constituents contained in phytoenetic feed additives used as feed additives (Gião et al., 2009a) and reduction of particle sizes has been demonstrated as an effective method to increase poorly dissoluble drugs. Zhao et al. (2010) also found that solubility and utilization of bioactive compounds in AMP were improved by reducing AMP particle sizes from 300 to 37 μm. This increase of solubility and absorption would enhance the efficacy of effective compounds in AMP exerting their effects on improving antioxidant status of broilers observed in this study. Zhang et al. (2009a) also found a linear improvement in the serum antioxidant status of broilers with reducing the particle size of ginger root from 300 to 37 μm. The increased contents of TP, albumin, and globulin in the serum of broilers by inclusion of AMP and by reducing AMP particle sizes indicated both treatments...

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>AMP300</th>
<th>AMP149</th>
<th>AMP74</th>
<th>AMP37</th>
<th>SEM</th>
<th>Control vs. AMP (P)</th>
<th>Effect of particle size (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein, g/L</td>
<td>21 d</td>
<td>28.0c</td>
<td>28.2c</td>
<td>28.9bc</td>
<td>29.8ab</td>
<td>30.7a</td>
<td>0.33</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>30.0bc</td>
<td>29.9b</td>
<td>30.8b</td>
<td>32.0a</td>
<td>32.8a</td>
<td>0.29</td>
<td>0.053</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>21 d</td>
<td>13.6b</td>
<td>13.6b</td>
<td>13.6b</td>
<td>14.5b</td>
<td>14.8a</td>
<td>0.19</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>15.3b</td>
<td>15.3b</td>
<td>15.3b</td>
<td>16.4a</td>
<td>16.7a</td>
<td>0.10</td>
<td>0.113</td>
</tr>
<tr>
<td>Globulin, g/L</td>
<td>21 d</td>
<td>14.5b</td>
<td>14.6b</td>
<td>15.4ab</td>
<td>15.4ab</td>
<td>16.0a</td>
<td>0.45</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>14.8bc</td>
<td>14.6c</td>
<td>15.6ab</td>
<td>15.4ab</td>
<td>16.4a</td>
<td>0.29</td>
<td>0.078</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>21 d</td>
<td>3.25a</td>
<td>3.09b</td>
<td>3.07b</td>
<td>3.00c</td>
<td>3.03bc</td>
<td>0.019</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>42 d</td>
<td>2.86a</td>
<td>2.81b</td>
<td>2.79b</td>
<td>2.80b</td>
<td>2.76c</td>
<td>0.010</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a–cMeans within a row with different letters differ (P < 0.05).

Data are means of 4 replicate cages of 2 chicks per cage.

AMP300, AMP149, AMP74, or AMP37 represents that AMP processed to particle sizes of 300, 149, 74, or 37 μm, respectively, and all were supplemented at a dose of 5 g/kg of diet.

Comparison of the control with pooled AMP treatments.
affected protein metabolism, which is consistent with the observation of enhanced serum antioxidant enzyme activity and increased carcass yield. The increased serum concentration of globulin and albumin as well as enhanced antioxidant status may also be indicative of enhanced immune system as the serum concentration of these 2 proteins antioxidant status are regarded as the direct reference to the body immune function (Zhou et al., 1994). Cho and Leung (2007) reported that effective compounds isolated from AMP improved body immune function in vitro and in vivo (Cho and Leung, 2007). It needs to be pointed out, however, that the enhanced antioxidant status and protein metabolism by AMP did not affect broilers’ growth performance. It therefore appears that response of serum antioxidant status and metabolites to dietary AMP supplementation is more sensitive than that of growth performance in broilers.

In conclusion, addition of AMP processed to particle sizes of 300 to 37 μm to a broiler diet at a dose of 5 g/kg of diet improved serum antioxidant status, but had no effect on growth rate, feed intake, or feed conversion rate. The efficacy of antioxidant-enhancing effect of AMP was linearly increased by reducing its particle sizes from 300 to 37 μm. The results indicate that AMP is a potential source of antioxidant additives to broiler chicken.

REFERENCES


