Effect of Chinese Herbal Medicine on Growth Performance, Immune Organ Index and Antioxidant Functions in Broiler Chickens

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Abstract

This study was conducted to investigate the effect of five different Chinese herbal medicine separated supplements on growth performance, immune organ index and antioxidant functions in broilers. One-day-old male Arbor Acres broilers (n=420) were equally allotted to seven treatment groups: basal diet (control) group or basal diet with additional 0.1% Monensin or one kind of Chinese herbal medicine (Cynanchum atratum; CA; Radices Paeoniae Alba; RPA; Morus alba L.; MAL; Astragalus membranaceus; AM; or Eucommia ulmoides Oliver; EUO). The results show that CA significantly increased the average weight gain and bursa index and decreased the average daily feed intake and feed/meat ratio (F/G) (P < 0.05), which was similar to that mediated by Monensin. Superoxide dismutase (SOD) concentration in kidney had a increasing tendency in the CA treatment group compared with control (P > 0.05). While MAL supplementation increased the F/G and decreased the survival rate and SOD levels in liver and kidney (P < 0.05). Growth performance was not significantly altered with RPA, AM and EUO supplement to the diet (P > 0.05). Whereas, EUO treatment significantly reduced SOD concentration in heart and liver (P < 0.05), and RPA treatment decreased the SOD concentration in heart and kidney (P < 0.05). In conclusion, these data suggest that the CA is a beneficial Chinese herbal medicine to improve the growth performance in broilers and can be used as an antibiotic alternative in poultry industry. © 2018 Friends Science Publishers

Keywords: Chinese herbal medicine; Cynanchum atratum; Growth performance; Antibiotic

Introduction

In the poultry industry, broilers can be threatened by heat stress and infectious diseases that impair growth performance and immunity (Han et al., 2010; Hoerr, 2010; Khan et al., 2012). Generally, antibiotics were widely used to improve growth performance and feed utilization efficiency. However, the extensive use of antibiotics in animal production leads to a variety of problems (Li et al., 2016). Therefore, the use of antibiotic substitutes has been considered to be an efficient means of improving performance in the poultry industry (Willis et al., 2007; Zhang et al., 2009; Khan et al., 2012).

Chinese herbal medicine (CHM) is a rarity of traditional medicine (Olmedo et al., 2014; Otoni et al., 2014). A series of studies indicated that the CHM can improve the immune efficacy and increase growth performance due to their antioxidant activity (Chen et al., 2013; Kallon et al., 2013; Cao et al., 2014; Kirkpinar et al., 2014), while scarce data exist describing these effects. Zhang et al. (2007) reported that dairy cows fed a diet supplemented with a Chinese herbal formula increased their milk yield by 14%. Supplementation with CHM mixture in the diet of laying hens significantly increased egg production rate and the superoxide dismutase levels under heat stress conditions (Diao et al., 2012).

Nevertheless, currently, no research has been conducted in broiler chickens to investigate the efficacy of adding single Chinese herbal medicine, such as Cynanchum atratum (CA), Radices Paeoniae Alba (RPA), Morus alba L. (MAL), AM and Eucommia ulmoides Oliver (EUO), which are widely used due to their functions in enhancing immunity (Chang et al., 2015; Wang et al., 2016; Choi et al., 2017; Hong et al., 2018). Therefore, it is essential to utilize different herbal resources as feed ingredient and understand their alone potential economic value in broiler production. The objective of this study was to research the effects of these CHM on growth performance, survival rate, immune organ index and superoxide dismutase concentrations in tissues in broiler chickens.

Material and Methods

Experimental Animals

A total of 420 healthy one-day-old male Arbor Acres (AA) broiler chicks were reared in the same environmentally controlled room. The temperature was maintained at 35°C (65% relative humidity) for the first 2 days and then gradually reduced to 30°C on day 7 and 26°C on day 21,
after which animals were maintained at room temperature. The light regime was 24 h illumination. All chicks received a starter diet with 21.5% crude protein and 12.33 MJ/kg of metabolizable energy (Zhao et al., 2009). All broilers had free access to food and water during the rearing period. The experimental procedures were approved by the Institutional Animal Care and Use Committees in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (Beijing, China).

### Treatment

The 420 broilers were randomly divided into 7 treatment groups, each of which included 3 replicates of 20 birds from 1 day of age. The experimental chicks were given a typical basal diet, supplemented with 0.1% CA, RPA, MAL, AM and EUO, respectively (shown in Table 1). All prepared CHM were dried, crushed and sifted through # 60 mesh and processed in conformity with national drug standards. The basal diet was fed to the control group as a negative control and an antibiotic group (supplemented with 0.1% Monensin, Mon) was used as a positive control. Dietary nutrient levels were based on National Research Council recommended nutrient requirements for broiler chickens (Yang et al., 2009), which was shown in Table 2. On day 1, 14, 21, 35 and 42, body weight and feed consumption for every group were recorded, and the average feed intake, weight gain and feed/gain ratio (F/G) were calculated. Deaths and culls in each pen were recorded daily.

### Sample Collection and Assays

On day 42, the chickens were deprived of feed for 12 h and weighted, and two broilers were randomly selected from each replicates and sacrificed. Hearts, spleens, bursa of fabricius and thymus were obtained at the time of slaughter, and then they were weighted and stored at -20°C until analyzed. The superoxide dismutase (SOD) concentration in heart, liver and kidney were measured with detection kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer’s protocol.

### Statistical Analysis

The data were presented as the mean ± SEM. The homogeneity among the treatments were confirmed using Bartlett’s test. All of the data were subjected to a one-way ANOVA analysis to test the main effect of the treatment (n = 6). P < 0.05 was considered statistically significant.

### Results

#### Survival Rate

There was no significant difference between CHM group, control and Mon group on survival rate during the experiment in broilers (Table 3, P > 0.05).
Table 3: Survival rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>CA</th>
<th>RPA</th>
<th>MAL</th>
<th>AM</th>
<th>EUO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0 to 14</td>
<td>96.82±0.02\textsuperscript{a}</td>
<td>98.34±0.05\textsuperscript{a}</td>
<td>100.00±0.01\textsuperscript{a}</td>
<td>100.00±0.10\textsuperscript{b}</td>
<td>97.0±0.09\textsuperscript{b}</td>
<td>96.7±0.09\textsuperscript{b}</td>
<td>100.00±0.01\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Abbreviation: Mon, Monensin; CA, Cynanchum atratum; RPA, Radices Paoniea Alba; MAL, Morus alba L.; AM, Astragalus membranaceus; EUO, Eucommia ulmoides Oliver

Table 4: Performance of Arbor Acres broilers fed diets containing different CHM

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Mon</th>
<th>CA</th>
<th>RPA</th>
<th>MAL</th>
<th>AM</th>
<th>EUO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0 to 14</td>
<td>15.57±0.83\textsuperscript{c}</td>
<td>29.64±0.29\textsuperscript{c}</td>
<td>27.08±0.24\textsuperscript{c}</td>
<td>25.60±0.51\textsuperscript{bc}</td>
<td>23.91±0.67\textsuperscript{c}</td>
<td>24.94±0.51\textsuperscript{cd}</td>
<td>26.55±0.21\textsuperscript{bc}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>d 15 to 28</td>
<td>21.75±0.74\textsuperscript{ab}</td>
<td>34.73±0.39\textsuperscript{a}</td>
<td>33.57±0.31\textsuperscript{a}</td>
<td>32.17±1.20\textsuperscript{b}</td>
<td>32.18±0.83\textsuperscript{a}</td>
<td>33.17±1.28\textsuperscript{b}</td>
<td>33.32±1.08\textsuperscript{a}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F/G</td>
<td>1.40±0.03\textsuperscript{a}</td>
<td>1.17±0.01\textsuperscript{c}</td>
<td>1.24±0.02\textsuperscript{c}</td>
<td>1.26±0.02\textsuperscript{bc}</td>
<td>1.35±0.01\textsuperscript{ab}</td>
<td>1.33±0.00\textsuperscript{ab}</td>
<td>1.26±0.05\textsuperscript{bc}</td>
<td>0.0008</td>
</tr>
<tr>
<td>d 29 to 42</td>
<td>48.58±2.13\textsuperscript{c}</td>
<td>67.68±1.43\textsuperscript{ab}</td>
<td>68.87±1.74\textsuperscript{c}</td>
<td>65.51±1.69\textsuperscript{bc}</td>
<td>61.77±1.67\textsuperscript{b}</td>
<td>64.29±2.53\textsuperscript{b}</td>
<td>64.58±1.81\textsuperscript{bc}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ADGI (g/d)</td>
<td>69.41±2.64\textsuperscript{a}</td>
<td>98.93±1.77\textsuperscript{a}</td>
<td>99.46±1.86\textsuperscript{a}</td>
<td>93.10±1.81\textsuperscript{b}</td>
<td>92.89±1.38\textsuperscript{a}</td>
<td>92.74±1.11\textsuperscript{a}</td>
<td>93.04±1.34\textsuperscript{b}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F/G</td>
<td>1.43±0.01\textsuperscript{b}</td>
<td>1.46±0.02\textsuperscript{b}</td>
<td>1.44±0.02\textsuperscript{b}</td>
<td>1.42±0.02\textsuperscript{b}</td>
<td>1.50±0.02\textsuperscript{a}</td>
<td>1.44±0.01\textsuperscript{b}</td>
<td>1.44±0.02\textsuperscript{b}</td>
<td>0.0856</td>
</tr>
<tr>
<td>Overall (d 0 to 42)</td>
<td>67.38±1.18\textsuperscript{c}</td>
<td>89.76±1.85\textsuperscript{a}</td>
<td>88.91±2.60\textsuperscript{a}</td>
<td>84.56±1.48\textsuperscript{a}</td>
<td>76.48±3.56\textsuperscript{b}</td>
<td>82.10±2.55\textsuperscript{b}</td>
<td>82.74±2.14\textsuperscript{a}</td>
<td>0.0071</td>
</tr>
</tbody>
</table>
| Values are the means of 3 replicates of 20 chickens. Data presented as mean ± SE (n = 6). Means within a line with different letters differ significantly (P < 0.05). Abbreviation: Mon, Monensin; CA, Cynanchum atratum; RPA, Radices Paoniea Alba; MAL, Morus alba L.; AM, Astragalus membranaceus; EUO, Eucommia ulmoides Oliver

Table 6: Effect of CHM supplementation on immune organ index of broilers at 42 days (g/kg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen index</th>
<th>Thymus index</th>
<th>Bursa index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.34±0.04\textsuperscript{a}</td>
<td>2.65±0.29\textsuperscript{a}</td>
<td>1.20±0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>Mon</td>
<td>1.28±0.14\textsuperscript{ab}</td>
<td>3.26±0.32\textsuperscript{a}</td>
<td>1.64±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>RPA</td>
<td>1.35±0.03\textsuperscript{ab}</td>
<td>2.82±0.24\textsuperscript{b}</td>
<td>1.63±0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>MAL</td>
<td>1.12±0.11\textsuperscript{ab}</td>
<td>2.60±0.24\textsuperscript{a}</td>
<td>1.76±0.10\textsuperscript{a}</td>
</tr>
<tr>
<td>AM</td>
<td>1.23±0.11\textsuperscript{ab}</td>
<td>2.33±0.24\textsuperscript{a}</td>
<td>1.78±0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>EUO</td>
<td>1.33±0.09\textsuperscript{ab}</td>
<td>3.23±0.37\textsuperscript{a}</td>
<td>1.97±0.18\textsuperscript{a}</td>
</tr>
<tr>
<td>P value</td>
<td>0.1302</td>
<td>0.1934</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Table 5: Effect of CHM supplement on immune organ index of broilers at 42 days (g/kg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.29±0.20\textsuperscript{a}</td>
<td>7.62±0.31\textsuperscript{a}</td>
<td>7.83±0.21\textsuperscript{a}</td>
</tr>
<tr>
<td>Mon</td>
<td>7.50±0.20\textsuperscript{a}</td>
<td>7.74±0.36\textsuperscript{a}</td>
<td>8.10±0.27\textsuperscript{a}</td>
</tr>
<tr>
<td>CA</td>
<td>6.78±0.41\textsuperscript{ab}</td>
<td>6.79±0.22\textsuperscript{a}</td>
<td>7.73±0.43\textsuperscript{a}</td>
</tr>
<tr>
<td>RPA</td>
<td>6.18±0.43\textsuperscript{ab}</td>
<td>7.34±0.29\textsuperscript{a}</td>
<td>7.72±0.53\textsuperscript{a}</td>
</tr>
<tr>
<td>MAL</td>
<td>6.64±0.40\textsuperscript{ab}</td>
<td>5.74±0.30\textsuperscript{a}</td>
<td>6.27±0.43\textsuperscript{a}</td>
</tr>
<tr>
<td>AM</td>
<td>7.55±0.35\textsuperscript{a}</td>
<td>7.84±0.27\textsuperscript{a}</td>
<td>7.55±0.45\textsuperscript{a}</td>
</tr>
<tr>
<td>EUO</td>
<td>5.10±0.42\textsuperscript{b}</td>
<td>5.81±0.25\textsuperscript{b}</td>
<td>5.62±0.34\textsuperscript{a}</td>
</tr>
<tr>
<td>P value</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

In conclusion, 0.1% CA supplementation in broiler dietary could get the similar effect to that of Monensin, which could contribute to the substitution or decrement of antibiotics in

**Immune Organ Index**

Compared with control, dietary supplemented with 0.1% CA, RPA, MAL, and EUO had no effect on spleen index and thymus index (Table 5, P > 0.05), but significantly increased the bursa index (Table 5, P < 0.05).

**SOD Concentrations in Heart, Liver and Kidney**

The effect of CHM supplementation on SOD concentrations in heart, liver, and kidney were shown in Table 6. Compared with control group, 0.1% Mon and AM supplementation increased while EUO supplementation decreased the SOD concentrations in heart (P < 0.05); 0.1% MAL and EUO supplementation decreased the concentration of SOD in liver (P < 0.05); 0.1% RPA and MAL supplementation decreased the SOD concentration in kidney (P < 0.05); 0.1% CA supplementation had no significant effect on SOD concentrations in heart, liver and kidney (P > 0.05).

**Discussion**

The effect of CHM on growth performance, immune organ index and SOD concentration in broilers are somewhat variable due to the varieties and ingredients of CHM themselves. Overall, relative to the control diet, 0.1% CA supplementation significantly increased ADG by 40.56% and decreased the F/G by 25.58%, and it had no difference on survival rate, immune organ index and SOD concentration in heart, liver and kidney. It appears that CA supplementation in control diets could effectively improve the growth performance of broiler chickens, which is similar to that of Monensin supplementation.
broiler production. Future studies should evaluate the mechanisms underlying enhanced growth performance by CA in broilers.

**Effect of CHM on Performance of Broiler Chicken**

In recent years, CHM feed additives have attracted much interest as a natural growth supplements in poultry production (Li et al., 2016), and CHM-supplemented dietary increased the growth rate of animals (Guo et al., 2008; Chen et al., 2009). The present study found that 0.1% CHM additive increased the ADG and ADFI on days 0-42 when compared with the control diet, due to the oxidative equilibrium from CHM (Li et al., 2016). It showed that *Cynanchum atratum* additive increased the overall ADG and had no effect on ADFI on days 0-42, leading to the decrease of F/G. While RPA, MAL, AM and EUO increased overall ADFI and F/G. It seems that CHM diets improves the health status of broilers, which therefor increases the growth performance. It is possible that the growth-stimulating effects of CHM may be related to the dosage or supplementation level in the diets.

**Effect of CHM on Immune Organ Index**

Immune organs consist of the central immune organs (such as thymus and bursa) and peripheral immune organs (such as spleen). The former are the places of mature lymphocytes and the latter are the primary venues for the immune response (Madej et al., 2015). Thymus mainly affects cell-mediated immunity, while the bursa and spleen participate in humoral immunity. Therefore, the status of immune organs can determine immune function, but the preliminary indicators of immune organs are measured by the immune organ index and immune organ weight (Jiang et al., 2010). In our study, 0.1% CHM and Monensin additive had no effect on spleen and thymus index, while the all increased the bursa index, especially EUO, whose immune-enhancement effect had been identified (Wang et al., 2013). It is generally suggested that the bioactive components of CHM were saponins, antioxidants, peptides, polysaccharides and alkaloids, which are believed to have immune-stimulatory effect (Jo et al., 1995).

**Effect of CHM on SOD Level in Tissues**

Heat stress can reduce immune functions and induce oxidative injury (Mahmoud and Edens, 2003; Lin et al., 2008), while oxidative stress occurs when the subtle balance between the oxidation and antioxidant system is interfered in cells (Chauhan et al., 2014), and further affects poultry health and performance (Rhoads et al., 2013; Monson et al., 2017). As one of the major antioxidant enzymes in the antioxidant defense system, SOD ubiquitously exists innumerable cells of animals and is critical for protecting the cell against the toxic products of aerobic respiration (Perry et al., 2010; Yang et al., 2017; Zhang et al., 2017). Previous studies reported that AM was able to protect against lipid peroxidation by binding to SOD in the body in order to increase its activity (Ma et al., 2013; Song et al., 2013; Lv et al., 2015) and AM injection is able to attenuate oxidative stress (Xu et al., 2002; Gao et al., 2004; Zhou et al., 2014). In the present study, AM additive increase the SOD concentration in heart, which was consistent with previous study, as well as CA. However, MAL and EUO reduced SOD levels in liver and RPA and MAL decreased SOD concentration in kidney. While the regulatory mechanism is unclear and remains to be studied at the molecular level, these results suggested that CHM such as CA and AM, were able to protect against oxidation in broiler chickens.

Noteworthy is the fact that the Chinese herbs from different regions and seasons have different efficacies and contents of active substances. So far, it is difficult to evaluate the efficacy and supervise quality as no uniform quality standards and standard formula. Thus, further research seems to be essential to standardize methods for their extraction. Several Chinese herbs are usually used together to obtain a pharmacological effect, thereby it is extremely difficult to attribute the effect to a particular herb (Liu et al., 2011; Gao et al., 2015; Xu et al., 2015; Xie et al., 2017). Thus, it is very important to note that it is a need to define a systematic research strategy that may be suitable for evaluating the Chinese herbs. While this present study have contributed to the development of research strategy on Chinese herbal. For exposed animals, much attention should be paid to adverse health effects of Chinese herbal feed additives, especially in the case of an accidental overdose. The current experimental results seem to justify the assumption that Chinese herbal medicine additives may have the potential to be a candidate of substitute for antibiotic to promote production performance and productivity of broiler chicken.

**Acknowledgments**

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