Biological Function of Berberine and Its Effects on Growth and Immune Functions of Broilers

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Abstract
Berberine has many biological functions including antimicrobial, antiparasitic, antioxidant and anti-inflammatory, which can help to prevent and treat intestinal infections of livestock and poultry, maintain the balance of oxidation and reduction in the body, which is of great significance to improve the production performance of animals. In this paper, the authors analyze the biological functions of berberine and its effects on growth and immune functions of broilers. Adding astragalus polysaccharide to the feed improved the spleen index, increased the number of lymphoid nodules in cecum tonsil and thus improved the immunity of broilers. The results showed that dietary berberine significantly increased the levels of endogenous insulin and glucagon, and significantly decreased the levels of adrenocorticotropic hormone and blood sugar in broilers. Therefore, berberine shiyogn can increase the daily feed intake, daily gain of broilers and improve the performance of broilers. At the same time, the experiment also showed that adding berberine could improve the immune organ index of broilers in varying degrees, but the effect was not as obvious as adding astragalus polysaccharide.

Key words: Berberine, Broiler, growth, Immune functions, Infectious diseases, Drug resistance

1. Introduction
The massive use of antibiotics in animal production has led to extensive bacterial resistance. Natural active substances have a variety of biological functions and with no residues, not easy to cause bacterial resistance and have other advantages, which are favored by drug researchers[1]. Berberine, also known as berberine hydrochloride (C20H18CLNO4) belongs to isoquinoline alkaloids[2]. Berberine has multiple biological functions, such as anti-inflammation, anti-oxidation, anti-microbial, anti-aging, anti-cancer and so on. It is widely used in clinical medicine and animal production[3,4]. The biological functions of berberine, such as antibacterial, antioxidant, anti-inflammatory, hypoglycemic and lipid-lowering, and its application in animal production were analyzed to provide the theoretical basis for its further research and application[5].
Berberine has bacteriostatic effect on many Gram-positive bacteria and Gram-negative bacteria. It has definite therapeutic effect on intestinal infections, such as gastroenteritis and bacterial dysentery[6,7]. In recent years, berberine has also been found to have anti-tumor effect, therapeutic effect on intestinal diseases, diabetes mellitus and acute lung injury[8,9]. Studies have shown that berberine can enhance the immunity, feed intake and daily gain of broilers. Astragalus polysaccharides, as the main component of active substances of Astragalus membranaceus, have obvious immune regulation, anti-viral, anti-bacterial and other effects. Moreover, have some other advantages, including less toxic side effects, no residues, no drug resistance, etc. It has been widely used in livestock and poultry production[10]. The effects of berberine and Astragalus Polysaccharide on broiler production have been reported, but there are few studies on the effects of combination of berberine and Astragalus Polysaccharide on broiler performance[11]. This study focused on the effects of berberine and Astragalus Polysaccharide on the growth performance and immune functions of AA broilers, and provided the scientific basis for the application of berberine and Astragalus Polysaccharide in broiler production.

2. Biological functions of Berberine

2.1 Antibacterial effect
Berberine has a wide range of bacteriostatic and bactericidal effects. Peng's study found that berberine plays a bactericidal role by destroying the structure of cell membrane of Streptococcus agalactiae by inhibiting the synthesis of protein and DNA. Berberine can also reduce the secretion of virulence factors of bacteria, making the pathogenicity of bacteria reduced[12,13]. In addition, berberine can also destroy the biofilm of methicillin-resistant Staphylococcus aureus (MRSA) by inhibiting the aggregation of phenol soluble regulatory proteins (PSMs) into amyloid fibers, thus enabling other combined antibiotics to further kill MRSA[14]. At present, berberine is mostly used in combination. Due to increased serious problem related to bacterial resistance and multi-drug resistance in veterinary clinic, effective combination of drugs can reduce the emergence of bacterial resistance and improve the effectiveness of clinical treatment of antibiotics. The minimum inhibitory concentration (MIC) of berberine against chicken-derived Escherichia coli ranged from 375 ug/ml to 1 000 ug/ml[15]. When berberine was combined with Astragalus polysaccharide, the antimicrobial activity of berberine increased by 2-8 times. In addition, some studies have shown that berberine itself has low antimicrobial activity against MRSA, but when combined with fusidic acid (FA), berberine has synergistic antimicrobial activity against clinically isolated MRSA, the MIC is between 0.19 and 0.5[16,17].

![Figure 1. Structure of Berberine](image)

2.2 Antioxidant Activity
Berberine has good antioxidant activity, mainly by scavenging free radicals and activating the corresponding antioxidant pathways. Siow study found that berberine can scavenge free radicals by destroying free radical groups and nitric oxide (NO), scavenging highly active peroxynitrates and hydroxyl radicals.

Other studies have shown that berberine can restore superoxide dismutase (SOD) activity and prevent superoxide anion production in endotoxin-induced cell and animal models[18]. Li et al. found that berberine could inhibit oxidative stress-induced apoptosis by up-regulating the expression of phosphorylated protein kinase (p-Akt) and B lymphoma-2 (Bcl-2) protein and down-regulating the expression of B-cell lymphoma factor-related protein (Bax) and caspase-3 protein. Other studies have confirmed that berberine can activate the pathway of nuclear factor E2 related factor 2 (Nrf2)/heme oxygenase (HO-1), activate endogenous antioxidant capacity and inhibit oxidative stress injury induced by hydrogen peroxide in mouse myoblasts[19].

2.3 Anti-inflammatory effect
Berberine has obvious anti-inflammatory effects and its mechanisms have been widely studied. Its anti-inflammatory mechanism mainly involves regulating the expression of inflammatory related factors. Wang et al. found that berberine can improve collagen-induced inflammatory response by inhibiting the expression of TNF-a, IL-6, IL-17, vascular endothelial growth factor (VEGF) and highly glycosylated type I transmembrane glycoprotein (CD34). Recent studies have found that berberine has a good anti-inflammatory effect by...
regulating the balance between helper T (Th17) cells and regulatory T (Treg) cells. In addition, berberine can also regulate the corresponding pathways to play an anti-inflammatory effect. Studies have shown that berberine has anti-inflammatory effects on endotoxin-induced macrophages and mouse models by activating the cross-talk between adenylate-activated protein kinase (AMPK) and Nrf2 pathway. Zhang et al. confirmed that berberine could significantly inhibit the transforming growth factor (TGF)-beta, transforming growth factor kinase 1 (TAK1)/JNK and TAK1/NF-κB signaling pathways, and improve the inflammatory response after abdominal surgery in rats.

![Figure 2. Antioxidant and Anti-Inflammatory Activities of Berberine](image)

2.4 Hypoglycemia and Lipid Regulation

In recent years, many studies have shown that berberine can regulate glycolipid metabolism. This activity has been confirmed by clinical, animal and in vitro cell tests. Qiu Hongmei et al. confirmed that berberine can regulate blood lipid and anti-angiosclerosis by inhibiting the expression of inducible nitric oxide synthase (iNOS) and type B scavenger receptor (SR-B1) genes in high-fat rabbit model. Berberine may reduce plasma endotoxin level in diabetic rats induced by high-sugar and high-fat diet combined with streptozotocin (STZ), improve insulin resistance and play a hypoglycemic role by improving intestinal flora structure. Recent studies have found that berberine can improve insulin tolerance by inhibiting the activity of M1 macrophages in adipose tissue, thereby regulating glycolipid metabolism in vivo.

![Figure 3. Hypoglycemic and lipid regulation](image)

3. Experimental Design

3.1. Experimental diets

200 healthy broilers with similar body weight were randomly divided into four groups with 50 broilers in each group. There was no significant difference among groups (P=0.871). A group was the control group, fed with basal diet; B group was fed with 0.02% berberine along with basal diet; C group was fed with 0.2% Astragalus Polysaccharide along with basal diet; D group was fed with 0.02% berine + 0.2% Astragalus polysaccharide along with basal diet. The feeding period was 50 days.
3.2. Feeding management

Two hundred one-day-old broiler chickens were raised in cages until 7 days of age. Then 160 healthy broilers with similar body weight were selected as experimental chickens and reared in groups until 50 days of age. The chicken house was well ventilated, temperature, humidity, light and other conditions were controlled according to the feeding regulations and immunization was carried out according to the routine procedure.

3.3. Measurement index and method

At the age of 7 days, the fasting weight of broilers was measured. At the end of the experiment, the weight of broilers was measured after 12 hours of fasting (free drinking water). During the experiment, feed intake and leftovers were recorded in each repetitive group, the average daily gain, daily feed intake and feed-weight ratio were calculated. At the end of the experiment, three chickens of nearly average body weight were randomly selected from each repetitive group. Blood samples (10 mL) were collected from the subwinged vein. Serum was separated and stored at a temperature of 20°C for the determination of serum immunoglobulin content. After blood collection, broilers were slaughtered, led to bleed, spleen, thymus, bursa of Fabricius and other organs were taken out, adipose tissue was removed and fresh weight was quickly weighed for the calculation of immune organ index. Serum immunoglobulin content was determined by immunoturbidimetry and operated according to the kit instructions (Shanghai Kehua Bioengineering Co., Ltd.). The formula for calculating immune organ index is as follows: immune organ index = fresh weight of immune organ (g)/fasting weight of broiler (kg).

3.4 Statistical Analysis of Data

The test results were expressed as "mean (±) standard deviation". Single factor analysis of variance was performed by SPSS 19.0 statistical analysis software and multiple comparisons were made by LSD method.

4. Results

4.1. Changes in body weight and food intake

Table 1 shows that the weight of 50-day-old broilers in each experimental group is significantly higher than in the A group. The weight of 50-day-old broilers in B group was the heaviest, which was significantly higher than that in C group (P < 0.05). The daily gain of broilers in the B group was the highest, significantly higher than that in the A group, significantly higher than that in the C group, and there was no significant difference with the D group (P < 0.01). The daily intake of the D group was the highest, which was significantly higher than that of the A group and the three groups, and there was no significant difference between the B group (P < 0.05). There was no significant difference in feed weight ratio between groups.

4.2 Immune organ index

Table 2 shows that the spleen index, thymus index and bursa index of the three groups are the highest, and the three indexes of the A group are the lowest; the spleen index of each experimental group is significantly higher than that of the A group (P < 0.01), the C group was significantly higher than that of the B groups (P < 0.05), and there is no significant difference between the C group and the D group, between the D group and the B group (P > 0.05); the thymus index of the C group is significantly higher than that of the B group (P < 0.05), and there is no significant difference between the four groups (P < 0.05). There were significant differences among the C group > D group > B group > A group (P < 0.01).

4.3 Serum immunoglobulin

Table 3 shows that the levels of serum IgG, IgA and IgM in C and D group are significantly higher than those in A and B groups (P < 0.01). There are no significant differences among three and four groups (P > 0.05). The levels of serum IgX in group I are significantly higher than those in B group (P(0.05). The levels of serum IgG and IgA in A group and B groups are not significantly different (P > 0.05). Therefore, the addition of Astragalus Polysaccharide in feed can improve the content of serum immunoglobulin.

Table 1. Effects on broiler performance

<table>
<thead>
<tr>
<th>group</th>
<th>After 50 days weight</th>
<th>Daily weight gain (g)</th>
<th>Daily feed intake (g)</th>
<th>Feed-weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2146.71±61.24</td>
<td>47.12±2.05</td>
<td>113.34±0.84**</td>
<td>2.54±0.06</td>
</tr>
<tr>
<td>Group B</td>
<td>2516.46±174.16*</td>
<td>54.71±2.91*</td>
<td>115.62±18.74**</td>
<td>2.45±0.41</td>
</tr>
<tr>
<td>Group C</td>
<td>2940.24±257.06</td>
<td>52.14±3.45*</td>
<td>113.41±2.46**</td>
<td>2.40±0.22</td>
</tr>
<tr>
<td>Group D</td>
<td>2484.34±215.62*</td>
<td>53.15±4.01**</td>
<td>125.15±3.54**</td>
<td>2.41±0.17</td>
</tr>
</tbody>
</table>
Note: * indicated significant difference between groups (P < 0.05), ** indicated significant difference between groups (P < 0.01).

### Table 2. Effects on immune organ index of Broilers

<table>
<thead>
<tr>
<th>Group</th>
<th>Splenic index (g/kg)</th>
<th>Thymus index (g/kg)</th>
<th>Bursa index (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.41±0.07</td>
<td>3.45±0.26</td>
<td>1.40±0.05</td>
</tr>
<tr>
<td>Group B</td>
<td>1.58±0.12*</td>
<td>3.57±0.61</td>
<td>1.58±0.08</td>
</tr>
<tr>
<td>Group C</td>
<td>1.61±0.11</td>
<td>3.81±0.12</td>
<td>1.62±0.05**</td>
</tr>
<tr>
<td>Group D</td>
<td>1.54±0.13*</td>
<td>3.74±0.43</td>
<td>1.69±0.11**</td>
</tr>
</tbody>
</table>

### Table 3. Effect of Serum Immunoglobulin in Broilers

<table>
<thead>
<tr>
<th>Group</th>
<th>IgG(g/L)</th>
<th>IgA(g/L)</th>
<th>IgM(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2.64±0.07</td>
<td>1.20±0.15</td>
<td>1.87±0.18</td>
</tr>
<tr>
<td>Group B</td>
<td>2.69±0.08*</td>
<td>1.16±0.07*</td>
<td>1.62±0.14</td>
</tr>
<tr>
<td>Group C</td>
<td>2.74±0.06*</td>
<td>1.51±0.12</td>
<td>1.81±0.12</td>
</tr>
<tr>
<td>Group D</td>
<td>2.71±0.09</td>
<td>1.54±0.14*</td>
<td>1.79±0.08</td>
</tr>
</tbody>
</table>

5. Discussion

Berberine has the biological functions of antimicrobial, antiparasitic, antioxidant and anti-inflammatory, which can help prevent and treat intestinal infections of livestock and poultry, maintain the redox balance in the body, which is of great significance to improve the production performance of animals. Researchers found that adding berberine to the diet could significantly increase the levels of endogenous insulin and glucagon, significantly reduce the levels of adrenocorticotropic hormone and blood sugar, increase the daily feed intake and daily gain of broilers, and improve the performance of broilers. Reducing fat deposition in livestock and poultry farming is helpful to improve the quality and nutritional content of meat products. It is also of great significance to control the high risk of obesity in today's society. Researchers such as Li Liangxiao found that berberine could reduce the liver fat rate and intramuscular fat content of leg muscle, decrease the contents of triglyceride in breast muscle and leg muscle, and regulate the lipid metabolism of broilers by increasing the insulin content in serum and the activity of liver lipase.

Berberine can promote the release of endogenous nitric oxide in rat intestinal mucosal vascular endothelial cells, mediate the endothelium-dependent relaxation of intestinal mucosal microvessels, improve local intestinal microcirculation, prevent and treat intestinal diseases, thereby improving the growth performance of rats. According to Sun Zhenping's research, adding high doses of berberine in feed can significantly increase the levels of endogenous insulin and glucagon, significantly reduce the levels of corticotropin and blood sugar, increase feed intake and daily gain of broilers and improve the performance of broilers. The effects of Astragalus Polysaccharides on broilers’ performance were different. Wei believed that the effect of Astragalus Polysaccharide on broiler performance depended on the feeding environment and the amount of additives. Adding less than 0.6% astragalus polysaccharide and under better feeding and management conditions, the effect of Astragalus Polysaccharide on broiler performance was not significant. Research showed that adding crude extract of Astragalus membranaceus could significantly promote the growth of broilers and could significantly increase the body weight and daily gain of broilers. The results showed that 0.02% berberine could significantly increase the body weight, daily gain and feed intake of AA broilers at 50 days of age. Adding 0.2% astragalus polysaccharide could also increase the body weight, daily gain and feed intake of AA broilers at 50 days of age, but the effect was not as obvious as adding 0.02% berine. The effect of berberine + Astragalus Polysaccharide on growth performance of broilers has not been reported yet. This study found that the effect of berberine + Astragalus Polysaccharide on growth performance of broilers and the effect of adding berberine alone were not significant, but higher than that of adding Astragalus Polysaccharide alone.

Spleen, thymus and bursa of Fabricius are important immune organs of poultry and they are important organs for poultry to exert immune function. Their weight is closely related to the immunity of chickens. Immune organ index is the ratio of immune organ weight to chicken weight and it is an important parameter to measure the immune function of chickens. The addition of Astragalus Polysaccharide in feed could improve the spleen index of broilers, increase the number of lymphatic nodules in cecum tonsil, and thus improve the immunity of laying hens. It was found that Astragalus polysaccharide could improve the immune organ index of broilers at later stage of the experiment. The results also showed that adding astragalus polysaccharides to the diet could significantly improve the immune organ index of broilers, which was consistent with the above results. At the same time, the experiment also showed that adding berberine could improve the immune organ
Adding berberine and Astragalus Polyascharide in diet could significantly improve spleen index and thymus index, but there was no statistical difference between the effect of adding astragalus polysaccharide alone and the effect of adding berberine and Astragalus Polyascharide in diet.

6. Conclusion
Adding berberine and astragalus polysaccharide to the diet of broilers at the same time can increase the 50-day-old body weight and daily gain of broilers, shorten the feeding cycle of broilers with the same weight, significantly improve the immune index and serum immunoglobulin content of broilers and improve the disease resistance in broilers. In conclusion, berberine can improve animal growth performance, meat quality and disease resistance by exerting various biological functions such as antimicrobial, antioxidant, anti-inflammatory, regulating sugar metabolism and controlling fat synthesis. However, its application in animal production needs further study.

References