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The Effects of Rare Earth-Chitosan on Growth Performances and Serum Components in Sichuan Bone-Black Chicken

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Abstract: The experiment was conducted to study the effects of different dose levels of Rare earth-chitosan on growth performances and serum components in Sichuan bone-black chicken. A total of 192 one day old Sichuan bone-black chickens were sampled and randomly allotted to four dietary treatments (12 birds per pen with 4 pens per treatment); the experiment period were 56 days. After determining the appropriate treatment dose, we studied further the effect on these traits by adding Rare-earth, Chitosan and Rare earth-chitosan, separately. The further study included 192 one day old Sichuan bone-black chickens, sampled and randomly allotted to 4 dietary treatments, 12 per pen with 4 pens per treatment and period were 56 days. The results showed that Rare earth-chitosan significantly elevated the average weight and average daily gain in Sichuan bone-black chicken and the fitting addition dose was 0.10%. As to the serum components, both the Rare earth-chitosan and Rare-earth, improved the serum of calcium, decreased the concentration of serum cholesterol and alkaline phosphatase ($p<0.05$) and the Rare earth-chitosan treatment had better results. Based on these results, we concluded that the fittest addition dose of Rare earth-chitosan is 0.10 %; the effects of Rare earth-chitosan on growth performances and Serum components in Sichuan bone-black chicken are better than adding Rare-earth and Chitosan separately.

Key words: Rare earth-chitosan, growth, serum parameters, sichuan bone-black chick

INTRODUCTION

The Rare earth-chitosan is an organic and giant molecule polysaccharide coordination compound, combining the Rare earth with chitosan by special electrochemistry and sequestration reaction. Rare earth element is the generic name of lanthanoid and has the same chemical characteristics (such as scandium). Chitosan is an amino-group, linear chain and polycose derivate. Its chemical name is (1, 4)-2-amid-2-deoxy-B-D-glucose. Some researches showed that by adding the Rare earth-chitosan into hens' basal diet, it could elevate the egg weight and laying rate, improve the quality of eggs and degrade cholesterol level of egg (Wang *et al*., 2005; Song and Zhang, 2006). Rare earth-chitosan could elevate daily weight gain and prompted the economic efficiency. So far, there have no reports about the effects on Growth performance and Serum components in Sichuan bone-black chicken by adding the Rare earth-chitosan into the basal diet, the experiment was conducted to cast more insights on this subject. After determining the fitting addition dose, we studied further the effect on these traits by adding Rare-earth, Chitosan and Rare earth-chitosan, separately.

MATERIALS AND METHODS

Animals and treatments: We collected a total of 192 one day old Sichuan bone-black chickens and randomly allocated to 4 dietary treatments (12 birds per pen with 4 pens per treatment); the experiment period was designed by 56 days period. The basal diet was conducted on the base of corn-bean diet by referencing the nutrition requirement parameters. The treatment group 1 (control group) was treated only with basal diet; in contrast, the treatment groups 2, 3 and 4 were designed by adding the Rare earth-chitosan into the basal diet with 0.10, 0.15 and 0.20% dosage, respectively. After determining the fitting addition dose, an another total of 192 one day old Sichuan bone-black chickens were further sampled and randomly allotted to four dietary treatments as described above; the experiment period were 56 days. The treatment group I (control group) was treated only with basal diet. On the contrary, the treatment groups II, III and IV were conducted by adding 0.10 % Rare earth-chitosan, 0.08% Chitosan and 0.02% Rare-earth rinto the basal diet, respectively. The designed component and its corresponding proportion were shown in Table 1.

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Table l: Composition of basal diet

0-4 Week		5-8 Week	
Ingredients (%)	Trophic levels	Ingredients (%)	Trophic levels
Corn (59.20)	ME (12.34 MJ K^{-1})	Corn (64.90)	ME(12.55MJK^{-1})
Soybean (29.50)	CP (21.00%)	Soybean (24.30)	CP (19.00%)
Rapeseed cake (5.00)	Ca (1.00%)	Rapeseed cake (5.00)	Ca (0.95%)
Cloza oil (2.06)	AP (0.45%)	Cloza oil (1.94)	AP (0.40%)
CaCO3 (1.26)	DLys (1.04%)	CaCO3 (1.35)	DLys (0.93%)
CaHPO4 (1.70)	DMet (0.49%)	CaHPO4 (1.43)	DMet (0.37%)
L-Lys.Hcl (0.15)	DThr (0.71%)	L-Lys.Hcl (0.16)	DThr (0.64%)
DL-Met (0.22)	DTrp (0.22%)	DL-Met (0.11)	DTrp (0.19%)
Becholine (0.15)		Becholine (0.15)	
Microingredients (0.30)[a]		Microingredients (0.30)[a]	
Salt (0.45)		Salt (0.45)	
Total (100.00)		Total (100.00)	

[a]Supplied per kilogram feed: Fe (80mg kg^{-1}), Zn (40mg kg^{-1}), Cu (20 mg kg^{-1}), Mn (60 mg kg), I (0.35 mg kg^{-1}), Se (0.15 mg kg^{-1}); VA (27000I U kg^{-1}), VD (35400I U k^{-1}), VE (9.0I U kg^{-1}), VK (32.5 mg kg^{-1}), VB_1 (11.0 mg kg^{-1}), VB_2 (27.5 mg kg^{-1}), VB_{12} (1215 ug kg^{-1}), VM (8 mg kg^{-1}), VP (25 mg kg^{-1})

Breeding and cultivation: The experiment was conducted in the Animal Nutrition Institution of Sichuan Agriculture University. The tactics of free-taking for the diet and water was adopted. The other daily breeding and cultivation measures were conducted according to the widely accepted description.

Sampling for the serum components analysis: On the 56th days old, we randomly sampled the 5 mL vein blood from 8 individuals per treatment (one female and one male per repeat). The blood serum was immediately isolated by 3, 000 r min^{-1} and stored at -20°C.

We measured live body weight on the 0th, 28th and 56th days old to explain the growth performances. The dosages of serum Calcium (Ca), serum P, Total Cholesterol (TC), Urea Nitrogen (UN) and Triglyceride (TG) were detected using GPO-PAP method; (alkaline phosphatase) AKP and (alanine aminotransferase) ALT were detected using Enzymic method using the Technicon RA1000 (American).

Statistical analysis: The variance analyses were conducted using the General Linear Models (GLM) procedure in SAS/STAT software and multiple treatment means were compared with Tukey's studentized range test (HSD). Statements of significance were based on $p \leq 0.05$ and the results were denoted by the "mean±standard error" format.

RESULTS AND DISCUSSION

The effect on growth performance of Rare earth-chitosan: As shown in Table 2, the different addition dosages of Rare earth-chitosan into the basal diet had significant influences on the growth performance of Sichuan bone-black chicken. By adding the Rare earth-chitosan, it increased significantly the average body weight and average daily gain on the 28th and 56th days old. Compared with the control group, the average body weight differed significantly in the 0.10% addition dosage group and 0.15% addition dosage group ($p<0.05$); however, the 0.20% addition dosage group differed insignificantly. Among the three treatment groups, the best growth performance appeared in the 0.10% addition dosage group; the average body weight and average daily gain were higher than the control group with 9.54 and 10.17%, respectively. Judging from the average daily gain, 0.10% addition dosage group and 0.15% addition dosage group were significantly higher than control group among the 0-4 weeks, 5-8 weeks and 0-8 weeks periods; and the former groups had better performance. In contrast, the 0.20% addition dosage group had no significant difference compared with the control group.

The effects of Rare earth-chitosan, Chitosan and Rare earth on the growth performances of Sichuan bone-black chicken were listed in Table 3. We can see that the addition of Rare earth-chitosan elevated the average body weight on the 28th and 56th days old. The average body weight in the treatment group II Rare earth-chitosan was significantly higher compared with the control group (23.61 % VS 16.86 %, $p<0.05$); however, it was lower in the Chitosan addition group to control group. Furthermore, among the three different treatment groups, the Rare earth-chitosan group had the best growth performances, followed by Rare earth treatment and Chitosan treatment ($p<0.05$). In the 0 - 4 weeks period, the average daily gain was higher than the remaining two treatment groups ($p<0.05$); the average daily gain in the Rare earth group and Chitosan were also higher than control group, but differed insignificantly ($p>0.05$); the same results appeared in the 5-8 weeks period. In addition, during the 0-8 weeks, the average daily gain in Rare earth-chitosan group was significantly higher than other treatment groups ($p<0.05$).

Table 2: Effect of different doses of Rare earth-chitosan on average weight and daily gain of Sichuan bone-black chicken

	Average weight (g)			Daily gain (g day^{-1})		
Dose (%)	0 day	28 day	56 day	0-4 week	5-8 week	0-8 week
0.00	32.53±0.39^{a}	302.80±14.83^{c}	918.05±41.25^{c}	9.65±0.52^{b}	21.97±1.27^{c}	15.81±0.73^{b}
0.10	32.68±0.67^{a}	331.68±14.30^{a}	1011.45±54.45^{a}	10.68±0.51^{a}	24.28±1.55^{a}	17.48±0.98^{a}
0.15	33.10±0.99^{a}	331.78±6.40^{ab}	1000.55±40.50^{ab}	10.67±0.25^{a}	23.88±1.43^{bc}	17.28±0.71^{a}
0.20	32.65±0.70^{a}	302.90±10.77^{c}	915.50±59.68^{c}	9.65±0.39^{b}	21.88±0.65^{c}	15.77±0.50^{b}

Means in the same rank bearing different superscript differ significantly (p = 0.05) using Tukey's Procedure

Table 3: Effect of Rare earth-chitosan, chitosan and rare earth on average weight and daily gain of Sichuan bone-black chicken

	Average weight(g)			Daily gain(g d^{-1})		
Treatments	0 day	28 day	56 day	0-4 week	5-8 week	0-8 week
Control	37.40±0.12^{a}	288.88±19.67^{b}	905.78±18.00^{b}	8.98±0.71^{b}	22.03±0.42^{b}	15.51±0.32^{b}
Rare earth-chitosan	37.60±0.12^{a}	357.08±19.03^{a}	1058.47±10.68^{a}	11.40±0.68^{a}	25.00±0.39^{a}	18.20±0.95^{a}
Chitosan	37.45±0.19^{a}	299.00±24.62^{b}	886.53±52.42^{b}	9.34±0.87^{b}	20.98±1.00^{bc}	15.16±0.93^{b}
Rareearth	37.50±0.00^{a}	305.00±6.81^{bc}	919.23±39.97^{bc}	9.55±0.24^{b}	21.94±1.23^{b}	15.74±0.71^{b}

Means in the same rank bearing different superscript differ significantly (p≤0.05) using Tukey's Procedure

Table 4: Effect of different doses of Rare earth-chitosan on serum biochemical parameters of Sichuan bone-black chicken

Doses (%)	Ca (mmol L^{-1})	P (mmol L^{-1})	BU (mmol L^{-1})	TG (mmol L^{-1})	TC (mmol L^{-1})	AKP (mmol L^{-1})
0.00	2.32±0.06^{b}	2.48±0.15^{a}	0.08±0.01^{a}	0.26±0.12^{a}	3.86±0.07^{b}	1682.00±770.51^{a}
0.10	2.63±0.04^{a}	2.50±0.25^{a}	0.06±0.02^{a}	0.25±0.15^{a}	3.14±0.46^{a}	1088.25±163.30^{a}
0.15	2.31±0.04^{b}	2.47±0.05^{a}	0.03±0.01^{a}	0.25±0.12^{a}	3.10±0.55^{a}	1272.75±414.13^{a}
0.20	2.37±0.05^{b}	2.40±0.24^{a}	0.05±0.01^{a}	0.27±0.13^{a}	2.98±0.39^{a}	1500.00±633.85^{a}

Means in the same rank bearing different superscript differ significantly (p≤0.05) using Tukey's Procedure

Table 5: Effect of Rare earth-chitosan, Chitosan and Rare earth on serum biochemical parameters of Sichuan bone-black chicken

Treatments	Ca (mmol L^{-1})	P (mmol L^{-1})	UN (mmol L^{-1})	TG (mmol L^{-1})	TC (mmol L^{-1})	AKP (mmol L^{-1})
Control	2.61±0.05^{b}	0.57±0.19^{a}	0.56±0.19^{a}	3.55±0.12^{a}	3.33±1.15^{a}	3626.00±192.69^{a}
Rare earth-chitosan	2.74±0.01^{a}	0.59±0.33^{a}	0.51±0.20^{a}	3.15±0.46^{a}	4.66±2.08^{a}	1479.00±413.98^{bc}
Chitosan	2.62±0.02^{b}	0.54±0.13^{a}	0.61±0.18^{a}	3.40±0.53^{a}	3.67±2.08^{a}	2653.00±1202.01^{ab}
Rare earth	2.75±0.06^{a}	0.52±0.03^{a}	0.77±0.28^{a}	3.51±0.15^{a}	1.67±1.53^{a}	1090.00±409.88^{c}

Means in the same rank bearing different superscript differ significantly (p≤0.05) using Tukey's Procedure

The effect on serum components of Rare earth-chitosan: In Table 4, the serum Ca and Total Cholesterol (TC) of Sichuan bone-black chicken differed significantly among the different dosage treatments of Rare earth-chitosan ($p<0.05$). The level of serum Ca in 0.10% treatment group was higher than control group, but with lower AKP level than control group.

As shown in Table 5, the effect of Rare earth-chitosan, Chitosan and Rare earth on the UN, TG, TC and ALT were not significantly different between the treatment groups and control group ($p<0.05$). In Rare earth-chitosan group, the serum Ca level was higher than the other groups; on the contrary, the AKP level had no significant difference compared with control group. There were no significant difference of these parameters between the Chitosan group and control group showed no significant difference between these. There was no significant difference between the Rare earth-chitosan treatment, Chitosan treatment and Rare earth treatment of the concentration of serum cholesterol.

The metal irons, such as Cu, Fe, Zn, Co and Re, can be combined to the Chitosan and form a soluble sequestration, which enhance the absorptivity and utilization of these micronutrients (Otha, 1995; Delzenne, 1995). So, the addition into the basal diet with Chitosan could promote the growth performances of domestic animals (Huang *et al*., 2005). Many published reports showed that the egg production number and average egg weight significantly increased after adding the Rare earth-chitosan into the basal diet and growth rate and feed efficiency increased. The same effects of Rare earth-chitosan had also been determined in pig (Gu and Song, 1994). In this study, by adding a dosage of 0.10% Rare earth-chitosan into basal diet, it had significantly elevated the average daily gain of Sichuan bone-black chicken. Interestingly, when the addition dosage increased to 0.20%, the average body weight and average daily gain decreased contrarily and were lower than the control group. We concluded that the addition of Rare earth-chitosan could significantly elevate the average weight and average daily gain in Sichuan bone-black chicken and the 0.10% addition dosage would be the better choice, which are consistent with our results (Xei and Huang, 1996; Zhang and Jie, 1991). The mechanism of prompting the growth for domestic animals is controversial.

In this study, adding chitosan separately made no difference of average weight and average daily weight gain in Sichuan bone-black chicken, the same effects of chitosan had also been determined in broiler (Kobayashi *et al*., 2002). Huang *et al.* (2005) reported that adding Rare earth could elevate average daily gain and feed efficiency, which are consistent with our results.

Our results showed that the different dosage addition of Rare earth-chitosan could significantly influence serum Ca, total cholesterol and AKP levels in Sichuan bone-black chicken compared with the control group, but had no significant difference on the other serum component parameters. And Shimada reported that Rare earth could elevate the active of AKP (Shimada *et al*., 1996). Xing and She (2001) reported that the addition of the Rare earth into the basal diet of meat chicken had only influenced the serum P level and which are consistent with the results in this study. Rare earth can effect the growth and development of animals and also affect the dose of trace elements (Nakagawa *et al*., 1997; Wang and Fisher, 1999). One supposition for such results was that the Rare earth-chitosan has no influence on the metabolism of protein and fat. The Rare earth-chitosan could decrease the level of cholesterol and the potential mechanism would be referred to that the Chitosan could selectively absorb the much low density lipoprotein cholesterol and bile acid. Razdan reported that the addition of Chitosan into the meat chicken basal diet down-regulated the serum cholesterol level (Razdan *et al*., 1997). Zhu and Song (2002) suggested that the chitosan was characteristic of degrading the hyperlipemia, cholesterol and low density lipoprotein, which were caused by yolk milk and high-fat Feedstuff. Some researches showed that the right addition dosage of Chitosan had lowered the serum cholesterol in broiler (Kobayashi *et al*., 2002; Deuchi *et al*., 1995), which was supported by this study. The rare earth element could influence the metabolism of fat, by decreasing the blood cholesterol level and regulating the process of citric acid cycle. These results in this study showed that there was no significant difference of serum cholesterol level between the Rare earth group and control group. Compared with control group, the serum cholesterol in Rare earth group decreased by 5.70%, but differed insignificantly; At a certain extent, the Rare earth-chitosan could down-regulate the serum cholesterol in Sichuan bone-black chicken, but the reliable mechanism are veiled and more work should be done.

CONCLUSION

The research indicated that the fittest addition dose of Rare earth-chitosan is 0.10%; it can increase the growth performance of Sichuan bone-black chicken, the effects of Rare earth-chitosan on growth performances and Serum components in Sichuan bone-black chicken are better than adding Rare-earth and Chitosan separately.

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