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*Short Communication*

# Effects of *Gynura procumbens* Extract on Egg Quality, Serum Biochemistry, and Immunity in Laying Hens

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**Abstract**

**Background**: Herbal medicine is increasingly recognized as a potential alternative to antibiotics in animal feed, offering both health benefits and the ability to mitigate antimicrobial resistance. This study aimed to evaluated the effects of dietary supplementation with Sambung Nyawa (*Gynura procumbens* (Lour.) Merr) extracts (GPLEs) on the production performance, egg quality, serum biochemistry, antioxidant and immune response in laying hens. **Methods**: Hens aged 45 weeks were randomly assigned to diets supplemented with 0 mg/kg, 1000 mg/kg, 2000 mg/kg, and 3000 mg/kg GPLEs for 8 weeks. **Results**: Initially, supplementation improved egg weight and yolk color without negatively affecting feed intake, laying rate, and feed conversion ratio. Additionally, higher inclusion levels of GPLEs significantly modified yolk composition, particularly amino acids and fatty acids. Moreover, serum biochemical and antioxidant markers showed beneficial changes, alongside positive modulation of immune indices, thereby highlighting their potential of GPLEs to enhance egg nutritional value and the health status of hens. **Conclusions**: GPLEs represented a promising phytogenic additive for poultry diets and a potential alternative to antibiotic supplementation.

**Keywords:** Sambung Nyawa; laying hens; egg quality; immunity; antioxidants

## 1. Introduction

For several decades, antibiotics have been extensively employed in poultry production to prevent infectious diseases and to enhance growth performance [1]. Nevertheless, the indiscriminate and prolonged application of these agents has precipitated the emergence of multidrug-resistant pathogens, led to the desorption of antibiotic residues in animal-derived products and the environment, and the perturbation of commensal microbial communities. Collectively, these consequences pose substantial risks to public health, animal welfare and ecological stability [2–4]. In response, numerous countries, including European countries, America, and China, have enacted regulatory frameworks prohibiting the non-therapeutic use of antibiotics in animal husbandry and restricting their use as growth-promoting additives. Consequently, there is an urgent imperative for the poultry industry to identify and implement efficacious alternative growth-promoting strategies that sustain productivity while supporting the transition toward antibiotic-free, environmentally responsible and production systems [5].

As substitutes to antibiotics, a wide spectrum of feed additives, particularly those derived from medicinal herbs, have garnered increasing academic and industrial interest for their potential to modulate intestinal microbiota, suppress inflammatory processes, bolster antioxidant defenses and stimulate immune function in poultry [6–8]. Herbal medicines are rich reservoirs of bioactive constituents such as polysaccharides, polyphenols, alkaloids and flavonoids, which collectively enhance metabolic efficiency and mitigate both infectious and metabolic disorders, thereby fostering avian health and productivity. Empirical evidences further demonstrated that phytogenic interventions could augment systemic antioxidant capacity and beneficially reconfigure gut microbial communities in weaned piglets and growing pigs [9,10]. Moreover, dietary incorporation of herbal formulations in laying hens has been reported to substantially elevate immune responsiveness, improve egg quality traits and optimize overall production performance.

Sambung Nyawa, botanically classified as *Gynura procumbens* (Lour.) Merr. (GPL), is a medicinal and edible plant within the genus *Gynura* [11]. It is predominantly distributed across several African nations, as well as Vietnam, Thailand, Indonesia and the southern regions of China. Phytochemical investigations have revealed that GPL is enriched with a wide spectrum of bioactive constituents, including polysaccharides, flavonoids and organic acids, which underpin its lipid-lowering, antioxidative and anti-inflammatory properties [12]. In parallel with the increasing global restriction on antibiotic growth pro-

moters, medicinal herbs have attracted considerable attention as functional feed additives in poultry nutrition, owing to their documented capacity to improve productivity and health outcomes [13]. For example, *Puerariae*, a well-recognized Chinese herbal medicine, has been extensively investigated and utilized in livestock and poultry husbandry due to its abundance of bioactive compounds and favorable safety profile [14]. To date, most studies on GPL extracts (GPLEs) have concentrated on its phytochemical constituents and pharmacological activities, particularly in vitro and in rodent models. However, its application in animal production systems remains scarcely documented, with only a limited number of investigations in poultry and even fewer specifically in laying hens [15]. This paucity of evidence represented a notable lacuna in the literature, especially given the growing interest in natural alternatives to antibiotics in livestock production. GPLEs has shown the promise as a potential feed additive that could enhance growth performance and health without the risks associated with antibiotic usage. Accordingly, the present study was designed to systematically evaluate, for the first time, the effects of dietary GPLEs supplementation on egg production, egg quality, antioxidant capacity and serum biochemical parameters in laying hens. By addressing this underexplored area, the study not only advanced the scientific understanding of GPLEs in avian species, but also contributed to the broader pursuit of sustainable, antibiotic-free strategies in poultry production.

## 2. Material and Methods

The trial was carried out in a farm setting at Institute of Animal Husbandry and Veterinary Science, Jiangxi Academy of Agricultural Sciences. All animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals established by the Jiangxi Academy of Agricultural Sciences.

### *2.1 Experimental Diets and Animal*

The basal diet for laying hens was formulated in accordance with the recommendations of the National Research Council (NRC, 1994) and the Chinese Chicken Feeding Standards (NY/T 33-2004). The formulation consisted of 62% corn, 24% soybean meal, 8.5% stone meal, 0.5% soybean oil, and 5% premix. Each kilogram of premix contained: Vitamin A 160,000 IU, Vitamin D3 80,000 IU, Vitamin $B_2$ 93 mg, Vitamin E 430 mg, Vitamin $K_3$ 50 mg, nicotinamide 600 mg, calcium pantothenate 290 mg, biotin 7 mg, calcium 15%, total phosphorus 3%, salt 7%, Mn 2000 mg, Fe 1400 mg, Zn 2000 mg, Cu 400 mg, Se 8 mg, and I 35 mg.

A total of 576 laying hens, 45 weeks of age and exhibiting comparable baseline egg-laying performance, were randomly allocated into four experimental groups, each comprising six replicates with 24 per replicate. The control group received only the basal diet, whereas the test groups were supplemented with GPLEs at inclusion level of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg, respectively. The trial included a one-week adaptation period followed by eight weeks feeding phase (GPLEs was obtained via water extraction, within the content of polysaccharides (55.67%) and total flavonoids (0.89%) in the extract were determined). The experimental hens were housed in a closed structure with three-layer, three-dimensional cages, under constant light for 16 hours, controlled by an automatic system. Artificial feeding was employed, with free access to food and water. The henhouse was disinfected and managed for epidemic prevention in compliance with established protocols. During the test period, the health and feeding status of the hens were observed daily [16].

### *2.2 Data Collection*

Eggs were collected manually on a daily basis, and records were maintained for the total number and weight of eggs, as well as the number and weight of defective eggs, throughout the experimental period. Further, the egg production rate, daily egg production, average egg weight, and qualified egg rate were calculated. Concurrently, feed intake was continuously monitored, allowing for the determination of average daily feed intake and feed-to-egg ratio.

At the end of the feeding trial, five eggs were randomly selected from each replicate, four of which underwent routine egg quality analyses, including measures of egg shape index, eggshell strength, eggshell thickness, egg yolk color and ratio, egg white height and Haugh units. The remaining egg was used to assess the nutritional quality, measuring moisture, crude protein, crude fat, cholesterol, trimethylamine, amino acid content and fatty acid composition.

After the experiment, two hens were randomly chosen from each replicate. After a 12-hour period of feed withdrawal, approximately 5 mL of blood were collected from the wing vein in the morning, after which the serum was separated for the assessment of biochemical, immune, and antioxidant indicators.

### *2.3 Data Analysis*

Data analysis was performed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA), employing one-way ANOVA, with the Least Significant Difference (LSD) test utilized for multiple comparisons among groups. Results are presented as the mean $\pm$ standard error of the mean (SEM), with $p$-values less than 0.05 denoting significant differences.

## 3. Results

### *3.1 Production Performance*

It can be clearly seen that the incorporating GPLEs into the diet of laying hens significantly increased the average egg weight ($p < 0.05$), compared to the control group (Table 1). By contrast, no discernible effects were observed

**Table 1. Effects of GPLEs on production performance of laying hens%.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Daily egg weight/(g/d) | 50.57 | 51.24 | 51.32 | 51.22 | 0.21 | 0.664 |
| Average egg weight/g | 62.63[b] | 63.04[a] | 63.06[a] | 62.94[a] | 0.05 | 0.003 |
| Qualified egg rate/% | 99.94 | 99.97 | 99.92 | 99.95 | 0.01 | 0.620 |
| Daily egg production/(egg/d) | 0.81 | 0.81 | 0.81 | 0.81 | 0.00 | 0.924 |
| Egg production rate/% | 80.74 | 81.29 | 81.39 | 81.39 | 0.34 | 0.928 |
| Average daily feed intake/(g/d) | 109.31 | 109.16 | 109.23 | 108.99 | 0.09 | 0.722 |
| Feed to egg ratio | 2.16 | 2.13 | 2.13 | 2.13 | 0.01 | 0.575 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$). GPLEs, (*Gynura procumbens* (Lour.) Merr) extracts; SEM, standard error of the mean.

on daily egg production, qualified egg rate, or overall egg-laying performance parameters. Similarly, the egg production rate, average daily feed intake and feed-to-egg conversion ratio GPLEs group did not differ significantly from the control group ($p > 0.05$). Notably, GPLEs has previously been reported as a supplement capable of supporting growth performance in laying hens.

The observed increase in egg weight may be attributable to the bioactive compounds of GPLEs, particularly flavonoids and saponins, which have been reported to enhance nutrient absorption and improve gastrointestinal integrity in poultry. More efficient nutrient uptake facilitated superior nutrient partitioning toward egg formation, thereby contributing to increased egg mass. Additionally, GPLEs possesses antioxidant and immunomodulatory properties [17], which may contribute to improved metabolic efficiency and overall laying performance. The hepatoprotective activity of certain GPLEs constituents may also augment hepatic function, thereby optimizing the synthesis of yolk precursors and albumen proteins that are indispensable for egg development. These findings are consistent with previous research demonstrating that dietary phytogenic additives can positively influence egg weight by modulating gut health, nutrient metabolism, and endocrine activity involved in egg production.

### *3.2 Egg Quality*

As presented in Table 2, dietary supplementation with GPLEs significantly enhanced yolk pigmentation in laying hens compared with the control group ($p < 0.05$). No other egg quality parameters were significantly affected ($p > 0.05$). Egg quality was generally evaluated through both internal and external attributes. The internal quality is determined by factors such as yolk characteristics, yolk color, and Haugh unit, whereas external quality encompasses egg shape index, eggshell strength, and egg ratio. The Haugh unit, a widely used indicator of freshness, is closely associated with shelf life. This study observed significant differences in yolk color, while other indicators did not.

Egg quality encompasses both external characteristics (e.g., shell strength, weight) and internal attributes (e.g., albumen viscosity, yolk color, nutrient composition). Yolk color is largely governed by the deposition of dietary carotenoids and other chromophoric compounds. GPLEs contains various phytochemicals, including flavonoids and chlorophyll-related compounds, which may contribute to enhanced yolks' pigmentation. Additionally, certain plant-based additives have been shown to augment the deposition of xanthophylls and related pigments in egg yolks, thereby intensifying yolk color without exerting measurable effects on other quality parameters. The absence of significant alterations in additional indices suggests that GPLEs, at the tested inclusion levels, does not perturb the physiological processes underpinning egg formation or structure integrity. These results corroborate previous findings indicating that herbal feed additives can selectively enhance yolk pigmentation while preserving overall egg quality [18]. Thus, GPLEs supplementation may represent a viable strategy to improve the aesthetic and commercial value of eggs through natural pigment enrichment, without adversely affecting other quality characteristics.

### *3.3 Egg Yolk Nutrition*

The evaluation of egg yolk nutrition is an essential parameter for assessing the effect of GPLEs supplementation in laying hens. As presented in Table 3, dietary inclusion of GPLEs significantly reduced yolk cholesterol content compared with the control group ($p < 0.05$), whereas moisture, crude protein, crude fat, and trimethylamine levels remained unaffected ($p > 0.05$). The hypocholesterolemic effect of GPLEs may be ascribed to its bioactive constituents, notably flavonoids and saponins, which have been reported to inhibit intestinal cholesterol absorption and enhance hepatic cholesterol metabolism [19]. Flavonoids can modulate lipid metabolism by influencing enzymes such as HMG-CoA reductase and increasing bile acid excretion, thereby reducing circulating cholesterol levels. The absence of significant alternations in other yolk components suggests that

**Table 2. Effect of GPLEs on egg quality.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| egg shape index | 1.33 | 1.35 | 1.35 | 1.35 | 0.00 | 0.236 |
| Eggshell strength/(N/m$^2$) | 39.89 | 41.91 | 42.00 | 42.75 | 0.66 | 0.564 |
| Eggshell thickness/mm | 0.39 | 0.39 | 0.38 | 0.38 | 0.00 | 0.188 |
| Haugh unit | 87.92 | 88.49 | 87.28 | 88.02 | 0.67 | 0.931 |
| Yolk color | 11.04$^b$ | 11.37$^a$ | 11.49$^a$ | 11.38$^a$ | 0.05 | 0.038 |
| Egg yolk ratio/% | 27.82 | 28.21 | 28.30 | 28.30 | 0.13 | 0.600 |
| Egg white ratio/% | 61.83 | 61.22 | 61.41 | 61.09 | 0.15 | 0.405 |
| Eggshell ratio/% | 10.35 | 10.57 | 10.28 | 10.62 | 0.08 | 0.451 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

**Table 3. Effect of GPLEs on conventional chemical components of egg yolk.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Moisture (%) | 49.57 | 49.75 | 49.25 | 49.61 | 0.10 | 0.410 |
| Crude protein (%) | 17.70 | 18.00 | 17.88 | 17.96 | 0.07 | 0.420 |
| Crude fat (%) | 10.61 | 10.53 | 10.52 | 10.38 | 0.06 | 0.646 |
| Cholesterol (mg/g) | 13.48$^a$ | 12.83$^b$ | 12.53$^b$ | 12.42$^b$ | 0.12 | <0.001 |
| Trimethylamine (μg/g) | 3.15 | 3.13 | 2.99 | 3.04 | 0.05 | 0.739 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

GPLEs may selectively lipid metabolic pathways without disrupting basic nutrient deposition. These findings were consistent with previous reports demonstrating that phytogenic additives can lower yolk cholesterol while maintaining overall egg quality and nutritional integrity, supporting the utility of GPLEs as a natural functional feed additive for the production of health-promoting eggs.

Furthermore, Table 4 indicated that the supplementation with 1000 mg/kg GPLEs did not significantly affect the amino acid content in egg yolks ($p > 0.05$). However, inclusion of 2000 mg/kg GPLEs significantly increased the serine content and reduced the glutamic acid content in egg yolks ($p < 0.05$). At 3000 mg/kg, GPLEs significantly elevated the levels of serine, alanine, and total amino acids in egg yolks ($p < 0.05$), yet it did not significantly influence the content of other amino acids ($p > 0.05$). These modifications may be attributed to the metabolic and physiological effects of the bioactive compounds in GPLEs, particularly flavonoids and phenolic compounds, which have been shown to influence amino acid metabolism and protein synthesis in animals [20]. The observed increase in serine and alanine levels may reflect enhanced hepatic synthesis or altered transamination reactions, as both amino acids are intimately linked to glucose and energy metabolism pathways. In addition, the antioxidant and hepatoprotective properties of GPLEs could optimize hepatic protein turnover, thereby modulating amino acid deposition into yolks. The reduction of glutamic acid at 2000 mg/kg may indicate a regulatory shift in nitrogen metabolism, potentially mediated by altered expression of amino acid transporters or feedback mechanisms governing amino acid utilization in the reproductive tract.

As it can be seen from Table 5, dietary supplementation with 1000 mg/kg GPLEs exerted no significant effect on the majority of yolk fatty acids, aside from modest effects on eicosanoid acid and arachidonic acid. By contrast, supplementation with 2000 and 3000 mg/kg GPLEs significantly increased yolk concentrations of palmitoleic acid, stearic acid, linoleic acid, $\alpha$-linolenic acid, arachidonic acid, DPA, total unsaturated fatty acids, polyunsaturated fatty acids, n3 polyunsaturated fatty acids, and the total amount of n6 polyunsaturated fatty acids and essential fatty acids while obviously reduced the total amount of palmitic acid, arachidic acid, and saturated fatty acids ($p < 0.05$), promoting the nutritional and health value of eggs. To our best knowledge, limited data were available regarding the impact of GPLEs on egg yolk lipid profiles in laying hens, underscoring the novelty of the present findings.

The observed alterations in egg yolk fatty acid composition may be mechanistically attributable to the bioactive components in GPLEs, such as flavonoids, phenolic acids, and saponins, which have been reported to modulate lipid

**Table 4. Effect of GPLEs on the amino acid content of egg yolks.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Aspartic acid (ASP), mg/g | 13.06 | 12.69 | 13.34 | 13.30 | 0.11 | 0.183 |
| Threonine (THR), mg/g | 6.80 | 6.71 | 6.81 | 6.91 | 0.05 | 0.599 |
| Serine (SER), mg/g | $11.06^{b}$ | $11.16^{b}$ | $11.69^{a}$ | $11.89^{a}$ | 0.09 | <0.001 |
| Glutamic acid (GLU), mg/g | $18.48^{a}$ | $18.42^{a}$ | $18.00^{b}$ | $18.49^{a}$ | 0.07 | 0.039 |
| Proline (PRO), mg/g | 5.30 | 5.33 | 5.42 | 5.21 | 0.04 | 0.226 |
| Glycine (GLY), mg/g | 4.42 | 4.72 | 4.57 | 4.71 | 0.06 | 0.253 |
| Alanine (ALA), mg/g | $7.35^{b}$ | $7.15^{b}$ | $7.26^{b}$ | $7.80^{a}$ | 0.07 | 0.001 |
| Cystine (CYS), mg/g | 2.47 | 2.50 | 2.42 | 2.52 | 0.02 | 0.557 |
| Valine (VAL), mg/g | 8.00 | 7.82 | 7.88 | 8.08 | 0.05 | 0.287 |
| Methionine (MET), mg/g | 3.90 | 4.13 | 3.99 | 3.98 | 0.04 | 0.206 |
| Isoleucine (ILE), mg/g | 6.52 | 6.12 | 6.54 | 6.48 | 0.14 | 0.758 |
| Leucine (LEU), mg/g | 12.00 | 11.95 | 12.05 | 12.11 | 0.04 | 0.667 |
| Tyrosine (TYR), mg/g | 4.82 | 4.78 | 4.82 | 4.62 | 0.03 | 0.075 |
| Phenylalanine (PHE), mg/g | 6.40 | 6.30 | 6.57 | 6.46 | 0.05 | 0.256 |
| Lysine (LYS), mg/g | 10.28 | 10.33 | 10.46 | 10.45 | 0.06 | 0.668 |
| Histidine (HIS), mg/g | 3.48 | 3.11 | 3.42 | 3.39 | 0.06 | 0.214 |
| Arginine (ARG), mg/g | 9.67 | 9.45 | 9.51 | 9.50 | 0.06 | 0.579 |
| Total amino acids (TAA), mg/g | $133.98^{bc}$ | $132.68^{c}$ | $134.75^{ab}$ | $135.90^{a}$ | 0.39 | 0.017 |
| Essential amino acid (EAA), mg/g | 67.03 | 65.92 | 67.23 | 67.36 | 0.22 | 0.093 |
| Flavor amino acid (FAA), mg/g | 54.52 | 54.07 | 54.55 | 55.39 | 0.19 | 0.096 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

**Table 5. Effect of GPLEs on the fatty acid composition of egg yolks.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Palmitic acid C16:0 | $24.822^{a}$ | $24.682^{a}$ | $23.824^{b}$ | $23.960^{b}$ | 0.122 | 0.001 |
| Palmitoleic acid C16:1n9 | $5.430^{c}$ | $5.460^{bc}$ | $5.652^{ab}$ | $5.698^{a}$ | 0.039 | 0.013 |
| Stearic acid C18:0 | $6.292^{b}$ | $6.308^{b}$ | $6.414^{a}$ | $6.523^{a}$ | 0.029 | 0.003 |
| Oleic acid C18:1n9 | 51.962 | 51.850 | 51.916 | 51.567 | 0.090 | 0.397 |
| Linoleic acid C18:2n6 | $9.375^{b}$ | $9.494^{b}$ | $10.012^{a}$ | $10.047^{a}$ | 0.074 | <0.001 |
| Alpha-linolenic acid C18:3n3 | $0.250^{c}$ | $0.268^{c}$ | $0.304^{b}$ | $0.328^{a}$ | 0.008 | <0.001 |
| Arachidic acid C20:0 | $0.120^{a}$ | $0.110^{b}$ | $0.098^{c}$ | $0.085^{d}$ | 0.003 | <0.001 |
| Eicosatrienoic acid methyl ester C20:3n3 | 0.302 | 0.306 | 0.298 | 0.305 | 0.004 | 0.916 |
| Arachidonic acid C20:4n6 | $0.578^{b}$ | $0.620^{a}$ | $0.620^{a}$ | $0.628^{a}$ | 0.007 | 0.022 |
| Docosadienoic acid C22:2 | 0.057 | 0.066 | 0.058 | 0.058 | 0.002 | 0.143 |
| DPA C22:5n6 | $0.157^{b}$ | $0.170^{ab}$ | $0.184^{a}$ | $0.178^{a}$ | 0.003 | 0.015 |
| DHA C22:6n3 | 0.133 | 0.134 | 0.142 | 0.142 | 0.002 | 0.283 |
| C24:1 | $0.058^{ab}$ | $0.064^{a}$ | $0.052^{b}$ | $0.060^{a}$ | 0.002 | 0.044 |
| Saturated fatty acids (SFA) | $31.698^{a}$ | $31.568^{a}$ | $30.782^{b}$ | $30.988^{b}$ | 0.120 | 0.007 |
| Unsaturated fatty acid (UFA) | $68.302^{b}$ | $68.432^{b}$ | $69.238^{a}$ | $69.012^{a}$ | 0.120 | 0.005 |
| Monounsaturated fatty acid (MUFA) | 57.450 | 57.374 | 57.620 | 57.325 | 0.087 | 0.690 |
| Polyunsaturated fatty acids (PUFA) | $10.852^{c}$ | $11.058^{bc}$ | $11.618^{ab}$ | $11.687^{a}$ | 0.087 | <0.001 |
| n3 polyunsaturated fatty acids | $0.685^{c}$ | $0.708^{bc}$ | $0.744^{ab}$ | $0.775^{a}$ | 0.010 | <0.001 |
| n6 polyunsaturated fatty acid | $10.110^{b}$ | $10.284^{b}$ | $10.816^{a}$ | $10.853^{a}$ | 0.079 | <0.001 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

metabolism at both the enzymatic and gene expression levels. Moreover, the antioxidant and anti-inflammatory properties of GPLEs may alleviate oxidative stress in hepatic tissues, thereby facilitating more efficient fatty acid elongation and desaturation pathways [21]. The increase in n-3 and n-6 PUFAs, including $\alpha$-linolenic acid and arachi-

**Table 6. Effect of GPLEs on biochemical indicators of laying hens in serum.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Total protein/(g/L) | 48.41 | 51.53 | 51.77 | 49.08 | 0.84 | 0.390 |
| Albumin/(g/L) | 15.60 | 16.08 | 16.08 | 15.37 | 0.20 | 0.541 |
| Globulin/(g/L) | 32.81 | 35.46 | 35.68 | 33.71 | 0.71 | 0.431 |
| Urea nitrogen/(mmol/L) | 0.33 | 0.36 | 0.35 | 0.34 | 0.01 | 0.770 |
| Total cholesterol/(mmol/L) | $2.48^{a}$ | $2.29^{ab}$ | $2.16^{b}$ | $1.86^{c}$ | 0.06 | 0.001 |
| Triglyceride/(mmol/L) | 7.77 | 7.48 | 6.71 | 6.14 | 0.25 | 0.097 |
| HDL/(mmol/L) | 0.52 | 0.69 | 0.67 | 0.64 | 0.03 | 0.090 |
| Low density lipoprotein/(mmol/L) | $1.08^{a}$ | $1.07^{a}$ | $0.88^{b}$ | $0.76^{b}$ | 0.03 | $<0.001$ |
| Alanine aminotransferase/(U/L) | $2.48^{a}$ | $2.37^{b}$ | $2.26^{c}$ | $2.26^{c}$ | 0.02 | $<0.001$ |
| Aspartate aminotransferase/(U/L) | $207.27^{a}$ | $195.08^{ab}$ | $181.50^{bc}$ | $178.83^{c}$ | 2.90 | 0.001 |
| Alkaline phosphatase/(U/L) | $5804.15^{b}$ | $5919.88^{a}$ | $5935.02^{a}$ | $5942.68^{a}$ | 18.20 | 0.022 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

donic acid, may also reflect enhanced intestinal absorption or greater mobilization of these fatty acids into yolks via circulatory transport. Collectively, these results highlight the potential of GPLEs to improve the nutritional quality of eggs by selectively modulating lipid and amino acid metabolism.

### *3.4 Serum Biochemical Indicators, Antioxidant Properties and Immune Performance*

As illustrated in Table 6, dietary supplementation with GPLEs exerted significant effects on the serum biochemical profile of laying hens. Inclusion of 1000 mg/kg GPLEs markedly reduced alanine aminotransferase (ALT) activity while elevating alkaline phosphatase (ALP) activity relative to the unsupplemented control. Higher supplementation levels (2000 and 3000 mg/kg) specifically decreased total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), while concomitantly increasing the activities of ALT, aspartate aminotransferase (AST), and ALP in serum ($p < 0.05$). As shown in Table 7 that the GPLEs can significantly promote the immune function and antioxidant performance of laying hens. Furthermore, the contents of immunoglobulin IgA, IgG, and glutathione peroxidase (GSH-Px) are significantly higher than those in the control group, while the content of malondialdehyde (MDA) was significantly lower than that in the control group ($p < 0.05$). Moreover, the effect was more significant when the addition level was at 2000 mg/kg and 3000 mg/kg, respectively, which could not only significantly reduce tumor necrosis factor $\alpha$ (TNF-$\alpha$) content, but also increase the activity of total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) ($p < 0.05$) in serum.

Flavonoids have been shown to stabilize hepatic enzyme activity, attenuate oxidative insults within the livers and modulate lipid metabolism by downregulating cholesterol synthesis pathways and promoting bile acid excretion. The observed reductions in serum cholesterol and LDL-C may be due to the inhibition of HMG-CoA reductase and enhanced clearance of circulating lipoproteins. The improved immune indices (IgA and IgG) may be attributed to the immunomodulatory effects of GPLEs, which can enhance B-cell activity and cytokine signaling. Moreover, the elevation in antioxidant enzymes such as GSH-Px and SOD, along with increased T-AOC, reflects enhanced systemic antioxidant defense, likely through the activation of Nrf2 signaling pathways and suppression of oxidative damage. The concomitant declines in MDA and TNF-$\alpha$ levels further substantiate the anti-inflammatory and anti-lipid peroxidation properties of GPLEs. These mechanisms are consistent with prior studies indicating that phytogenic feed additives can support both immune competence and oxidative homeostasis in poultry [21]. Taken together, the data provide compelling evidence that GPLEs supplementation confers multifaceted benefits, including modulation of hepatic function, improvement of serum lipid metabolism, reinforcement of immune competence, and augmentation of systemic antioxidant capacity. Collectively, these effects underscore the potential of GPLEs as a phytogenic feed additive that promotes metabolic health, immune resilience and oxidative homeostasis in laying hens.

## 4. Discussion

The current results suggested a dose-dependent association between dietary GPLEs supplementation and average egg weight, with significant differences observed at higher inclusion levels. In contrast to the present results, some previous reports stated that there are no relationships between egg production and the dose of GPLEs as well as no clear effect on the food intake [22]. The most plausible explanation is that the constituents (flavonoids and organic acids) in GPLEs may slow the progress of growth performance of laying hens. On the other aspect, these observed results may be due to the adaption of laying hens in different environments. Briefly, similar results mentioned above

**Table 7. Effect of GPLEs on immune and antioxidant properties of laying hens serum.**

| Items | Addition of GPLEs (mg/kg) | | | | SEM | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 3000 | | |
| Immunoglobulin A (IgA)/(g/L) | $0.21^{b}$ | $0.31^{a}$ | $0.34^{a}$ | $0.35^{a}$ | 0.01 | <0.001 |
| Immunoglobulin G (IgG)/(g/L) | $1.54^{b}$ | $2.17^{a}$ | $2.38^{a}$ | $2.47^{a}$ | 0.09 | <0.001 |
| Immunoglobulin M (IgM)/(g/L) | 0.12 | 0.12 | 0.12 | 0.12 | 0.00 | 0.952 |
| Cytokine 1$\beta$ (IL-1$\beta$)/(pg/mL) | 0.24 | 0.23 | 0.24 | 0.23 | 0.01 | 0.892 |
| Cytokine 6 (IL-6)/(pg/mL) | 1.32 | 1.32 | 1.29 | 1.27 | 0.03 | 0.924 |
| Tumor necrosis factor $\alpha$ (TNF-$\alpha$)/(pg/mL) | $2.19^{a}$ | $2.11^{ab}$ | $2.02^{bc}$ | $1.91^{c}$ | 0.03 | <0.001 |
| Total antioxidant capacity (T-AOC)/(U/mL) | $2.02^{b}$ | $2.05^{b}$ | $2.22^{ab}$ | $2.42^{a}$ | 0.05 | 0.007 |
| Superoxide dismutase (SOD)/(U/mL) | $82.87^{b}$ | $83.54^{b}$ | $84.68^{a}$ | $89.96^{a}$ | 0.84 | 0.007 |
| Glutathione peroxidase (GSH-Px)/(U/mL) | $1228.27^{b}$ | $1306.33^{a}$ | $1322.83^{a}$ | $1327.33^{a}$ | 12.61 | 0.017 |
| Malondialdehyde (MDA)/(μmol/mL) | $10.12^{a}$ | $8.25^{b}$ | $8.21^{b}$ | $8.00^{b}$ | 0.18 | <0.001 |

Note: In the same row, values without superscripts or with the same superscripts mean no significant difference ($p > 0.05$), while with different small letter superscripts mean significant difference ($p < 0.05$).

who reported that GPLEs had no effect on growth performance of laying hens, which is synchronized with results of the present study. Compared to other feed additives, GPLEs is an important alternative to antibiotics in animal diets as well as essential oils, chelates, spirulina, and saffron extracts as a feed additive in poultry nutrition, which provides demonstrable advantages in laying hens [23].

Previously, few reports demonstrated that GPLEs could affect the egg quality, which was possibly first found in the present study [24]. Specifically, yolk pigmentation was enhanced, while other traits such as the Haugh unit and egg shape index remained unaffected, suggesting a neutral mechanism on structural egg formation. These findings are in agreement with earlier studies that the GPLEs used in broiler chicken. As a result, the significant difference in egg quality was present in current study.

Proximate analysis revealed no difference in yolk moisture, crude protein, and fat between treatments, aside from a non-significant decline in crude fat content, consistent with reports that GPLEs administered via drinking water can modestly influence lipid deposition. In contrast, it has been reported that the protein content in eggs was increased due to GPLEs supplementation, which might be due to the high level of tested extracts that were used. The dietary treatments in this study significantly reduced the cholesterol levels in egg yolk. As cholesterol is synthesized in the liver, secreted as very-low-density lipoprotein (LPL), and deposited into the yolk via receptor-mediated endocytosis [25]. Thus, the decrease in cholesterol by GPLEs supplementation might be due to the consequences of altered endogenous lipoproteins (production or secretion) and/or cholesterol metabolism (i.e., synthesis, degradation, and distribution). Amino acid profiling further demonstrated that serine concentrations increased significantly with dietary GPLE, while glutamic acid levels declined at 2000 mg/kg [26]. What's more important, the level of total amino acids was clearly increased by the incorporating 3000 mg/kg GPLEs to diet. In addition, the fatty acids involved in egg yolks were also studied. Most of fatty acids with the supplementation at 2000 mg/kg and 3000 mg/kg existed statistical differences, compared to the control group. It can be concluded that GPLEs has the influence on the synthesis of fatty acid in egg yolks where the richness of flavones, polysaccharides and organic acids in GPLEs may regulate the metabolic pathway of fatty acid in laying hens. However, no reports explain the mechanism of GPLEs that affects fatty acids while other herbs used as supplementation in poultry have been announced that flavones can reduce proportion of SFA and increase PUFA [27]. This suggested that there would be a dose-dependent relationship between flavones and fatty acids and that the nutritional regulation in the composition of egg yolks.

Secondary metabolites present in herbal plants, such as flavonoids, flavanones, phenols, and saponins, have been proven to enhance immune function and regulate lipid metabolism in animals. For example, *Pueraria lobate* indicated the properties for the regulation of immunity by its abundance of flavones as well as effecting on lipid metabolism that biochemical indicators showed significant differences with variable dose feeding of *Pueraria lobate*. In our study, the findings were consistent with the results mentioned in literatures that GPLEs can clearly reduce the level of total cholesterol in high dose feeding. In addition, the level of low-density lipoprotein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase also has statistical difference after feeding high dose of GPLEs. In the present study, the dietary supplementation of GPLEs improved the immune status of laying hens by increasing the concentration of serum IgA and IgG. Similarly, the production of SOD and GSH-Px was also higher in the supplemented groups, while the level of MDA was opposite. These results were concordant with prior findings that the flavonoids and phenols in various GPLEs can regulate blood indices, and enhance immunity and antioxidant [28,29]. Despite the studies on the effects of herbal plants applied in poultry, the precise mechanism of action of in-

dividual secondary metabolites is unknown. Hence, further research is warranted to disentangle these complex interactions and clarify the molecular pathways through which GPLEs and related phytogenics exert their diverse effects on avian physiology.

## 5. Conclusions

In summary, the present study suggested that GPLEs held considerable promise as a phytogenic supplement for laying hens. Its influence on productive performance was evident across multiple dimensions, with higher inclusion levels exerting more pronounced effects on egg quality, yolk nutrient composition, immunological indices, and systemic antioxidant capacity. Collectively, it can be safely concluded that GPLEs would be a promising feed additive with its significant functions and wide range of sources in poultry.

## Availability of Data and Materials

The datasets generated and analyzed during the present study are available on reasonable request.

## Author Contributions

DW designed the research study and wrote the original draft of the manuscript. QLS and XTC performed the data. PWX, MCL, JPG and ZHZ analyzed the data. WPH and XLC conceptualized and supervised the research, reviewed and edited the manuscript, and contributed to critical revision of the manuscript. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee for Jiangxi Academy of Agricultural Sciences (approval number: 2024-JXAAS-XM-10). All animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals established by the Jiangxi Academy of Agricultural Sciences.


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## Conflict of Interest

The authors declare no conflict of interest.

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