Effect of Pidotimod, Astragalus and Eachnicea on Immune Response and Growth Performance of Broiler Chicks

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Abstract

This experiment was done to investigate the effects of dietary Pidotimod, Astragalus and Eachnicea supplementation in water on blood analysis, immune response, growth performance and histopathological changes of broiler chicks. A total of 200 healthy one day-old Cobb chicks were divided into 4 treatment; dietary treatment groups were; control, Eachnicea, Pidotimod and Astragalus. The results revealed that, Pidotimod, Astragalus and Eachnicea treatment highly increased the growth performance and final body weight as compared to control as well as the immune response at 21 and 35 days. Histopathological investigation of lymphoid organs; bursa Fabricius, thymus and spleen showed the normal structure of lymphoid follicle proliferation and hyperplasia of lymphocytes in all the supplemented groups. It could be concluded that, Pidotimod, Astragalus and Eachnicea has immunostimulant with a good growth promotion in poultry farming.

Keywords: Pidotimod; Astragalus; Eachnicea; Immunostimulant; Broiler chicks

Introduction

Immunomodulators are substances which act on the host immune system and produce effect either increasing or decreasing the immune responses of the host. It also could be defined as, biological immunoregulators which act as drug leading to non-specific stimulation of immune system defence mechanisms [1].

Astragalus polysaccharide (APS) possesses main components such as mannose, D-glucose, D-galactose, xylose, and L-arabinose. This polysaccharide is used as an immunomodulating agent in mixed herbal decoctions to treat common cold, diarrhea, fatigue, and anorexia [2]. It can also stimulate cell proliferation, induce the expression of surface antigens on lymphocytes, and affect the expression of cytokines and promote the production of antibodies [3].

Pidotimod (Polimod®) is a synthetic dipeptide molecule which acts as a biological response modifier (BRM) with biological and immunological activity on both the adaptive and the innate immune responses [4,5].

The objectives of this work were to evaluate the effects of a commercial supplement including pidotimod, Echinacea and Astragalus as potential immunomodulation and growth promoters through evaluating their effects on: Broiler growth performance; some hematological and biochemical parameters.

Materials and Methods

Experimental design

The present experiment was conducted for 6 weeks. The chicks were assigned randomly into four groups (each of 50 chicks). All groups were kept under the same conditions and received the same management procedures and vaccination program, the feed and water were offered ad libium [6]. The time schedule for adding the feed supplement is detailed in following Table 1.

Growth performance parameters

Bodyweight/week: Average body weight of each group was determined weekly by weighting whole chicks of each group. The total weight (g) was divided on a number of weighted chicks (average body weight/chick).

Weight gain/week: The average weekly gain in body weight was calculated through the difference between body weights of each two successive weeks for each group.

Feed intake/week: The diets offered regularly three times at 6 am, 2 and 10 pm daily. The feed intake (g) was calculated weekly per bird by the difference between the weight of offered feed and the remained portion, then divided by the number of birds in each group per week and calculated to be per day.

Feed conversion ratio (FCR)/week: Feed conversion ratio was calculated as the unit of feed consumed per unit of body weight gain.

Total feed efficiency: FE=Total body weight gain (g)/Total feed intake (g).

Blood sampling

In the 35th day of age, two blood samples were collected from the wing vein (brachial vein) six birds from each group; About 5 mL of blood was collected from each bird into two sets of sterilized labelled sample tubes, one containing heparin and other tube without anti-coagulant. The blood samples with heparin were immediately used in the evaluation of different biochemical parameters. The sera was collected from the second set of tubes (without anti-coagulants) through, leaving blood to clot at room temperature then centrifuged for 10 minutes at 3000 rpm. The sera was collected in Eppendorf tubes and stored at -20°C to be used in the evaluation of different biochemical parameters.

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Differential leukocyte count (Diff.LC)

Blood films prepared and stained with Giemsa. 100 cells were counted, then, the percent of eosinophils, lymphocytes, basophils, heterophils and monocytes were recorded [7]. The heterophils/lymphocytes (H/L) ratio was calculated by dividing the number of heterophils by the number of lymphocytes [8].

Serum biochemical studies

Liver and kidney function: Aspartate and alanine aminotransferase (AST and ALT) were quantitatively estimated according to the method described by Reitman and Frankel [9], creatinine and uric acids were determined according to the methods of Caraway [10] and Young [11] respectively. These parameters were spectrophotometrically assayed using semi-automated spectrophotometer (Erba-Chem7, Germany) and using commercial kits purchased from (Spectrum, Cairo, Egypt).

Lipid profile: Triglycerides, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were spectrophotometrically assayed using semi-automated spectrophotometer (Erba-Chem7, Germany) and by using diagnostic reagent kits (Spectrum, Cairo, Egypt) as described by Kannan et al. [12].

Serum protein electrophoresis: The serum protein electrophoresis was performed according to Laemmli [13].

Immunological studies

Haemagglutination inhibition test: At 28 and 42 days, six serum samples were collected from each group humeral immune response was investigated by detecting serum antibody titer against ND by hemagglutination inhibition test as described by Shahir et al., Alexander and Chettle [14,15].

Determination of interleukin-6: This is a quick ELISA assay test is measured according to Nishimoto and Kishimoto, Dowlati et al. [16,17].

Determination of tumor necrosis factor: The test was drawn according to Dowlati et al. [17].

Statistical analysis

The obtained data were statistically analyzed by variance method (ANOVA) considering P<0.05 using MiniTab170 (Mini Tab 17, 2010) software. The significant differences were taken to Duncan multiple range tests to compare the means.

Results and Discussion

In the modern poultry industry production, go to broad application of immunomodulatory is a recent practical method for beneficial effect and correction of immune system by using immunostimulating agent, most of them play their role in intensifying the immune system by increasing T-cell immunity, decreasing or blocking the suppressor activity, stimulating the Natural Killer cells (NK cells) and interferon production as well as inducing specific cytokine production by activated target cells which enables the body to help itself, so, people start to realize the importance of healthy immune system and its popular in the worldwide natural health industry [18,19,20,21].

In the present study, the Pidotimod had the best effect on the body performance that showed a significant increase in the final body weight and total weight gain as well as a significant reduction in total FCR and total feed intake as compared to the Eachnicea and Astragalus-supplemented groups (Table 2). The proper effect of Pidotimod on the broiler performance mainly originated from the presence of a balanced mixture of vitamins, especially vitamin E and amino acids in its structure which cause a significant elevation of nitrogen utilization and amino acid digestibility and activation of intestinal villus epithelial cells [22]. Also, Liu Xianyong et al. [23] reported that, the middle Pidotimod dosage group is better than all the other groups in weight gain and survival rate.

The supplementation of Genix Eachnicea improved the final live body weight and weight gain. This result was in agreement with the findings [24-26]. This improvement could be attributed to the involvement of Eachnicea in some biological functions such as its role in increase of protein utilization by the cells. The reduction in feed intake by Eachnicea reflected on FCR due to the direct relationship between feed intake and FCR. This result was in agreement with the findings [27-29] who reported that, the EP extract significantly lowered the FCR in broilers. Also, this result agreed with Nasir and Grashorn [30] who stated that, the active ingredients and phenolic compounds present in the fermented juice of EP extract has an active effect on the enzymes and microflora in the digestive tract. The obtained results from Astragullus supplemented group showed a significant increase in total body weight and weight gain. In general, the improvements in feed efficiency and the positive responses of broilers body performance characteristics to Astragalus may be attributed to the essential oils, the active ingredients of Astragalus which stimulates the digestive system to increase the production of digestive enzymes and also improves the utilization of digestive products[31,32] revealed the Astragalus improve the nutrient digestibility in broiler due to enhancement of trypsin and amylase enzymes which improve feed intake and FCR, promoting a better sedimentation of muscle protein, so, improves the live body weight.

Concerning the effect of addition of studied Pidotimod, Astragalus and EP on the hematological parameters in 35 days (Table 3), showed a significant increase in total leukocyte count (TLC) and lymphocyte percentage as well as a non-significant changes (P<0.05) in heterophil percent, monocyte, heterophile, eosinophile and significant reduction in heterophil: lymphocyte (H/L) index when compared to the control. Regarding to the Eachnicea supplemented group, these results agreed with Dehkordi and Fallah [33] who reported that, there was a significant increase in TLC and lymphocytes and with EP suspension for six weeks at 0.5%. Furthermore, [34] reported that, the addition of phytochemical feed additive resulted in a significant (P<0.05) increase in the total leukocyte count than the other treatments. Wang et al. [2] reported that the Astragalus has been reported to contain various bioactive compounds, including Astragalosides, flavonoids, isoflavones, isoflavan, saponins, kumatakenin, choline, betaine, polysaccharides, 

Table 1: Experimental design for adding studied supplements in water.
reported that, the dietary Eachnicea supplementation had no effect 
supplemented with Eachnicea agreed with Speranda et al. [41] who 
provides evidence for the hepato and reno-protective effects of group 
affecting by the addition of Eachnicea, Pidotimod and Astragalus to the 
4) the creatinine and uric acid levels were non-significantly (P<0.05) 
protect cell against damage caused by free radical [40].

had a protective effect on liver tissue by increasing the GSH-PX, which 
reported that, the serum activity of ALT and AST remained unchanged 
38]. This obtained result in Eachnicea agreed with Wen et al. [39] who 
examination. These observations support the findings obtained [36- 
Groups. These results refer to the hepatoprotective effect of Eachnicea, 
revealed non-significant difference between supplemented and control

and glucuronic acid, and to possess antiinflammatory, anti-aging,anti-
infarction, hepatoprotective, immunomodulating, anti-inflammatory 
and antitumor effects. Recently the Astragalus polysaccharides used 
causes a significant decrease in the serum concentration of cholesterol 
Oligosccharides(XOS) and Gamma Amino Butyric Acid (GABA).
The obtained result of Astragalus provides evidence for the hepato 
Reno-protective effects of the Astragalus as one of its components. 
These results similar to the results obtained by 44. Fasamni et al. [44] 
who reported that, the addition of Astragalus either in broiler water 
or feed had no significant effect on the uric acid and creatinine levels. 
Recently, revealed that, the essential oils had Reno-protective effect on 
the broiler chicks [45].

Regarding the lipid profile, in this experiment (Table 5), there were 
supplements on the liver and kidney functions of broiler chicks at 35 days.

Table 4: Effects of studying supplements on the liver and kidney functions of broiler chicks at 35 days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1 Control</th>
<th>G2 Eachnicea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (u/l)</td>
<td>15.54 ± 0.20a</td>
<td>16.63 ± 0.43a</td>
<td>16.55 ± 0.63a</td>
<td>15.70 ± 0.91a</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td>15.17 ± 0.34c</td>
<td>16.80 ± 1.21c</td>
<td>16.44 ± 0.45c</td>
<td>14.55 ± 0.33c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.25 ± 0.02a</td>
<td>0.24 ± 0.02a</td>
<td>0.23 ± 0.01a</td>
<td>0.25 ± 0.02a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.65 ± 0.20a</td>
<td>2.43 ± 0.15a</td>
<td>2.21 ± 0.28a</td>
<td>2.74 ± 0.08a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); n=6. Means within the same row with different
Superscripts are significantly different (P<0.05).ALT: Aspartate Aminotransferase; AST: Alanine Aminotransferase.

Table 3: Effects of studying supplements on the hematological parameter of the broiler chicks at 35 days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1Control</th>
<th>G2 Eachnicea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte (%)</td>
<td>6.33 ± 0.52a</td>
<td>5.17 ± 0.30a</td>
<td>5.50 ± 1.05a</td>
<td>5.83 ± 0.40a</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>58.67 ± 0.96b</td>
<td>63.00 ± 0.52b</td>
<td>63.16 ± 0.79b</td>
<td>62.17 ± 0.70b</td>
</tr>
</tbody>
</table>

Table 2: Effects of studying supplements on the total body performance of broilers.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1Control</th>
<th>G2 Eachnicea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (1000/ul)</td>
<td>13.17 ± 0.65c</td>
<td>19.33 ± 0.56a</td>
<td>19.17 ± 0.31a</td>
<td>16.50 ± 0.76a</td>
</tr>
<tr>
<td>Heterophil (%)</td>
<td>32.67 ± 1.09a</td>
<td>30.50 ± 0.43a</td>
<td>30.00 ± 0.52a</td>
<td>30.67 ± 0.84a</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>58.67 ± 0.96b</td>
<td>63.00 ± 0.52b</td>
<td>63.16 ± 0.79b</td>
<td>62.17 ± 0.70b</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>6.33 ± 0.52a</td>
<td>5.17 ± 0.30a</td>
<td>5.50 ± 1.05a</td>
<td>5.83 ± 0.40a</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>2.33 ± 0.21a</td>
<td>1.33 ± 0.21a</td>
<td>1.34 ± 0.21a</td>
<td>1.33 ± 0.21a</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>0.57 ± 0.03c</td>
<td>0.48 ± 0.05c</td>
<td>0.47 ± 0.06c</td>
<td>0.49 ± 0.07c</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); n=6. Means within the same row with different
Superscripts are significantly different (P<0.05).WBCs: White Blood Cells; H/L ratio: Heterophil/Lymphocyte ratio.
Oligosaccharides(XOS) [47]. Thus, the reduction of serum triglyceride, cholesterol and LDL in the concurrent study may be attributed to the presence of vitamin E and XOS in its structure.

The reductions in cholesterol, triglycerides and LDL in the group supplemented by Astragalus agreement with Amad et al. [34] who reported that, the addition of AM Flexus(as a phytogenic feed supplement) in broiler diet caused a significant reduction in the total cholesterol. Other studies conducted by explained that, the ability of phytobiotic to reduce the cholesterol level could be attributed to their direct effect on the nutrient digestion in the broiler gut, such as fat, starch or/and protein digestibility of feeds [48,49]. Moreover, cholesterol lowering property of essential oil constituents has been ascribed to suppressing of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme that is considered to be limiting cholesterol synthesis rate [50].

Blood serum proteins play roles in the maintenance of colloid osmotic pressure, as a rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis, in the transport of minerals and hormones, in building of enzymes and in the immune system of the organism. Therefore, blood serum proteins have an exceptional significance in the homeostasis maintenance (Table 6). The total concentration of blood serum proteins of birds is about the same as half its value in mammals. In mammals, it is 50-70 g/L [51], while in birds it is approximately 40 g/L [52].

Regarding to the serum protein fractionation in this study, it was observed that, in the 35 days of the experimental period, there was a significant increase (P<0.05) in the levels of total plasma protein, total globulin and total gamma-globulin in all supplemented groups as compared to the control group. While, non-significant change was observed between Echinacea,Astragalus and control groups in total beta globulin (Table 7). These results indicating improvements in hepatic functions because of the hepatoprotective effect of Echinacea purpurea owing to the antioxidant effect of its higher contents of phytophenolic compounds as previously discussed. Also, there was a significant increase in serum alpha and beta globulins in infected treated groups with Echinacea purpurea compared to control group (group1) at 1st week post infection. The significant increase in serum gamma globulins of treated (Echinacea purpurea group) group compared to the control group at 1st and 2nd weeks. However, Echinacea purpurea fermented juice improve health and immunity of the birds by improving serum globulin contents and stabilizing serum creatine kinase activities (reducing the risk of sudden death syndrome) [30] (Table 8).

Regarding to the results of serum protein fractionation fed on Astragalus compared to control. High positive effect on total

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1 Control</th>
<th>G2 Echinacea</th>
<th>G3 pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>67.84 ± 2.64*</td>
<td>37.73 ± 1.03*</td>
<td>34.30 ± 0.78**</td>
<td>32.46 ± 0.86*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>118.50 ± 0.87*</td>
<td>118.59 ± 0.25*</td>
<td>112.22 ± 0.50*</td>
<td>115.89 ± 0.74*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>64.80 ± 1.04*</td>
<td>64.63 ± 1.2°</td>
<td>76.53 ± 1.0°</td>
<td>57.26 ± 1.2°</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>65.50 ± 1.7°</td>
<td>51.36 ± 2.0°</td>
<td>34.90 ± 2.0°</td>
<td>51.11 ± 1.7°</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); n=6. Means within the same row with different superscripts are significantly different (P<0.05). HDL: High Density Lipoprotein; LDL: Low density lipoprotein.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>G2 Echinacea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>3.96 ± 0.06*</td>
<td>4.31 ± 0.11*</td>
<td>4.82 ± 0.12*</td>
<td>4.42 ± 0.05*</td>
</tr>
<tr>
<td>Total globulin (g/dl)</td>
<td>2.77 ± 0.06*</td>
<td>3.05 ± 0.07*</td>
<td>3.72 ± 0.07*</td>
<td>3.18 ± 0.06*</td>
</tr>
<tr>
<td>Total gamma-globulin (g/dl)</td>
<td>0.85 ± 0.014*</td>
<td>0.99 ± 0.03*</td>
<td>1.56 ± 0.05*</td>
<td>1.21 ± 0.04*</td>
</tr>
<tr>
<td>Total beta-globulin (g/dl)</td>
<td>0.78 ± 0.02*</td>
<td>0.83 ± 0.023*</td>
<td>1.03 ± 0.03*</td>
<td>0.90 ± 0.024*</td>
</tr>
<tr>
<td>Total alpha-globulin (g/dl)</td>
<td>1.14 ± 0.03*</td>
<td>1.24 ± 0.03*</td>
<td>1.12 ± 0.02*</td>
<td>1.07 ± 0.10*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.19 ± 0.01*</td>
<td>1.27 ± 0.06*</td>
<td>1.21 ± 0.044*</td>
<td>1.24 ± 0.04*</td>
</tr>
<tr>
<td>Albumin/Globulin ratio</td>
<td>0.43 ± 0.01*</td>
<td>0.42 ± 0.02*</td>
<td>0.33 ± 0.01*</td>
<td>0.39 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); n=6. Means within the same row with different superscripts are significantly different (P<0.05).

<table>
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<tr>
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<th>G2 Echinacea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.33 ± 0.33*</td>
<td>5.77 ± 0.31°</td>
<td>6.17 ± 0.31*</td>
<td>5.63 ± 0.31*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.17 ± 0.17°</td>
<td>5.50 ± 0.34°</td>
<td>5.50 ± 0.22°</td>
<td>5.30 ± 0.37°</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); n=6. Means within the same row with different superscripts are significantly different (P<0.05). ND: Newcastle.

<table>
<thead>
<tr>
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<th>G2 Echinacea</th>
<th>G3 Pidotimod</th>
<th>G4 Astragalus</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>35ds</td>
<td>128.68 ± 1.20°</td>
<td>144.04 ± 1.40°</td>
<td>163.14 ± 2.59°</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>35ds</td>
<td>55.52 ± 0.40°</td>
<td>66.13 ± 0.87°</td>
<td>69.71 ± 1.00°</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard error (SE); 11° superscripts are significantly different (P<0.05).
protein, total globulin and A/G ratio are suggested to be due to the immunostimulant effect of Astragalus extract in their composition. In the matter of fact, higher levels of total globulin and low albumin/ globulin (A/G) ratio could be considered as a good indicator for efficient disease resistance and immune response which agreed [34,53-56].

The result showed that, antibody titers against ND were significantly increased (Ps 0.05) at both 21 days and 28 days, this finding assent to the findings [57-59]. The antibody titers against ND were significantly increased (Ps 0.05) in astragalus-fed groups in both 21 and 35 days than in control, The obtained result was in agreement with the findings [57-59] stated that, the phytobiotic might be attributed to the stimulation of the complement receptor mediated phagocytosis. Thus, led to a significant increase in the humoral antibody titers against the ND virus, they activate the immune system [60]. On the other hand, the phytobiotic failed to create a significant improvement in the humeral immunity of broiler chicks [61-63].

IL-6 functions as a mediator for notification of the occurrence of some emergent event. IL-6 is generated in an infectious lesion and sends out a warning signal to the entire body. The signature of exogenous pathogens, known as pathogen-associated molecular patterns, is recognized in the infected lesion by pathogen-recognition receptors (PRRs) of immune cells such as monocytes and macrophages [64]. TNF-a is known to be a key mediator for the induction of apoptosis and development of humoral immune response [65]. In the present study, IL-6 and TNF-a were significant increases in all supplemented groups in comparing with the control group, while group supplied with pidotimod(G3) significantly increase than other groups at 21 and 35 days, followed by Astragalus (G4) then Eachnicea (G2) fed groups. EP has an interferon (IFN) like effect, activating macrophages and inducing the production of interleukin (IL)-1 and IFN [66]. EP has been shown to increase phagocytosis, increased cytokine production and natural killer cell activity [67-69], Tang et al. [70] found APS and GPS to promote IL-2 bioactivity. Also, these results similarly to Liu Xianyong et al. [23] who showed that at 28days of age, the dose of pidotimod of peripheral blood lymphocytes secreted interleukin-2 and gamma interferon is higher than the control and drug control group.

Conclusion

In conclusion, the present study showed that, the Pidotimod, Astragalus and Eachnicea can be considered as immunostimulant and in the same time growth promoters. So, we recommended using the pidotimod and astragalus in poultry production.

References


