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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 labium [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 days 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 for determination of the differential leukocytic count. While sera were 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 were collected in Eppendorf tubes and stored at -20°C to be used in the evaluation of different biochemical parameters.

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Time/Group	1st week of experiment	2nd week of experiment	3rd week of experiment	4th week of experiment	5th week of experiment
G1 Control	No feed additives till end of Experiment				
G2 Eachnicea	Add at 4, 5 and 6 day.	Add at 9, 10, 11, 12 and 13 day.	Add at 15, 16, 17, 19, 20 and 21 days.	Add at 22 and 23, 25, 26 and27 days.	Add at 29, 30 and 31 day.
G3 Pidotimod	Add at 4, 5 and 6 days.	Add at 9, 10, 11, 12 and 13 days.	Add at 15, 16, 17, 19, 20 and 21 days.	Add at 22 and 23, 25, 26 and 27 days.	Add at 29, 30 and 31 day.
G4 Astragalus	Add at 4, 5 and 6 days.	Add at 9, 10, 11, 12 and 13 days.	Add at 15, 16, 17, 19, 20 and 21 days.	Add at 22 and 23, 25, 26 and 27 days.	Add at 29, 30 and 31 days.

Table 1: Experimental design for adding studied supplements in water.

Differential leukocyte count (Diff.LC)

Blood films prepared and stained with Giemsa. 100 cells were counted, then, the percents 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 by 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 titers 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 MiniTab17© (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 leukocytic count (TLC) and lymphocyte percentage as well as a non-significant changes ($P<0.05$) in heterophil percent, monocyte, heterophile, esinophile 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 phytogenic 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,

Group/ Parameter	Total body performance			
	G1 Control	G2 Eachnicea	G3 Pidotimod	G4 Astragalus
Total feed intake	4033.34 ± 3.90[a]	3970.33 ± 3.43[b]	3980.94 ± 2.35[b]	3954.08 ± 3.22[b]
Final body weight	2397.1 ± 32.0[c]	2521.6 ± 33.3[b]	2826.2 ± 32.6[a]	2560.1±41.5[b]
Total weight gain	2353.86 ± 9.42[d]	2477.40 ± 10.12[c]	2782.08 ± 13.40[a]	2516.98 ±11.10[b]
Total FCR	1.7 ± 0.09[a]	1.57 ± 0.04[b]	1.38 ± 0.03[c]	1.54 ± 0.05[b]
Total feed Efficiency	0.58 ± 0.003[c]	0.62 ± 0.005[b]	0.70 ± 0.008[a]	0.64 ± 0.005[b]
Values are expressed as means ± standard error (SE); n=6.Means within the same row with different [a,b,c,d] superscripts are significantly different (P<0.05).				

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

Groups / Parameters	Differential leukocytic count			
	G1Control	G2 Eachnicea	G3 Pidotimod	G4 Astragalus
WBCs (1000/ul)	13.17 ± 0.65[c]	19.33 ± 0.56[a]	19.17 ± 0.31[a]	16.50 ± 0.76[b]
Heterophil (%)	32.67 ± 1.09[a]	30.50 ± 0.43[a]	30.00 ± 0.52[a]	30.67 ± 0.84[a]
Lymphocyte (%)	58.67 ± 0.96[b]	63.00 ± 0.52[a]	63.16 ± 0.79[a]	62.17 ± 0.70[a]
Monocyte (%)	6.33 ± 0.52[a]	5.17 ± 0.30[a]	5.50 ± 1.05[a]	5.83 ± 0.40[a]
Esinophil (%)	2.33 ± 0.21[a]	1.33 ± 0.21[a]	1.34 ± 0.21[a]	1.33 ± 0.21[a]
Basophil (%)	0.00	0.00	0.00	0.00
H/L ratio	0.57 ± 0.03[a]	0.48 ± 0.05[b]	0.47 ± 0.06[b]	0.49 ± 0.07[b]
Values are expressed as means ± standard error (SE); n=6.Means within the same row with different [a,b,c]Superscripts are significantly different (P<0.05).WBCs: White Blood Cells; H/L ratio: Heterophil/Lymphocyte ratio.				

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

Groups / Parameters	Liver and kidney functions			
	G1 Control	G2 Eachnicea	G3 Pidotimod	G4 Astragalus
ALT (u/l)	15.54 ± 0.20[a]	16.63 ± 0.43[a]	16.55 ± 0.63[a]	15.70 ± 0.91[a]
AST (u/l)	15.17 ± 0.34[a]	16.80 ± 1.21[a]	16.44 ± 0.45[a]	14.55 ± 0.33[a]
Creatinine (mg/dl)	0.25 ± 0.02[a]	0.24 ± 0.02[a]	0.23 ± 0.01[a]	0.25 ± 0.02[a]
Uric acid (mg/dl)	2.65 ± 0.20[a]	2.43 ± 0.15[a]	2.21 ±0.28[a]	2.74 ± 0.08[a]
Values are expressed as means ± standard error (SE); n=6.Means within the same row with different [a]Superscripts are significantly different (P<0.05);AST: Aspartate Aminotransferase; ALT; Alanine Aminotransferase.				

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

and glucuronic acid, and to possess antinociceptive, anti-aging,anti-infarction, hepatoprotective, immunomodulating, anti-inflammatory and antitumor effects. Recently the Astragalus polysaccharides used intensively in poultry production due to its positive effect on the growth performance and immunity. Ghasemi et al. [35] showed that, prebiotic and phytobiotic have no effect on the leukocyte count and heterophil/lymphocyte ratio.

Regarding to the liver function test in this study (Table 4) chicken supplemented with Pidotimod, Eachnicea, and Astragalus showed significant reduction in serum AST and ALT at 35 days of age revealed non-significant difference between supplemented and control groups. These results refer to the hepatoprotective effect of Eachnicea, Pidotimod and AStragalus. These results confirmed by immunological examination. These observations support the findings obtained [36-38]. This obtained result in Eachnicea agreed with Wen et al. [39] who reported that, the serum activity of ALT and AST remained unchanged at Eachnicea dietary supplementation. Also, showed that, Eachnicea had a protective effect on liver tissue by increasing the GSH-PX, which protect cell against damage caused by free radical [40].

Concerning the renal function in the current study at 35 days (Table 4) the creatinine and uric acid levels were non-significantly (P<0.05) affected by the addition of Eachnicea, Pidotimod and Astragalus to the broiler drinking water when compared with the control group. This provides evidence for the hepato and reno-protective effects of group supplemented with Eachnicea agreed with Speranda et al. [41] who reported that, the dietary Eachnicea supplementation had no effect on uric acid and creatinine concentration. In contrast, recorded an increase in uric acid concentration in Eachnicea the supplemented group compared to the control chicks [42,43].

The obtained result of Astragalus provides evidence for the hepato and reno-protective effects of the Astragalus as one of its components. These results similar to the results obtained by 44. Fasanmi 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 significant (P<0.05) reduction in the serum triglycerides, cholesterol and LDL levels in Pidotimod and Astragalus supplemented groups in comparison to control. On the other hand, there was a significant increase in HDL of 35 days. Furthermore, the effect of Eachnicea was non-significant change in cholesterol, but the significant reduction was recorded in triglycerides and LDL at 35 days. In our study the chickens treated with Pidotimod showed a significant reduction in triglyceride, cholesterol and LDL in the serum. These obtained results mainly due the Pidotimod containing a mixture of vitamins as vitamin E, Xylo-Oligosccharideds(XOS) and Gamma Amino Butyric Acid (GABA). Concerning to vitamin E has a significant effect on the lipid profile that causes a significant decrease in the serum concentration of cholesterol and triglycerides [42,46]. And also, Xylo-Oligosccharideds(XOS) cause a significant reduction in thetriglycerides (TG), total cholesterol (TCHOL) and high density lipoprotein (HDL) in broilers fed on Xylo-

Oligosccharideds(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 Fleux(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 Eachnicea,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 Astragulus compared to control. High positive effect on total

Parameters \ Groups	Lipid profile			
	G1 Control	G2 Eachnicea	G3 pidotimod	G4 Astragalus
Triglycerides (mg/dl)	67.84 ± 2.64^{a}	37.73 ± 1.03^{b}	34.30 ± 0.78^{bc}	32.46 ± 0.86^{c}
Cholesterol (mg/dl)	118.50 ± 0.87^{a}	118.59 ± 0.25^{a}	112.22 ± 0.50^{c}	115.89 ± 0.74^{b}
HDL (mg/dl)	50.80 ± 1.0^{d}	64.63 ± 1.2^{b}	76.53 ± 1.0^{a}	57.26 ± 1.2^{c}
LDL (mg/dl)	65.50 ± 1.7^{a}	51.36 ± 2.7^{b}	34.90 ± 2.0^{c}	52.11 ± 1.7^{b}
Values are expressed as means ± standard error (SE); n=6.Means within the same row with different ^{a,b,c,d}Superscripts are significantly different (P<0.05).HDL: High Density Lipoprotein; LDL: Low density lipoprotein.				

Table 5: Effects of studying supplements on the lipid profile of broiler chicks at 35 days.

Parameters \ Groups	Serum protein fractionation (g/dl)			
	G1 Control	G2 Eachnicea	G3 Pidotimod	G4 Astragalus
Total protein(g/dl)	3.96 ± 0.06^{c}	4.31 ± 0.11^{b}	4.92 ± 0.12^{a}	4.42 ± 0.05^{b}
Total globulin(g/dl)	2.77 ± 0.06^{c}	3.05 ± 0.07^{b}	3.72 ± 0.07^{a}	3.18 ± 0.06^{b}
Total gamma-globulin(g/dl)	0.85 ± 0.014^{d}	0.99 ± 0.03^{c}	1.56 ± 0.05^{a}	1.21 ± 0.04^{b}
Total beta-globulin(g/dl)	0.78 ± 0.02^{c}	0.83 ± 0.023^{b}	1.03 ± 0.03^{a}	0.90 ± 0.024^{ab}
Total alpha-globulin(g/dl)	1.14 ± 0.03^{a}	1.24 ± 0.03^{a}	1.12 ± 0.02^{a}	1.07 ± 0.10^{a}
Albumin(g/dl)	1.19 ± 0.01^{a}	1.27 ± 0.06^{a}	1.21 ± 0.044^{a}	1.24 ± 0.04^{a}
Albumin/Globulin ratio	0.43 ± 0.01^{a}	0.42 ± 0.02^{a}	0.33 ± 0.01^{b}	0.39 ± 0.02^{b}
Values are expressed as means ± standard error (SE); n=6. Means within the same row with different ^{a,b,c}Superscripts are significantly different (P<0.05).				

Table 6: Effects of studied supplements on the serum protein fractionation (g/dl) of broiler chicks at 35 days.

Parameter \ Groups		Hemagglutination inhibition test			
		G1 Control	G2 Eachnicea	G3 Piotimod	G4 Astragalus
ND	28ds	4.33 ± 0.33^{b}	5.77 ± 0.31^{a}	6.17 ± 0.31^{a}	5.63 ± 0.31^{a}
	42ds	4.17 ± 0.17^{b}	5.50 ± 0.34^{a}	5.50 ± 0.22^{a}	5.30 ± 0.37^{a}
Values are expressed as means ± standard error (SE); n=6. Means within the same row with different ^{a,b}Superscripts are significantly different (P<0.05). ND: Newcastle.					

Table 7: Effects of studied supplements on humoral immune response estimated by detecting serum antibody titers against ND and AI viruses using a hemagglutination inhibition test.

Parameter \ Group		Interleukin-6 (IL-6) and Tumour necrosis factor-α (TNF-α)			
		G1 Control	G2 Eachnicea	G3 Pidotimod	G4 Astragalus
IL-6 (pg/ml)	35ds	128.68 ± 1.20^{d}	144.04 ± 1.40^{c}	163.14 ± 2.59^{a}	158.62 ± 2.13^{b}
TNF-α (pg/ml)	35ds	55.52 ± 0.40^{c}	66.13 ± 0.87^{b}	69.71 ±1.00^{a}	66.37 ± 0.60^{b}
Values are expressed as means ± standard error (SE); ^{a,b,cd}superscripts are significantly different (P<0.05).					

Table 8: Effects of studied supplements on the interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) production in serum.

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 (P≤ 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 (P≤ 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-α is known to be a key mediator for the induction of apoptosis and development of humeral immune response [65]. In the present study, IL-6 and TNF-α 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 immumostimulant and in the same time growth promoters. So, we recommended using the pidotimod and astragalus in poultry production.

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