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Effects of Pueraria lobata leaf powder as a feed additive on the immune system and growth performance of broiler chickens

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Abstract: The present research assessed the effect of different levels of Pueraria lobate leaf (PLL) on growth performance, immunity, antioxidant capacity, and blood index in broiler feed. A total of 384 at 21 days old chicks were assigned to 1 of 4 dietary treatments to examine the benefits of basal diet, basal diet +1% PLL, basal diet +2% PLL and basal diet +3% PLL. The trial was divided into 2 time periods, including starter from days 21– 49 and finisher from days 50–70. The addition of 2% and 3% PLL powder to diets increased total protein content, triglycerides, albumin, and low-density lipoprotein ($p < 0.05$). In contrast, total cholesterol, aspartate aminotransferase, urea nitrogen, and high-density lipoprotein in serum were reduced ($p < 0.05$). The levels of total antioxidants, superoxide dismutase, IgM, and IgG, on the other aspect, were significantly increased, nevertheless, the content of malondialdehyde and interleukin was decreased. Although dietary supplementation with PLL did not have an effect on the growth and slaughter performance, clear improvements in the thymus organ index, antioxidant performance, and immune functions ($p < 0.05$) were observed. The leaf of *P. lobate* is a strong candidate as a potential feed additive due to its wide range of sources and well-established record of safety, in addition to its efficacy in improving total protein, albumin, low-density lipoprotein, total antioxidant capacity, superoxide dismutase, Ig M and Ig G without any adverse effects on growth and slaughter performance. In conclusion, the leaf of *P. lobate* can be regarded as a novel resource for broiler feed additive to replace antibiotics.

Keywords: *Pueraria lobate*, growth performance, blood index, immune system, broiler

1. Introduction

The poultry industry serves a critical role in providing animal meat protein and eggs for daily consumption for humans. Sinclair et al. [1] reported that the poultry industry produces nearly 80% of meat from the total meat production and increased to 83% yearly over the past 20 years. The increasing demand for chicken meat necessitated that farmers expand their production capacity to meet consumer demand. However, increasing feed prices has the potential to stagnate this growth [2]. A chicken's diet consists primarily of corn and soya beans, so new additives to enhance nutritional quality and decrease costs are highly desirable.

Recently, the problem of antibiotic use in poultry and livestock industries or growth promotion, which has resulted in the emergence of antibiotic-resistant pathogens and the resulting decrease in human and animal health, has been brought to the attention of the public [3]. As a result, the Ministry of Agriculture and Rural Affairs of China issued announcement No. 194 in January of 2020. This notification requires the cessation of all growth-promoting drugs and feed additive varieties except traditional Chinese medicines (TCM) in order to strengthen food safety. As a result, veterinary drug production and importing would be severely restricted. Thus, it is essential to explore safe and innovative alternatives to antibiotics for the poultry industry.

There still exists a thirst to seek new resources for replacing antibiotics. A medicinal and edible plant named *Pueraria lobate* was widely used in China for the treatment of various illnesses and also for food provision. In this study, the leaf of Pueraria lobate was explored as a feed supplement in broiler production. Interestingly, several previously published studies have demonstrated the

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content of crude protein in *Pueraria lobate* leaf (PLL) was roughly double that of any other feed additives such as *Pennisetum purpureum* Schum while having a similar low crude fiber content [4–13]. Therefore, the current study focused on the affection of different amounts of PLL as an additive to growth performance, immunity, antioxidant capacity, and blood index in growing broilers.

2. Materials and method

The trial was carried out in a farm setting at the Institute of Animal Husbandry and Veterinary Medicine of the Jiangxi Academy of Agricultural Sciences.

2.1. Experimental diets and animals

Powdered PLL was prepared from fresh PLL via a series of steps including drying, selecting, coarse crushing, and passing through 8 mesh sieves. The nutritional composition of PLL is presented in Table 1. A total of 384 chicks at 21 days of age were selected and randomly allocated into 4 dietary treatment groups. Each group consisted of 6 replicates with 16 birds per replicate. Dietary formulations were designated control, PLL-1, PLL – 2, and PLL - 3, which correspond to 0%, 1%, 2%, or 3% PLL, respectively. All diets were prepared following the recommendations provided by the NRC (1994), and the percentage inclusion of PLL was considered as the final concentration in the basal feed containing soya beans, and corn. Other nutrients were complemented as essential to suit the nutrient requirements of the broilers. The feed trials were divided into 2 age periods, including the starter phase from days 21 to 49, and the finisher phase from days 50 to 70. All broilers were maintained in identical husbandry conditions for the duration of the study.

2.2. Data collection

Throughout the trial, the amount of feed delivered, residual feed amount, and the beginning and end animal weights were all recorded. The average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (F/G) of the test chickens were determined.

Prior to blood collection (5 mL) from the wing vein at the end of the trial, the broilers were fasted for 12 h. The

Table 1. Composition and nutrient contents of basal diets (air-dry basis) %.

Items	Control		Additive contents of LPL powder					
			1%		2%		3%	
	Early	Later	Early	Later	Early	Later	Early	Later
Ingredients								
Corn	60.23	67.97	58.70	66.69	57.16	65.41	55.63	64.13
Soybean meal	31.13	22.82	31.05	22.69	30.96	22.55	30.88	22.42
Soybean oil	3.64	4.21	4.26	4.62	4.87	5.04	5.49	5.45
PLL	0.00	0.00	1.00	1.00	2.00	2.00	3.00	3.00
Premix[1)]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Total	100.00	100	100.00	100	100.00	100	100.00	100
Nutrient levels[2)]								
DE/(MJ/kg)	12.56	12.90	12.56	12.90	12.56	12.89	12.56	12.89
CP	19.00	16.01	19.01	16.01	19.02	16.02	19.02	16.02
Ca	0.90	0.80	0.90	0.80	0.90	0.80	0.90	0.80
TP	0.65	0.62	0.65	0.62	0.65	0.62	0.65	0.62
Lys	0.98	0.90	0.98	0.90	0.98	0.90	0.98	0.90
Met	0.40	0.35	0.40	0.35	0.40	0.35	0.40	0.35
Met+Cys	0.73	0.65	0.73	0.65	0.73	0.65	0.73	0.65

[1]The premix provided the following per kg of diets: VA 5 000 IU, VD_2 1 000 IU, VE 10 IU, VK_3 0.5 mg, VB_1 2 mg, VB_2 5 mg, VB_6 4 mg, VB_{12} 0.02 mg, *D*-pantothenic acid 10 mg, Niacin 30.mg, Biotin 0.15 mg, folic acid 0.5 mg, Choline 650 mg, Fe 100 mg, Cu 8 mg, Zn 80 mg, Mn 100 mg, I 0.5 mg, Se 0.14 mg, Phytase 0.02 mg

CP: crude protein; TP: total phosphorus; Met: methionine; Early: growth stage 21 to 49 days; Later: growth stage 50 to 70 days.

Early and later represent 21 to 49 days of age and 50 to 70 days of age, respectively. We added the description at the bottom of the table.

blood was centrifuged at 3000 r/min after a 4-h incubation at room temperature. The serum was decanted and kept at −80 °C. Total protein, albumin, urea nitrogen, triglycerides, cholesterol, alkaline phosphatase, alanine aminotransferase, aspartate amino acid and other biochemical indicators, glutathione peroxidase (GSH-Px) activity, super oxidation antioxidant capacity indexes such as SOD, malondialdehyde (MDA), total antioxidant capacity (T-AOC) and immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and other immune performance indicators were measured.

The determination of slaughter rate, semicleaning rate, full-cleaning rate, pectoral muscle rate, leg muscle rate, and abdominal fat rate were performed on the "People's Republic of China agriculture profession standard (NY/T 823-2020)".

Immune organs such as the spleen, bursa of fabrics, and thymus were collected immediately after performing dissections. Excess visceral fat and blood were removed with filter paper. The immune organ index was calculated using the following formula: immune organ index = immune organ weight/live weight before slaughter × 100%.

2.3. Data analysis

SPSS 21.0 statistical software was used to organize the data, one-way ANOVA and LSD multiple comparisons were used to compare results between groups. All results are expressed as mean ± standard deviation.

3. Results

3.1. Growth performance

As can be seen in Table 2, PLL supplementation did not have a significant effect on the average daily weight gain, average daily feed intake, and feed conversion ratio of broilers ($p < 0.05$).

3.2. Serum biochemical indicators

In Table 3, there was no discernible influence in serum biochemical indicators with additive 1% PLL powder ($p > 0.05$). However, supplementation with 2% and 3% PLL powder resulted in an increase in broiler serum albumin and low-density lipoprotein ($p < 0.05$). Furthermore, 3% PLL powder produced a significant reduction in total cholesterol, urea nitrogen, high-density lipoprotein, and alanine aminotransferase. In contrast, 2% PLL powder merely decreased the content of albumin and triglycerides ($p < 0.05$).

3.3. Serum antioxidant properties

There was an increase in the total antioxidant capacity and glutathione peroxidase activity in the serum of broiler chickens (Table 4). These improvements were observed for all supplementation levels ($p < 0.05$). Conversely, a significant reduction in serum malondialdehyde concentrations was observed ($p < 0.05$) in animals receiving 3% PLL powder supplementation. Meanwhile, serum superoxide dismutase was not significantly altered by adding 1%–3% PLL powder in diets ($p > 0.05$).

Table 2. Effects of PLL powder on growth performance of broilers.

Growth stage	Item	Control group	Additive contents of LPL powder		
			1%	2%	3%
21–49 d	21 d Body weight/g	324.67 ± 1.21	325.17 ± 1.72	323.67 ± 1.63	325.50 ± 2.51
	49 d Body weight/g	1150.52 ± 30.07	1134.90 ± 47.94	1117.71 ± 49.83	1172.57 ± 41.48
	ADG/(g/d)	29.50 ± 1.07	28.93 ± 1.75	28.36 ± 1.81	30.26 ± 1.49
	ADFI/(g/d)	66.87 ± 3.79	67.09 ± 4.29	63.94 ± 3.28	68.51 ± 3.39
	FCR	2.27 ± 0.07	2.32 ± 0.09	2.26 ± 0.13	2.27 ± 0.04
50–70 d	70 d Body weight/g	1745.08 ± 71.45	1740.74 ± 49.79	1741.11 ± 76.29	1742.86 ± 69.37
	ADG/(g/d)	29.73 ± 3.46	30.29 ± 1.83	31.17 ± 3.58	28.52 ± 2.29
	ADFI/(g/d)	100.38 ± 6.03	98.34 ± 6.12	100.15 ± 11.29	100.10 ± 8.40
	FCR	3.40 ± 0.31	3.25 ± 0.20	3.24 ± 0.38	3.52 ± 0.20
21–70 d	ADG/(g/d)	29.60 ± 1.48	29.50 ± 1.06	29.53 ± 1.60	29.53 ± 1.43
	ADFI/(g/d)	80.83 ± 2.74	80.11 ± 3.33	79.02 ± 3.74	81.67 ± 3.80
	FCR	2.74 ± 0.10	2.72 ± 0.06	2.68 ± 0.10	2.77 ± 0.07

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).
FCR: feed conversion ratio

Table 3. Effects of PLL powder on serum biochemical index of broilers.

Item	Control group	Additive contents of LPL powder		
		1%	2%	3%
Total protein/(g/L)	28.84 ± 1.86[b]	29.26 ± 1.80[b]	30.01 ± 1.71[ab]	30.99 ± 1.86[a]
Albumin/(g/L)	12.87 ± 0.60[b]	13.06 ± 0.50[b]	13.60 ± 0.41[a]	13.89 ± 0.46[a]
Triglycerides/(mmol/L)	2.78 ± 0.16[a]	2.71 ± 0.10[a]	2.52 ± 0.13[b]	2.42 ± 0.12[b]
Total cholesterol/(mmol/L)	2.99 ± 0.33[a]	2.88 ± 0.15[ab]	2.85 ± 0.13[ab]	2.76 ± 0.16[b]
Aspartate aminotransferase (U/L)	135.83 ± 6.52[a]	134.13 ± 3.49[a]	128.28 ± 3.44[b]	126.09 ± 4.72[b]
Alanine aminotransferase (U/L)	2.40 ± 0.14[a]	2.34 ± 0.17[a]	2.33 ± 0.11[a]	2.16 ± 0.11[b]
Alkaline phosphatase (U/L)	5780.36 ± 179.53	5815.20 ± 128.46	5805.70 ± 95.34	5887.33 ± 123.01
Urea Nitrogen (μmol/L)	412.89 ± 8.07[a]	409.85 ± 8.15[a]	409.03 ± 6.50[a]	399.20 ± 11.06[b]
High density lipoprotein (mmol/L)	2.50 ± 0.18[a]	2.47 ± 0.10[a]	2.38 ± 0.15[ab]	2.29 ± 0.10[b]
Low density lipoprotein (mmol/L)	1.81 ± 0.11[b]	1.85 ± 0.12[b]	1.98 ± 0.08[a]	1.99 ± 0.11[a]

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

3.4. Serum immune performance

Supplementation with all concentrations (1%–3% PLL) produced a significant increase in broiler serum IgM titer. However, adding 2%–3% PLL powder in diets resulted in a reduction of the serum proinflammatory cytokine IL-1β. In addition, 3% PLL not only increased serum IgG titers but also reduced IL-2 concentrations ($p < 0.05$). Data on immune parameters are described in Table 5.

3.5. Slaughter performance and Organ Index

Adding PLL powder to broiler diets had no effect on the slaughter performance of broilers, as shown in Table 6 ($p > 0.05$). There was no observable effect on the bursal, heart, liver, and gastric indices ($p > 0.05$). Conversely, PLL supplementation significantly increased the thymus index ($p < 0.05$) at all concentrations tested (Table 7).

4. Discussion

4.1. Effects of PLL powder on growth performance of broilers

Puerarin, the main active ingredient in *P. lobata* exerts estrogen-like effects and has been demonstrated to promote the growth and development of the body [14]. At present, few studies have been published examining the effect of *P. lobata* on animal performance; the existing reports also present inconsistent data. Peng et al. [15] demonstrated that the addition of 250 mg/kg and 1250 mg/kg total flavonoids extracted from *P. lobata* to the basic diet of broilers did not have a significant effect on growth performance. Peng et al. [15] reported that puerarin improved the performance and meat quality of beef cattle exposed to heat stress conditions. Moreover, studies have shown that puerarin can significantly inhibit appetite [16] and reduce production performance [17]. In this experiment, the addition of 1%–3% PLL powder to the broiler diet had no significant effect on the average daily weight gain, average daily feed intake, and feed conversion ratio of the broilers. However, these observed results may be due to long-term heat stress. The stress state causes the body to be undernourished, and the body uses its stored nutrients to maintain the body's heat balance [18], resulting in no change in growth performance. Similar to the findings mentioned above, the extract of P. lobata had no influence on broiler growth performance, which was compatible with the results of the current research.

4.2. Effects of PLL powder on serum biochemical indices of broilers

The processes of protein synthesis and metabolism can be estimated by measuring serum total protein (TP) and albumin (ALB), and is related with immunity [19]. The increase or decrease of the content of triglycerides, lipoproteins, and total cholesterol in animal serum can be used as indicators of fat metabolism, and blood urea nitrogen levels can accurately reflect the degree of protein catabolism and renal function in the body. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are important transaminases in the body, affecting the metabolic activity of a variety of amino acids. In this study, the results of the trial found that adding a high proportion of PLL powder significantly increased broiler serum total protein, albumin and low-density lipoprotein content, significantly reduced a series of factors including triglyceride, total cholesterol, urea nitrogen and high-density lipoprotein content, AST and ALT. Most of these indexes were affected with 3% PLL additive in daily diet.

Table 4. Effects of PLL powder on serum antioxidant indexes in broilers.

Item	Control group	Additive contents of LPL powder		
		1%	2%	3%
Total antioxidant capacity (T-AOC) (U/mL)	3.68 ± 0.24[c]	3.86 ± 0.15[b]	3.95 ± 0.15[ab]	4.03 ± 0.15[a]
Superoxide dismutase (SOD) (U/mL)	109.56 ± 5.19	110.91 ± 3.94	111.27 ± 7.64	113.89 ± 5.34
Glutathione peroxidase (GSH-Px) (U/mL)	1393.64 ± 87.44[c]	1454.17 ± 81.85[b]	1512.73 ± 60.18[ab]	1566.67 ± 48.68[a]
Malondialdehyde (MDA) (μmol/mL)	8.71 ± 0.64[a]	8.56 ± 0.35[ab]	8.40 ± 0.38[ab]	8.27 ± 0.53[b]

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

Table 5. Effect of PLL powder on serum immune indexes in broilers.

Item	Control group	Additive contents of LPL powder		
		1%	2%	3%
IgA(g/L)	2.15 ± 0.14	2.23 ± 0.13	2.35 ± 0.19	2.30 ± 0.69
IgG(g/L)	4.05 ± 0.05[b]	4.11 ± 0.11[ab]	4.11 ± 0.07[ab]	4.14 ± 0.13[a]
IgM(g/L)	1.51 ± 0.10[c]	1.62 ± 0.06[b]	1.62 ± 0.08[b]	1.73 ± 0.09[a]
Tumor necrosis factor (mg/mL)	0.66 ± 0.04	0.66 ± 0.03	0.68 ± 0.04	0.68 ± 0.03
Interleukin 1β(mg/mL)	0.21 ± 0.01[a]	0.21 ± 0.01[a]	0.19 ± 0.01[b]	0.19 ± 0.01[b]
Interleukin 6(mg/mL)	1.25 ± 0.14	1.24 ± 0.11	1.20 ± 0.13	1.18 ± 0.10
Interleukin 2(mg/mL)	3.92 ± 0.16[a]	3.87 ± 0.12[a]	3.78 ± 0.20[a]	3.58 ± 0.22[b]

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

Taken together, these results suggest that PLL enhances protein and fat metabolism, and strengthens the immune system while reducing generalized inflammation at the same time, which were the desired effects of PLL supplementation.

4.3. Effects of PLL powder on the antioxidant and immune functions in broiler serum

The normal oxygen-consuming metabolism of animals produces a large amount of reactive oxygen species (ROS), which leads to the peroxidation of lipids, producing malondialdehyde (MDA), hydroperoxyl groups. These molecules damage protein structures of the cell membrane, impairing cellular functioning. Thus, ROSs need to be eliminated by the body's antioxidant system [20]. GSH can directly remove oxygen free radicals and repair the damage caused by free radicals to protect cells; superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) play a crucial part in the body's antioxidant process because they are the principal antioxidant enzymes for scavenging free radicals in the body's cells. These enzymes, SOD, and GSH-Px, are the main endogenous antioxidants that scavenge free radicals within the body, maintaining the body in a state of oxidative balance. Chen et al. [21] reported that puerarin can significantly reduce the content of serum MDA and significantly increase the activity of serum SOD in aging mice. Wu et al. [22] reported that the addition of puerarin isoflavones to sow diets can significantly reduce the MDA content in the serum of pigs. The results of this experiment found that the addition of 1%–3% PLL powder can significantly increase the total antioxidant capacity (T-AOC) and GSH-Px activity in broiler serum. Furthermore, the addition of a high proportion of PLL powder significantly reduced the serum MDA content. These observations are consistent with those reported by the above-mentioned studies. One cause could be the high content of isoflavone in PLL, which can boost the body's antioxidant capacity dramatically. Another interpretation could be linked to high quantities of components in the additives. Of course, more research is needed to figure out the specific mechanisms in the future.

The leaf of *P. lobata* also affects the immune function of broilers. In this experiment, the addition of PLL powder

Table 6. Effects of PLL powder on slaughter performance of broilers.

Item	Control group	Additive contents of LPL powder		
		1%	2%	3%
Slaughter rate	90.41 ± 1.66	90.52 ± 1.08	90.51 ± 0.99	91.37 ± 1.11
Semieviscerated rate	81.57 ± 1.79	81.64 ± 2.06	81.38 ± 0.66	81.89 ± 1.31
Eviscerated rate	67.95 ± 1.51	66.76 ± 2.31	67.08 ± 1.09	67.45 ± 1.40
Breast muscle rate	16.55 ± 1.58	16.69 ± 0.92	16.82 ± 1.52	17.20 ± 1.12
Leg muscle rate	22.54 ± 1.36	22.32 ± 0.82	22.23 ± 0.90	22.60 ± 1.14
Abdominal fat rate	3.15 ± 0.41	2.89 ± 0.64	2.88 ± 0.48	2.79 ± 0.59

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

Table 7. Effects of PLL powder on viscera indices of broilers.

Item	Control group	Addition ratios of PLL powder		
		1%	2%	3%
Spleen index	1.60 ± 0.28	1.64 ± 0.33	1.73 ± 0.34	1.74 ± 0.19
Bursa index	1.41 ± 0.36	1.52 ± 0.42	1.53 ± 0.25	1.53 ± 0.26
Thymus index	3.49 ± 0.64^{b}	4.27 ± 0.72^{a}	4.28 ± 0.48^{a}	4.33 ± 0.82^{a}
Heart index	4.99 ± 0.86	5.00 ± 0.78	4.91 ± 0.50	4.92 ± 0.87
Hepatic index	18.59 ± 1.45	18.98 ± 1.42	18.64 ± 2.37	18.64 ± 1.60
Stomach Index	17.24 ± 1.52	17.84 ± 1.99	17.74 ± 3.53	18.09 ± 3.09

Note: In the same row, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

significantly increased the serum IgG and IgM content of broilers. With the increase in the proportion of addition, the effect on interleukins also changed from a single interleukin to a variety of interleukins, and its content level was significantly reduced. Studies by Peng et al. [15] showed that the addition of 0.1% puerarin extract to the basic diet, significantly increased IgG and IgM titers in beef cattle serum by 9.45% and 8.33%, respectively. The immune function also has been shown to exert a growth-promoting effect. Those observations are corroborated here, which together suggest that adding PLL to the diet can improve broiler immune performance. The results also are partly consistent with the studies mentioned above.

4.4. Effects of PLL powder on the organ indices and slaughter performance of broilers

The spleen is the main immune organ of the animal body, and the center of humoral immunity and cellular immunity. Both organ indices may be reflective of the strength of the body's immune function. At present, there is very little research on the effect of PLL on an animal's organ index. The results of this trial show that the addition of PLL powder to the broiler diet can significantly increase the thymus index of broilers, while not affecting other organ indices. Moreover, these findings indicate that the addition of PLL to the diet can promote the immune function of broilers. However, the specific role of PLL here remains unclear, and further research is needed.

Additionally, in this experiment, the addition of PLL powder to the broiler diet did not have a clear effect on the slaughter performance of broilers. However, the abdominal fat percentage of broilers showed a downward tendency with the increase of the proportion of PLL in feed. Grgic [23] reported that the addition of isoflavones in the diet of livestock can affect the metabolism and other aspects such as growth performance and laying performance. The results of this experiment are in agreement with those reported here. In a nutshell, it is believed that adding PLL to the feed can promote slaughter performance.

5. Conclusions

In brief, the current findings suggest that *P. lobata* leaves have the potential as an alternative feed ingredient for broiler chickens. This is because, the addition of PLL powder to broiler feed rations significantly enhances the thymus organ index, antioxidant performance, and immune function. Diets supplemented with PLL powder showed no clear impact on the growth and slaughter performance of broilers. Taken together, the leaf of *P. lobata* is a promising feed additive with its wide range of sources and its security.

Acknowledgement/Disclaimers/Conflict of interest

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