Effects of *Moringa Oleifera* Leaf Polysaccharide on Growth Performance and Immune Response of Broiler Chickens

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Research Article

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Abstract

*Moringa oleifera* (MO) is a widely used as the nutritious and non-traditional feed supplementation containing kinds of bioactive substances. However, the enhancement effect of *Moringa oleifera* leaf Polysaccharide (MOLP) as a feed additive in broilers growth performance and immunity remains unclear. In this study, MOLP was obtained by water extraction and alcohol precipitation method, then purified with Trichloroacetic acid (TCA) assay. Chickens were randomly divided into 4 groups, to receive different doses of MOLP (0, 0.1, 0.2, 0.4g/kg) in feed for 3 weeks. The body weight gain (BWG) and feed consumption were recorded for feed conversion ratio (FCR) and average daily feed intake (ADFI) calculation. Broiler chickens were sacrificed and sampled on day 14, 21, 28 (D 14, D 21, and D 28) respectively. Serological indicators, including total protein (TP), albumin (ALB), globulin (GLO), and creatinine (CREA) were detected. ELISA kits were applied for detecting the levels of immunoglobulin A (IgA), immunoglobulin G (IgG), interleukin-2 (IL-2), and tumor necrosis factor (TNF-α). From D 21 to D 28, the results showed that middle dose of MOLP signicantly increased BWG and ADFI as well as liver and bursa indexes when compared with the control group. In addition, TP and GLO were also increased ($P<0.05$). All MOLP treatments enhanced the serum concentrations of IgG and IL-2 ($P<0.01$). Furthermore, results of quantitative RT-PCR showed that high dose of MOLP treatment significantly increased ($P<0.001$) the mRNA expression levels of IL-2 and TNF-α of chickens relative to the control group. In conclusion, the results showed that MOLP supplementation contributed to improve growth performance and immune response in broiler chickens, and MOLP could be considered as a promising feed additive.

Introduction

*Moringa Oleifera* (MO) is a native plant from South Asia, South Western Africa, Madagascar and Arabia, and belongs to the family Moringaceae (Alnidawi et al., 2016; Anwar et al., 2017; Fahey et al., 2005; Moyo et al., 2016). MO is now used as a crucial nutraceutical and medicinal herb because of its abundant phenolic compounds and polysaccharides. *Moringa Oleifera* leaves (MOL) are evidenced as the most utilized part (Leone et al., 2015). MOL supplemented diets could enhance the growth performance in broilers poultry (Abdulsalam et al., 2015). In recent years, some studies reported that the benefits of plant-derived bioactive compounds including polysaccharides have attracted increasing attention due to their potential biological properties, which can improve growth performance, enhancing immune response, and intestinal health in poultry production (Long et al., 2020; Wu, 2018; Zhang et al., 2014).

*Acanthopanax senticosus* polysaccharide (ASP) is reported to strengthen cellular and humoral immune responses by modulating the production of immunocytes, cytokines, and antibodies (Kong et al., 2007). Additionally, ASP has antioxidant (Zhao et al., 2013), and anti-inflammatory (Han et al., 2016) activities. *Lycium barbarum* polysaccharide (LBP) is promoting the expression of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) proteins in rat serum, when mixed in the feeds as an additive (Ren et al., 2017). Meanwhile, *enteromorpha algae*-derived polysaccharide (ADP) exertion significant effects on anti-inflammation against microbial pathogens, digestive functions, immunological regulation, and free-radical scavenging effects (Duan et al., 2015; Du et al., 2019; Guo et al., 2020).
Serum immunoglobulins are considered as important indicators of an animal's immune functions (Wang et al., 2017). Some studies confirm that the usage of Moringa leaf can improve the immunoglobulins including total protein (TP), albumin (ALB), and globulin (GLO), while enhance the metabolism of protein in the chicken organs (Bush, 1991; Melesse et al., 2013; Sirvydis et al., 2006). The improvement of immune status may be because of antioxidant activities of some components of MOL like the capacity of plants extracts to modulate the immune system (Dong et al., 2007; Diallo et al., 2009). Generally, pro-inflammatory cytokines like interleukin-2 (IL-2), interferon-γ (IFN-γ) and TNF-α genes, mediate immune response in chickens as they pose potential effects on systemic inflammatory status. However, the potential effects of MOL polysaccharide (MOLP) on the avian growth performance and above-mentioned pro-inflammatory cytokines have not been investigated.

Therefore, the objective of the present study to evaluate the effects of MOLP supplementation on poultry production and immune response, and the possibility to be as an effective feed additive for chickens.

**Materials And Methods**

Moringa oleifera leaf polysaccharide extract preparation

MOL was obtained from the Yunnan Ruziniu Biotechnology, China. They were dried and ground as 75g powder then boiled with 1000 mL ddH$_2$O for 2 h, and repeated boiled powder for 2 times. All the supernatants were collected, mixed and evaporated using the rotary evaporator until the supernatant decreased to a proper volume 400 mL. The polysaccharides were precipitated by added slowly Ethanol 95% into the concentrated solution until the alcohol content reached 80%, then stored and incubated at 4°C overnight, after that, washed with absolute ethyl alcohol, acetone, petroleum ether, acetone, ethyl alcohol, respectively. Then dissolved in 500 mL ddH$_2$O and boiled until the supernatant concentrated to 200 mL, collected and freeze-dried at -108°C overnight, deproteinated with Trichloroacetic acid (TCA) assay. Freeze-dried again, then the content of polysaccharide was determined by phenol-sulfuric method (Nkukwana et al., 2014). The final product was kept in a dry place for further use.

Animals and Preparation of house

One-day-old Chinese yellow broiler chickens were obtained from the Institute of Poultry Science, Chinese Academy of Agricultural Sciences. And brood under standard conditions for 7 days before commencement of the study. Birds were housed in individual cages with proper lighting, heat and free access to feed and water. All animal experiments were performed in accordance with the guidelines approved by the Animal Care and Use Committee of Yangzhou University, China.

**Experimental Design**

60 chickens were randomly divided into 4 groups with 15 birds in each group. A total of 15 birds were placed in one cage. All chickens, except for those in the control group, were fed with MOLP. Groups MOLP-
L (low dose), MOLP-M (middle dose), and MOLP-H (high dose) fed with 0.1, 0.2, 0.4 g/kg (feed weight) respectively, from day 7 and lasting for 3 weeks.

Average daily feed intake (ADFI) and feed Conversion Ratio (FCR)

The feed was weighed every day and divided by the number of chickens in each group and totalized to be per week to determine the ADFI. FCR was calculated by dividing the amount of feed consumed (g) with body weight gained (g), following is the formula:

Feed Intake = Introduced parts of food – Residual parts of food

FCR = Feed intake (g) /chick / week ÷ Body weight gain (g) /chick/ week

Blood Samples and Contents Collection

On day 14, 21 and 28, five chickens were randomly selected from each replicate cage of all the groups (15 chickens per group) for each time point, respectively. Blood samples were collected from the jugular vein and centrifuged at 2000 rpm/10 min. After centrifugation, serum was stored at -20°C for further analysis. The viscera were removed and separated, and the organs (bursa, spleen, liver) were collected and placed into micro tubes in a samples box with liquid nitrogen. All the samples were stored at -80°C for further detection.

Determination of Serum Immunoglobulin and Cytokine Levels by ELISA

The serum levels of IgA, IgG, IL-2, and TNF-α were measured using ready-to-use sandwich ELISA kits according to the manufacturer’s instructions (Biosamite Biotechnology Co., Ltd., Shanghai, China).

RNA isolation, cDNA synthesis and qRT-PCR

The mRNA expression levels of IL-2, IFN-γ and TNF-α in thymus were determined by the quantitative RT-PCR.

Total RNA was extracted from each thymus sample using Trizol reagent, according to the manufacturer’s instructions. After measuring the concentration and purity, first-strand cDNA was synthesized according to the Hifair® III1st strand cDNA synthesis SuperMix (11123ES). Quantitative RT-PCR was performed on the CFX96™ connect real-time PCR system (Bio-Rad, USA) by using SYBR® Green Master Mix Kit (Cat No.11137ES). In addition, 18 sRNA was selected as an internal control (housekeeping gene). Results of genes are presented relative to the control average values. The specific primer sequences for target genes were listed in Table 1.

Statistical Analysis

Data are expressed as mean ± standard error of the mean. Statistical analyses were performed by one-way ANOVA test in GraphPad Prism (version 7.0). Values of P<0.05 stand for a significant difference.
Results

Growth Performance

The content of polysaccharides of MOLP was 71.7%. Effects of MOLP on growth performance of broilers at different phases are presented in Table 2. From D 14 to D 28, there were no significant difference ($P>0.05$) between MOLP-L and MOLP-H. However, a significant increment in BW and ADFI was noted in MOLP-M group ($P<0.05, P<0.01$) compared with birds in the control group.

The values of AWG and FCR are shown in Table 3. For the FCR, from D 14 to D 28, there were slight changes in all three MOLP groups, although these changes were not significant ($P>0.05$) relative to the control. However, for the AWG from D 21 to D 28, there was a significant increment in MOLP-M group ($P<0.05$) compared with the control group.

The results suggested broilers fed with MOLP-M (0.2 g/kg) had superior feed efficiencies.

Effects of MOLP on relative organs weight

The effects of MOLP supplementation on relative organs weight (bursa, spleen and liver) at different phases are presented in Table 4. From D 14 to D 21, among the treated groups of MOLP, bursa and spleen weights were also slightly different. However, these changes were not significant ($P>0.05$). While, at D 14, there were significant differences ($P<0.01$) in MOLP-M group on liver weight; at D 28, a significant increment ($P<0.01$) on bursa weight could also be observed in the same group relative to the control group.

Blood biochemical analysis

With the increasing dose of MOLP, levels of TP and GLO showed slight changes in MOLP groups, although these changes were not significant ($P>0.05$) at D 14. Whereas, from D 21 to D 28, a significant increase ($P<0.05$) of TP and GLO levels could be found in MOLP-M group. However, there were no significant difference ($P>0.05$) of TP and GLO levels between MOLP-L and MOLP-H groups.

Additionally, from D 14 to D 28, the values of ALB, ALB/GLO and CREA in each group of MOLP were not significant ($P>0.05$) relative to the control group (Table 5). These results indicated that MOLP has no obvious side-effects to chickens.

Serum Concentrations of Immunoglobulin and Cytokine

From D 14 to D 28, the values of IgA among the treated groups of MOLP were not significant ($P>0.05$). While, at D 21, a significant increase of IgG ($P<0.05$) could be found in MOLP-H group. Meanwhile, the IL-2 levels were increased ($P<0.05, P<0.01, P<0.001$) in MOLP-L, MOLP-M and MOLP-H groups, respectively. However, the levels of TNF-α were not remarkably altered by different doses of MOLP compared to that of the control ($P>0.05$) (Fig. 1-4).
The mRNA expression levels of IL-2, IFN-γ and TNF-α in thymus

Quantitative RT-PCR analysis showed that IL-2 and TNF-α mRNA expressions were dramatically up-regulated ($P<0.001$, $P<0.0001$) in MOLP-H group from D 21 to D 28. Meanwhile, no significant alternatives ($P>0.05$) could be observed in the levels of IFN-γ mRNA expression in three MOLP groups relative to that of the control (Fig. 5-7).

Discussion

*Moringa oleifera* proved to be a valuable plant that is useful in broiler chickens feed formulation for cost reduction and enhancing the growth performance. In this study, supplementation of 0.2 g/kg (MOLP-M) could improve BW, AWG, and ADFI of chickens (Tables 2 and 3). This increment might be as the result of the nutritional properties (Carbohydrate, especially dietary fibers). Similarly, higher BW is also recorded by Khan et al. (2017), who used MOL powder as a dietary supplement at 1.2% levels in broilers. In line with this, MOL supplemented diets enhanced the growth performance at finisher period (Abdulsalam et al., 2015). In addition, birds in the current study, gave better FCR than the control group, which means better returns on investment. This assertion was similar to Banjo (2012) who reported that inclusion of MOL meal at 1, 2 and 3% in the diet did not significantly enhance FCR.

The relative weights of bursa and liver were obviously increased ($P<0.05$) in MOLP-M group (0.2 g/kg), indicating that MOLP improved the immune status and health condition in birds. This result is in agreement with a previous research on bursa relative weights in MOL meal diet (Nkukwana et al., 2014). However, spleen relative weights were not significant ($P>0.05$) in all MOLP treatment groups compared to the control. Inconsistent with our results, Ayo-Ajasa et al. (2016) and Alabi et al. (2017) got the significant data in MOL meal.

Our study showed that MOLP-M increased levels of TP and GLO, reflecting a more intensive metabolism of the protein in the chicken organs (Sirvydis et al., 2006). Also, the increase in TP may be due to the increase in GLO level. However, the decrease in TP always due to low level of ALB (Bush, 1991). This result is in agreement with Melesse et al. (2013) who noticed that inclusion of MOL meal in the diets significantly ($P<0.05$) influenced the most serum biochemical parameters of chickens.

Plant extracted natural polysaccharides supplementation is found to improve host immune functions (Li et al., 2018; Okwari et al., 2013). In our study, a high dose of MOLP increased concentrations of IgA, IgG and cytokine levels of IL-2, TNF-α in serum, as well as mRNA expression levels of IL-2, IFN-γ and TNF-α in thymus, which might account for the beneficial effects of MOLP on the health status and leading to enhance antibody production. Similar to previous researches by Qian (2019) and Zhang et al. (2014), who observed that MOL was effective in immune modulating activities through the activation of macrophages or lymphocytes to generate nitric oxide and promote cytokine secretion. Consequently, these results implied MOLP improved the immune response of broiler chickens.
Conclusion

This present study showed that MOLP supplementation contributed to improve BW gain and feed efficiency in chickens, as well as the immune status. These findings support a recommendation of supplementation with MOLP as an effective and beneficial feed additive for chickens.

Declarations

Acknowledgment

This work was funded by National Natural Science Foundation of China (32072911) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Ethics approval

This study was conducted according to the guidelines approved by the Animal Care and Use Committee of Yangzhou University, China

Research Data Policy and Data Availability

The data set is not available for public access.

Financial support statement

This research was funded by National Natural Science Foundation of China (32072911) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References


115, 90-97.


Tables
### Table 1. List of Primer sequences used for qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′- 3′)</th>
<th>Product (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 sRNA</td>
<td>F TGCTGTGTTCCCATCTATCG</td>
<td>148 bp</td>
</tr>
<tr>
<td></td>
<td>R TTGGTGACAATAACCGTGTCCA</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>F GCTAATGACTACAGCTTATGGAGCA</td>
<td>135 bp</td>
</tr>
<tr>
<td></td>
<td>R TGGGTTCAGTTGTGTGTAGAG</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>F ATCATACTGAGCCAGATTGTTTCG</td>
<td>140 bp</td>
</tr>
<tr>
<td></td>
<td>R TCTTTCAACCTTCCTCAGCCAT</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>F AGATGGGAAGGGGAATGAACC</td>
<td>268 bp</td>
</tr>
<tr>
<td></td>
<td>R CAGAGCATCAACGCAAAG</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

The effect of MOLP on body weight of chickens

<table>
<thead>
<tr>
<th>Groups</th>
<th>Con (Initial BW)</th>
<th>MOLP - L (0.1 g/kg)</th>
<th>MOLP - M (0.2 g/kg)</th>
<th>MOLP - H (0.4 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 d-old</td>
<td>149.02 ± 6.20</td>
<td>148.29 ± 6.01</td>
<td>153.94 ± 6.02</td>
<td>146.28 ± 4.57</td>
</tr>
<tr>
<td>21 d-old</td>
<td>199.61 ± 5.81a</td>
<td>227.80 ± 10.35ab</td>
<td>240.28 ± 8.08a</td>
<td>214.72 ± 9.28b</td>
</tr>
<tr>
<td>28 d-old</td>
<td>280.04 ± 17.83b</td>
<td>311.40 ± 15.36ab</td>
<td>344.42 ± 10.97a</td>
<td>253.90 ± 15.18c</td>
</tr>
</tbody>
</table>

*Note: values in a column with different superscripts (a–c) were significantly different (P<0.05)*

Table 3

The effects of MOLP on ADFI, AWG and FCR of chickens

<table>
<thead>
<tr>
<th>Groups</th>
<th>14-d-old</th>
<th></th>
<th>21-d-old</th>
<th></th>
<th>28-d-old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>MOLP-L (0.1 g/kg)</td>
<td>MOLP-M (0.2 g/kg)</td>
<td>MOLP-H (0.4 g/kg)</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>422.84</td>
<td>449.74</td>
<td>456.15</td>
<td>443.88</td>
<td>16.18</td>
<td></td>
</tr>
<tr>
<td>AWG (g)</td>
<td>87.60</td>
<td>87.14</td>
<td>92.81</td>
<td>85.15</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td>AFCR</td>
<td>4.82</td>
<td>5.16</td>
<td>4.91</td>
<td>5.21</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>382.00c</td>
<td>467.34ab</td>
<td>474.51a</td>
<td>397.01b</td>
<td>23.74</td>
<td></td>
</tr>
<tr>
<td>AWG (g)</td>
<td>138.19c</td>
<td>166.65ab</td>
<td>179.15a</td>
<td>153.59b</td>
<td>8.78</td>
<td></td>
</tr>
<tr>
<td>AFCR</td>
<td>2.76</td>
<td>2.80</td>
<td>2.64</td>
<td>2.58</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>309.40b</td>
<td>334.78ab</td>
<td>379.95a</td>
<td>244.35c</td>
<td>22.69</td>
<td></td>
</tr>
<tr>
<td>AWG (g)</td>
<td>170.92c</td>
<td>250.25ab</td>
<td>333.45a</td>
<td>192.77b</td>
<td>36.28</td>
<td></td>
</tr>
<tr>
<td>AFCR</td>
<td>1.81</td>
<td>1.51</td>
<td>1.00</td>
<td>1.26</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

Note: values in a column with different superscripts (a–c) were significantly different (P<0.05)

Table 4

The effects of MOLP on organ indexes of chickens

<table>
<thead>
<tr>
<th>Groups</th>
<th>Con</th>
<th>MOLP-L (0.1 g/kg)</th>
<th>MOLP-M (0.2 g/kg)</th>
<th>MOLP-H (0.4 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-d-old</td>
<td>Bursa</td>
<td>0.734 ± 0.074</td>
<td>0.529 ± 0.046</td>
<td>0.554 ± 0.073</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.242 ± 0.413</td>
<td>0.167 ± 0.020</td>
<td>0.218 ± 0.028</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4.563 ± 0.464&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.963 ± 0.179&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.400 ± 0.234&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>21-d-old</td>
<td>Bursa</td>
<td>0.940 ± 0.122</td>
<td>0.976 ± 0.224</td>
<td>1.273 ± 0.378</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.335 ± 0.047</td>
<td>0.282 ± 0.025</td>
<td>0.335 ± 0.041</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>6.188 ± 0.154</td>
<td>7.481 ± 0.736</td>
<td>8.166 ± 1.602</td>
</tr>
<tr>
<td>28-d-old</td>
<td>Bursa</td>
<td>1.382 ± 0.204&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.653 ± 0.163&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.736 ± 0.157&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.388 ± 0.020</td>
<td>0.425 ± 0.036</td>
<td>0.470 ± 0.043</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>7.251 ± 0.588</td>
<td>7.602 ± 0.222</td>
<td>7.477 ± 0.442</td>
</tr>
</tbody>
</table>

Note: values in a column with different superscripts (a–c) were significantly different (P<0.05)


Table 5

The effects of MOLP on Blood biochemical analysis
<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>MOLP-L (0.1 g/kg)</th>
<th>MOLP-M (0.2 g/kg)</th>
<th>MOLP-H (0.4 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-d-old TP (U/L)</td>
<td>39.8 ± 2.14</td>
<td>42.0 ± 3.31</td>
<td>41.4 ± 6.41</td>
<td>42.2 ± 4.54</td>
</tr>
<tr>
<td>ALB (U/L)</td>
<td>14.6 ± 2.40</td>
<td>12.0 ± 1.74</td>
<td>12.7 ± 3.47</td>
<td>13.4 ± 2.78</td>
</tr>
<tr>
<td>GLO (U/L)</td>
<td>25.2 ± 3.82</td>
<td>28.0 ± 2.58</td>
<td>27.7 ± 4.65</td>
<td>28.8 ± 3.55</td>
</tr>
<tr>
<td>ALB/GLO</td>
<td>0.7 ± 0.17</td>
<td>0.3 ± 0.45</td>
<td>0.4 ± 0.61</td>
<td>0.5 ± 0.12</td>
</tr>
<tr>
<td>CREA(µmol/L)</td>
<td>22.0 ± 2.54</td>
<td>21.4 ± 2.18</td>
<td>21.6 ± 2.58</td>
<td>21.1 ± 1.92</td>
</tr>
<tr>
<td>21-d-old TP (U/L)</td>
<td>34.83 ± 2.65</td>
<td>33.83 ± 1.07</td>
<td>33.16 ± 0.75</td>
<td>32.00 ± 0.30</td>
</tr>
<tr>
<td>ALB (U/L)</td>
<td>14.63 ± 0.29</td>
<td>14.06 ± 0.56</td>
<td>14.53 ± 0.31</td>
<td>13.76 ± 0.24</td>
</tr>
<tr>
<td>GLO (U/L)</td>
<td>18.63 ± 0.46²</td>
<td>19.76 ± 0.76²ab</td>
<td>26.20 ± 2.53¹a</td>
<td>18.23 ± 0.53²b</td>
</tr>
<tr>
<td>ALB/GLO</td>
<td>0.66 ± 0.06</td>
<td>0.73 ± 0.03</td>
<td>0.80 ± 0.00</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>CREA(µmol/L)</td>
<td>13.06 ± 1.31</td>
<td>15.56 ± 0.99</td>
<td>14.06 ± 1.13</td>
<td>11.40 ± 0.95</td>
</tr>
<tr>
<td>28-d-old TP (U/L)</td>
<td>34.93 ± 2.02²b</td>
<td>33.33 ± 1.08³c</td>
<td>43.46 ± 6.27¹a</td>
<td>35.60 ± 0.50²ab</td>
</tr>
<tr>
<td>ALB (U/L)</td>
<td>14.83 ± 0.88</td>
<td>15.06 ± 0.31</td>
<td>15.50 ± 0.40</td>
<td>13.46 ± 0.53</td>
</tr>
<tr>
<td>GLO (U/L)</td>
<td>20.10 ± 1.76</td>
<td>18.26 ± 0.80</td>
<td>20.10 ± 0.78</td>
<td>20.00 ± 5.98</td>
</tr>
<tr>
<td>ALB/GLO</td>
<td>0.74 ± 0.07</td>
<td>0.83 ± 0.03</td>
<td>0.78 ± 0.04</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td>CREA(µmol/L)</td>
<td>18.40 ± 9.67</td>
<td>16.20 ± 3.48</td>
<td>16.16 ± 1.96</td>
<td>17.46 ± 3.21</td>
</tr>
</tbody>
</table>

Note: values in a column with different superscripts (a–c) were significantly different (P<0.05)


**Figures**

**Figure 1**

Effect of MOLP on IgA concentration (A) 14 day, (B) 21 day, (C) 28 day after treatments. P>0.05 treatments vs control.
Figure 2

Effect of MOLP on IgG concentration (A) 14 day, (B) 21 day, (C) 28 day after treatments. *P<0.05 treatments vs control.

Figure 3

Effect of MOLP on IL-2 concentration (A) 14 day, (B) 21 day, (C) 28 day after treatments. *P<0.05, **P<0.01, ***P<0.001 treatments vs control.

Figure 4

Effect of MOLP on TNF-α concentration (A) 14 day, (B) 21 day, (C) 28 day after treatments. P>0.05 treatments vs control.

Figure 5
Effect of MOLP on relative mRNA expression levels of IL-2 gene in thymus tissue, (A) 14 day, (B) 21 day, (C) 28 day after treatment. ***P<0.001, ****P<0.0001 treatments vs control.

Figure 6

Effect of MOLP on relative mRNA expression levels of IFN-γ gene in thymus tissue, (A) 14 day, (B) 21 day, (C) 28 day after treatment. P>0.05, treatments vs control.

Figure 7

Effect of MOLP on relative mRNA expression levels of TNF-α gene in thymus tissue, (A) 14 day, (B) 21 day, (C) 28 day after treatment. *P<0.05, ***P<0.001 ****P<0.0001 treatments vs control.