RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 2: 326-337 (February 2026)

Effects of Pomegranate Peel Polyphenols on Egg Production and Health in Laying Hens

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10.18805/IJAR.BF-1913

ABSTRACT

Background: Pomegranate peel polyphenols (PPPs) is a by-product of pomegranate. It has many biological activities, but it has not been used in laying hens. The effect of pomegranate peel polyphenols on laying hens is unclear.

Methods: To understand the role of pomegranate peel polyphenols. We added different concentrations of pomegranate peel polyphenols to the diets of laying hens and studied the changes of growth performance, productivity performance and intestinal microbe during feeding.

Result: The PPPs were found to reduce the feed-to-egg ratio in laying hens. Medium (MP, 500 mg) and high (HP, 750 mg) concentrations of PPPs increased the apparent digestibility of crude protein and calcium. Compared to the control group (CON), all egg performances in the MP group showed significantly improvement. There was a significant increase in reproductive hormones in the MP group compared with the low concentration (LP, 250 mg) and CON groups. As the concentration of PPPs increased, lg Y, SOD and CAT activity increased. The PPPs increased Thyroxine levels significantly. In the LP and MP groups, TNF-α and IL-6 levels were significantly lower than in the CON and HP groups. The relative abundance of *Firmcutes* increased with the rise in PPPs addition. While *Bacteroidota* decreased with increasing PPPs concentration. It was concluded that PPPs had a promoting effect on the growth performance for laying hens. This study found the best effects of PPPs addition at the medium concentration because high concentrations would have adverse effects on laying hens' growth.

Key words: Growth performance, Gut microbiology, Laying hens, Pomegranate peel polyphenols, Reproductive hormones.

INTRODUCTION

Feed additives have been essential substances for the growth process of animals. Suitable feed additives have a significant effect on animal growth and disease resistance. Antibiotics used to be listed as feed additives in China and played an essential role in helping animals fight diseases (Yu *et al.,* 2018). China used to be a significant producer and consumer of antibiotics, but the misuse of antibiotics has adversely affected food safety in the farming industry (Hu *et al.,* 2018). Antibiotics are currently banned for use in farming industry in China (Li *et al.,* 2021), which is a massive challenge for the farming industry.

Plant extracts are environmentally friendly, safe, natural products with good biological activity and low toxicity (Zhou *et al.,* 2009). Studies have shown that plant extracts have the potential to replace antibiotics (Ding *et al.,* 2023) and have demonstrated promising results in improving animal immunity. Meanwhile, plant extracts also exhibit some advantages that antibiotics lack. Most plant extracts have shown sound growth-promoting effects (Lu *et al.,* 2022; Raissy *et al.,* 2022), attributed to their pro-feeding and pro-nutrient uptake abilities. Polyphenol is one of the essential plant extracts that has demonstrated good antioxidant capacity *in vitro* studies (Zhang *et al.,* 2022) due to its unique polyhydroxy structure. Several plant polyphenols have shown growth-promoting properties in animal production. Adding 2.34 g/kg of grape polyphenols to the diet increased chicken tocopherol concentrations and meat oxidative stability without impairing growth performance (Romero *et al.,* 2021). The addition of 350 or 500 mg/kg of resveratrol, an antioxidant polyphenolic compound found in plants, improved broiler growth performance under heat stress (He *et al.,* 2019). Over 8,000 polyphenols have been identified, but only a few are used for animal production.

Pomegranate peel is one of the essential by-products of pomegranate. Pomegranate peel extracts have multiple biofunctional activities (Peršurić *et al.,* 2020; Botteri *et al.,* 2022; Mo *et al.,* 2022), which are beneficial to human health and have potential applications in the fields of food, medicine, hygiene and nutraceuticals. Polyphenols are one of the main functional components of pomegranate peel (Gigliobianco *et al.,* 2022) and pomegranate peel

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How to cite this article: Hongrui, Xu., Huang, A., Guo, L., Nie, F., Hou, J., Zengxin, Lv. and Dang, M. (2026). Effects of Pomegranate Peel Polyphenols on Egg Production and Health in Laying Hens. *Indian Journal of Animal Research*. **60(2):** 326-337. doi: 10.18805/IJAR.BF-1913.

Submitted: 30-10-2024 **Accepted:** 12-03-2025 **Online:** 29-04-2025

polyphenols (PPPs) can be obtained by solvent extraction, ultrasound-assisted extraction and microwave-assisted extraction (Nayak *et al.,* 2015). China is currently the world's largest producer of pomegranates, with the country's total output in 2019 alone already reaching 1 million tons. The PPPs have high potential value, but few studies have been conducted on using PPPs in animal production. There is insufficient research to support PPPs as a safe feed additive. This research aimed to investigate the utilization of PPPs as feed additives in the diets of laying hens, assess their effect on the growth performance and microbiota and expedite the integration of PPPs into animal production practices.

MATERIALS AND METHODS

Preparation of polyphenols extract

The extraction method of pomegranate peel polyphenols was modified with reference to the method of Adithya Sridhar and Takahiro Tsujita (Adithya *et al.,* 2021; Takahiro *et al.,* 2011). The pomegranate peel was dried and ground using a pulverizer, then passed through a 40-mesh sieve. The powder was mixed with 30% ethanol at a solid-to-liquid ratio of 1:40. The mixture was stirred at 70°C for 2 h and the supernatant was collected after centrifugation. The residue was then re-mixed with 30% ethanol at the same solid-to-liquid ratio (1:20) and stirred for an additional 2 h. The supernatant from this second centrifugation was combined with the first supernatant. The concentrate was collected and freeze-dried to obtain pomegranate peel polyphenols. Under these conditions, the total phenol content was (232.016±3.15) mg GAE/g.

Experimental design

The experiment was conducted with 300 Jing Hong laying hens, 310 d of age, healthy and in the same body condition. The hens were divided into four groups of 75. There were 5 replicates per group and 15 hens per replicate.

The CON group (CON) were fed the normal diet, while experimental groups received additional low (LP, 250 mg), medium (MP, 500 mg) and high concentration (HP, 750 mg) of PPPs, respectively. The total experimental period was seven weeks, including a pre-test period of one week and a formal test period of six weeks. The basal diet was fed during the pre-test period and different concentrations of PPPs were added to the experiment groups during the formal test period.

Feeding management and diet formulation

Animal testing was conducted by Henan University of Animal Husbandry and Economy in 2023. The feeding experiments were carried out in the egg farm of Henan De An Agricultural. The laying hens in this experiment were raised in cages with water and feed ad libitum. The experimental house was equipped with a 4-layer stepped cage, with 72 cages in each layer and 3 hens in each cage and the feed was fed four times a day. The immunization procedures were carried out in accordance with the daily management of the farm. The daily feeding and management of laying hens was carried out in accordance with the procedures of commercial laying hens. The temperature, humidity, light and hygiene in the chicken house were in accordance with the agricultural industry standard of the People's Republic of China for experimental animals-environmental and facility standards: GB/T14925-1994 hygienic requirements for egg-laying hen rearing. The composition and nutritional levels of the basal diets are shown in Table 1.

Production performance

Record egg production at 9:00 a.m. daily and calculate egg production rate. The average daily feed intake (ADFI) and Feed-to-Egg (F/E) ratio of laying hens was calculated by counting their feed consumption, recycling and weighing leftover feed each week. Average egg weight calculated by dividing the total egg weight by the number of eggs. Before the end of the formal trial period, each laying hens was weighed once again.

Egg quality measurement

Eggs from the last three days of the formal trial period were collected. From each replicate, five eggs were randomly selected for egg quality determination. A multifunctional egg quality analyzer determined Haugh Unit and egg white height. Eggshell strength was determined by an eggshell strength tester (KQ-1A, Nannong Animal Husbandry Technology Co. Ltd.) and eggshell thickness, egg shape index and yolk index were determined by vernier caliper.

Determination of apparent digestibility

The metabolic experiment was conducted for 3 d before the end of the formal trial period. Three healthy laying hens were selected for each replication and clean fecal collection trays were placed under the cage. Fecal samples were collected using the total collection method (Oh *et al.,* 2020). Proteins were fixed by adding 10% sulfuric acid solution immediately after collection.

Table 1: Composition and nutrient levels of the basal diet (Dry basis).

Items	Content (%)
Corn	62.77
Soybean meal	20.91
Rapeseed meal	2.29
Soybean oil	1.99
Methionine	0.08
Mountain flour	8.96
Premix	3.00
Total	100
Nutritional levels	
ME/(MJ/kg)	13.9
CP	16.2
TP	0.4
Ca	3.6
Met	0.37
Lys	0.71

Note: The premix provided the following for per kg of the diets: VA 267 000 IU, VD3 50 000 IU, VE 25 IU, VK3 17 mg, VE 300 IU, Mn 2 g, Fe 2 g, Cu 0.3 g, Zn 2 g, I 10 mg, Se 10 mg, DL-Met 1 g. Nutrient levels were calculated.

The fecal and feed samples were dried at 105°C by oven and crushed through a 0.5 mm mesh screen and nutrients were measured on the feed and fecal samples to calculate the apparent digestibility.

$$\text{Apparent digestibility (\%)} = \frac{\text{Intake - Excretion}}{\text{Intake}} \times 100$$

Reproductive hormones and thyroxine estimation

At the end of the of the formal trial period, one hen from each replicate was randomly selected for blood collection and serum isolation to determine the levels of reproductive hormones and thyroxine, egg yolk immunoglobulin (IgY), estradiol (E2), luteinizing hormone (LH), triiodothyronine (T3) and thyroxine (T4), follicle stimulating hormone (FSH), were determined by ELISA kits (Shanghai, Enzyme-linked Biotechnology Co., Ltd).

Estimation of serum immunity indices

Levels of IL-6, TNF-α, lgA and lg M, which were all determined by ELISA kits (Shanghai, Enzyme-linked Biotechnology Co., Ltd).

Estimation of serum antioxidant indices

The GSH-px, CAT, SOD and MDA levels in serum were determined by commercial kits (Beijing, Solarbio Science and Technology Co., Ltd).

Serum enzyme activity and calcium estimation

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) were measured by commercial kits (Beijing, Solarbio Science and Technology Co., Ltd).

Serum calcium was determined by the methyl thymol blue colorimetric method (Yan *et al.,* 2022).

Microbial sequencing analysis

At the end of the formal trial period, one hen was randomly selected from each replicate for slaughtering. Colon contents were placed in sterile 5 mL freezing tubes, rapidly cooled with liquid nitrogen. Subsequently stored in a refrigerator at a temperature of -80°C. The colon contents were sent to Wuhan Bena Technology Co. for microbial sequencing.

Data analysis

Statistical analysis of data was performed using one-way ANOVA by SPSS 16.0 software. A $P<0.05$ was considered a significant difference. Graphics were drawn using Origin 8.0 software. Graphics for microbiological analysis were plotted using *R* 3.5.1 software.

RESULTS AND DISCUSSION

Effect of PPPs on growth performance

The addition of PPPs significantly reduced the ratio of F/E ($P<0.05$, Fig 1B). In the MP group, egg production was significantly higher than in the CON group ($P<0.05$, Fig 1C). Meanwhile, in the LP and HP groups, the final body weight (FBW) was significantly higher ($P<0.05$, Fig 1D).

Effect of PPPs on apparent digestibility

The PPPs significantly increased the apparent digestibility of crude protein (CP, $P<0.05$, Fig 2C). Additionally, the apparent digestibility of dry matter (DM) and calcium were significantly higher in the MP and HP groups compared to the CON and LP groups ($P<0.05$, Fig 2A, B).

Effect of PPPs on egg quality

The albumen height of the MP group was significantly higher than that of the LP and CON groups ($P<0.05$, Fig 3A). Additionally, compared to the HP and CON groups, the MP group had significantly higher egg weights ($P<0.05$, Fig 3B). Furthermore, the MP group exhibited significantly higher eggshell strength and thickness than the other experimental groups ($P<0.05$, Fig 3D, E). Moreover, the yolk height and haugh unit were significantly higher in the PPPs group than in the CON group ($P<0.05$, Fig 3C, F).

Effect of PPPs on serum enzyme activities and calcium levels

The figure shows that the MP group had significantly lower AKP levels than the other groups ($P<0.05$, Fig 4A). In contrast, the LP group had the highest AKP level ($P<0.05$, Fig 4A). Additionally, in the MP group, serum calcium levels were significantly higher than in the other groups ($P<0.05$, Fig 4B). Moreover, compared to the CON group, the LP and HP groups had significantly higher serum calcium levels ($P<0.05$, Fig 4B). Furthermore, serum ALT activity decreased with the addition of increasing PPPs and the serum AST activity was significantly higher in the MP group ($P<0.05$, Fig 4C,D).

Effect of PPPs on serum reproductive hormones

The figure illustrates that serum E2 was significantly higher in the MP group than in the other groups ($P<0.05$, Fig 5A). Additionally, FSH activity was significantly higher in the MP and HP groups than in the LP and CON groups ($P<0.05$, Fig 5B). Furthermore, LH levels in the LP and MP groups were significantly higher than in the other two groups ($P<0.05$, Fig 5C). However, lgY levels in the LP and MP groups were significantly lower than in the CON and HP groups ($P<0.05$, Fig 5D).

Effect of PPPs on serum thyroxine

There was a s significant difference in T3 levels in the MP group compared to the other three experimental groups ($P<0.05$, Fig 6A). Furthermore, the three PPPs groups had significantly higher T4 levels than the CON group ($P<0.05$, Fig 6B). Meanwhile, the MP group had significantly lower T4 levels than the LP and HP groups ($P<0.05$, Fig 6B).

Effect of PPPs on serum antioxidant capacity

As shown in Fig, CAT activity was significantly lower in the HP group ($P<0.05$, Fig 7A). Additionally, MDA levels in the MP group were significantly lower than those in the HP and CON groups ($P<0.05$, Fig 7B). Furthermore, SOD activity in the PPPs group increased with the increase of PPPs addition and the difference between the groups was significant ($P<0.05$, Fig 7D).

Effect of PPPs on serum immunity capacity

As depicted in figure, the LP and HP groups exhibited significantly higher levels of IgA than the CON group (P<0.05, Fig 8A). Additionally, when compared to the CON and HP groups, the LP group had a significantly higher level of IgM (P<0.05, Fig 8C). Conversely, IL-6 and TNF-α levels were significantly lower in the LP and MP groups than in the CON and HP groups (P<0.05, Fig 8B, D).

Effect of PPPs on microbial α-diversity

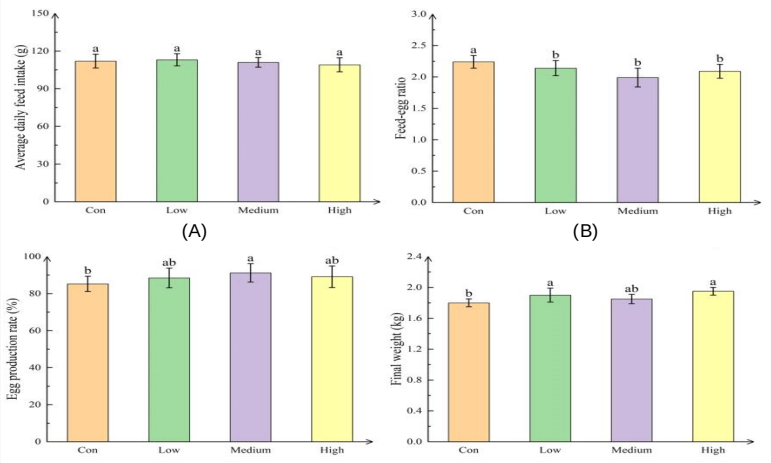
The figure illustrates that the LP group had significantly lower ACE index and Chao1 index than the other three groups (P<0.05, Fig 9A, C). The Simpson index was not significantly influenced by PPPs, but the Shannon index was significantly lower in the LP group than in the HP group (P<0.05, Fig 9B, D).

Effect of PPPs on microbial β-diversity

The Venn figure illustrates similarities in microbial structure among the four groups, with notable differences (Fig 10A). The total number of identical OTUs in the four groups was 618 and the number of OTUs unique to each group increased with the amounts of PPPs added. PCA analysis showed differences among the groups with varying concentrations of PPPs, while still maintaining some similarity with the CON group (Fig 10B).

Effect of pomegranate peel polyphenols on microbial community structure

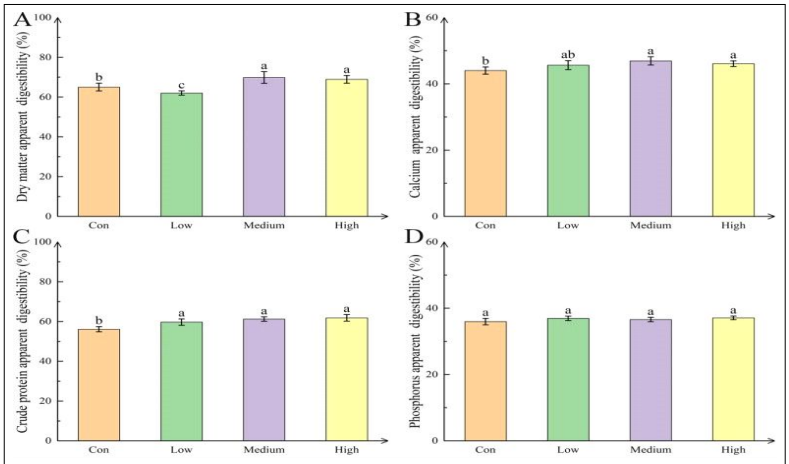
The figure shows that the relative abundance of *Firmcutes* increased with the rise in PPPs addition (Fig 11A). While Bacteroidota decreased with increasing PPPs concentration. The relative abundance of Fusobacteriota was significantly



(A) Average daily feed (B) Feed- egg ratio (C) Egg production (D) Final weight.

Note: The same lowercase letter expresses that the difference is not significant and different lowercase letters indicate that the difference is significant in Fig 1-9.

Fig 1: Effect of PPPs on the growth performance.

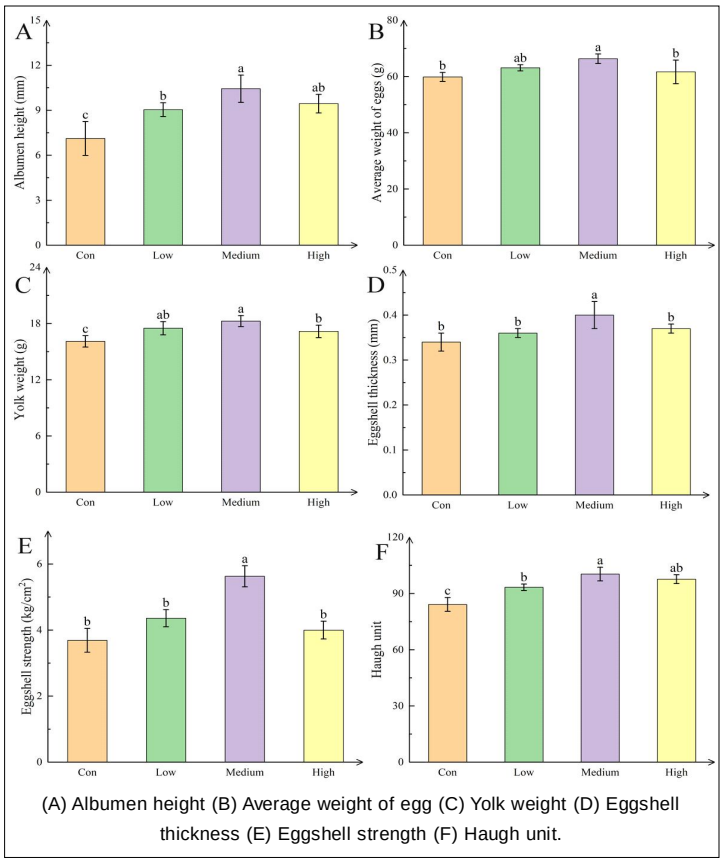


(A) Dry matter apparent digestibility (B) Calcium apparent digestibility (C) Crude protein apparent digestibility (D) Phosphorus apparent digestibility.

Fig 2: Effect of PPPs on apparent digestibility.

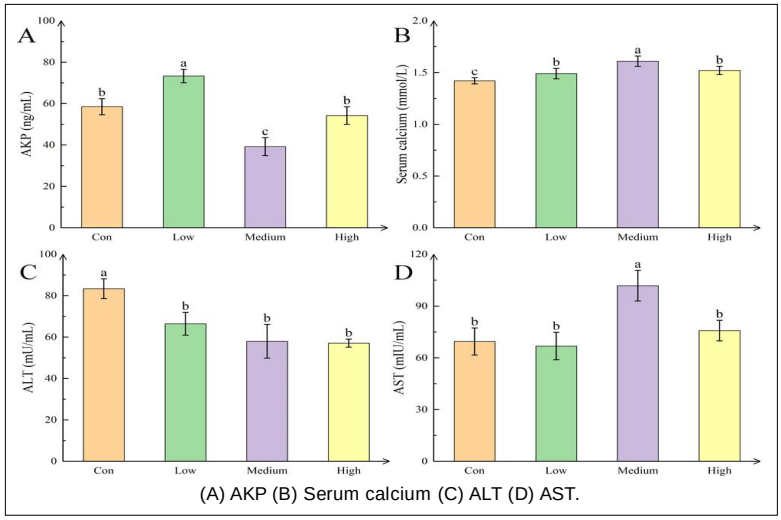
lower in the MP group than in the other groups. Meanwhile, the relative abundance of proteobacteria in the MP group was significantly higher than the other groups.

Microorganisms significantly affected by PPPs can bemore precisely identified at the species level (Fig 11B). The relative abundance of *Megamonas hypermegale*, *Bacteroides salanitronis*, *Bacteroides gallinaceum* and *Bacteroides caecigallinarum* in the MP group was significantly higher than the other groups. The relative abundance of *Desulfovibrio sp* and *Bacteroides plebeius* was significantly lower in the MP group than in the other groups. The relative abundance of *Firmicutes bacterium* and *Clostridium spiroforme* increased with increasing PPPs concentrations.



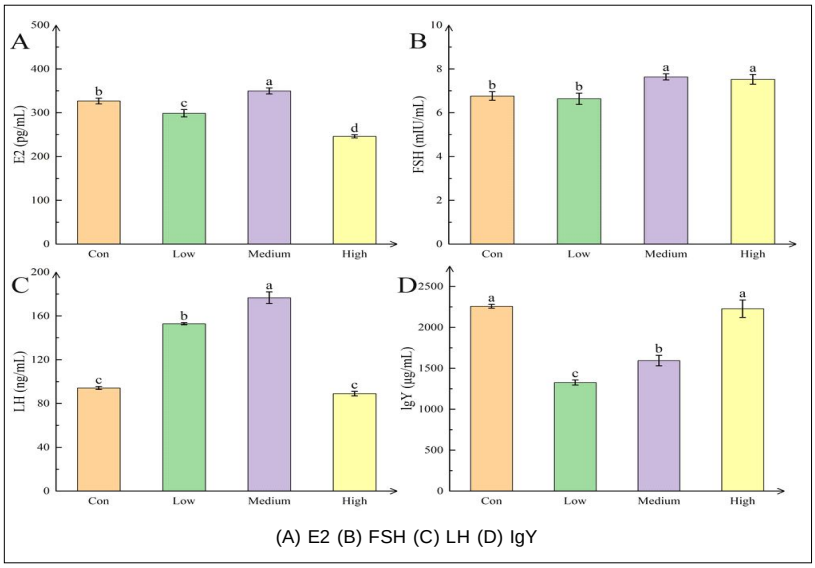
(A) Albumen height (B) Average weight of egg (C) Yolk weight (D) Eggshell thickness (E) Eggshell strength (F) Haugh unit.

Fig 3: Effect of PPPs on egg quality.



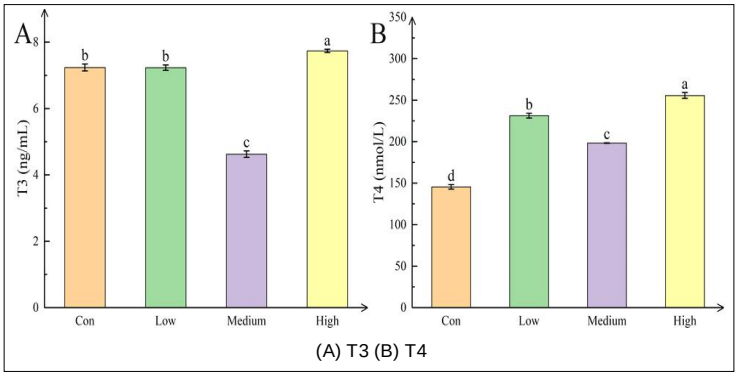
(A) AKP (B) Serum calcium (C) ALT (D) AST.

Fig 4: Effect of PPPs on serum enzyme activities and calcium levels.



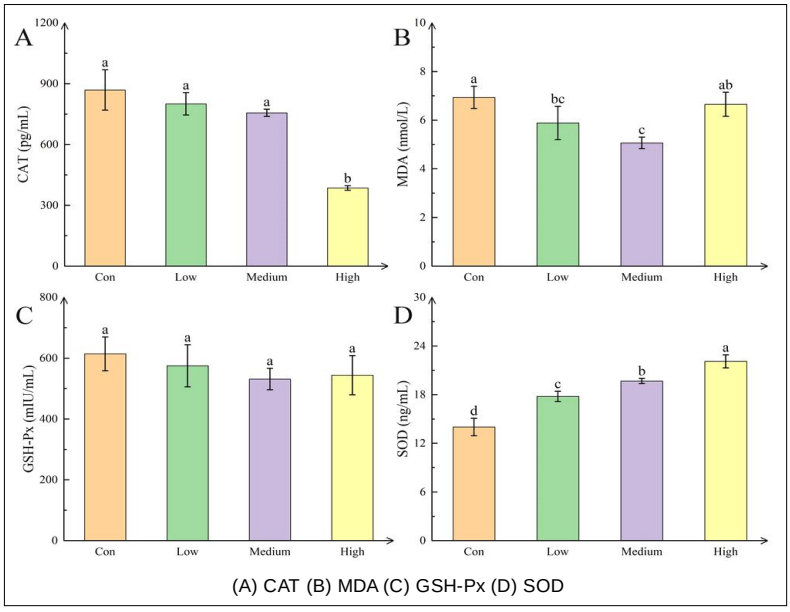
(A) E2 (B) FSH (C) LH (D) IgY

Fig 5: Effects of PPPs on reproductive hormones.



(A) T3 (B) T4

Fig 6: Effect of PPPs on thyroxine.



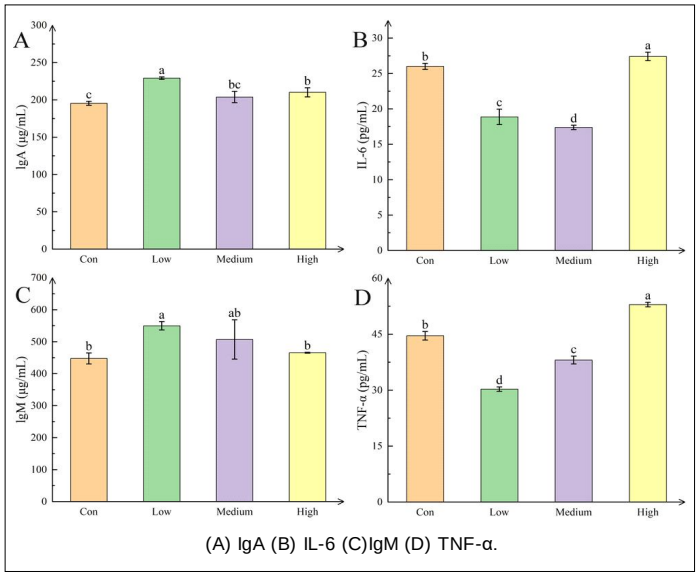
(A) CAT (B) MDA (C) GSH-Px (D) SOD

Fig 7: Effect of PPPs on serum antioxidant capacity.

Microbiological correlation analysis

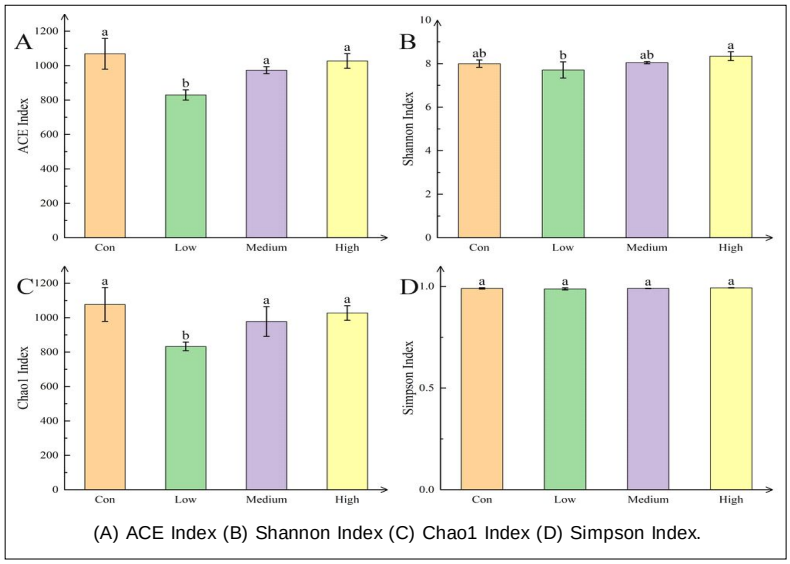
Spearman's correlation coefficient was used to analyse the correlation between microbial species levels and to generate heat maps. Fig 12 shows a significant correlation between antioxidant capacity and immuno competence and microorganisms in laying hens. There was a significant negative correlation between *Alloprevotella*, *Anaerotruncus*, *Candidatus vestibaculum*, *Succinatimonas* and IL6. The correlation between *Anaerostipes* and SOD, Ig A, Ig M was significantly negative but with Ig Y it was significantly positive. Between *Barnesiella* and MDA, TNF-α, Ig Y and IL6 is a significant positive correlation. A strong link can be seen between microorganisms and antioxidant and immune competence.

Plant polyphenols, as essential active plant extracts, are known to promote animal growth performance (Singh *et al.,* 2021; Chen *et al.,* 2024; Rao *et al.,* 2023). In the present study, the laying rate and final body weight of layinghens in the PPPs group were enhanced compared to the CON group. However, there was no significant difference in feed intake among the groups. Studies have shown that plant polyphenols can improve feed utilization in poultry (Shakeri *et al.,* 2020; Qin *et al.,* 2024). Therefore, we determined the apparent digestibility and found that the PPPs group had improved the apparent digestibility of CP and DM, consistent with previous studies. This means that PPPs promote nutrient uptake in laying hens to improve growth performance.



(A) IgA (B) IL-6 (C)IgM (D) TNF-α.

Fig 8: Effect of PPPs on serum immune indices.



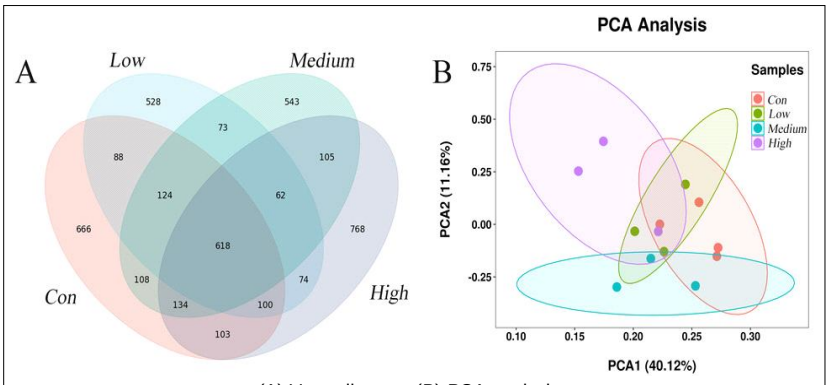
(A) ACE Index (B) Shannon Index (C) Chao1 Index (D) Simpson Index.

Fig 9: Effect of PPPs on microbial á-diversity.

Meanwhile, we found that the apparent digestibility of calcium was significantly higher in the MP and HP group than in the CON group. Calcium is eggshells' main building block and significantly affects eggshell strength and thickness (Vijay *et al.,* 2021). The increased serum calcium levels and apparent digestibility of calcium in laying hens in the PPPs group indicated that PPPs promoted calcium absorption in laying hens. This was confirmed by significantly higher eggshell strength and thickness in the MP group than the CON. The PPPs improved both egg white height, yolk height and haugh unit, with the best results obtained with MP group, which may be related to improved apparent digestibility of nutrients.

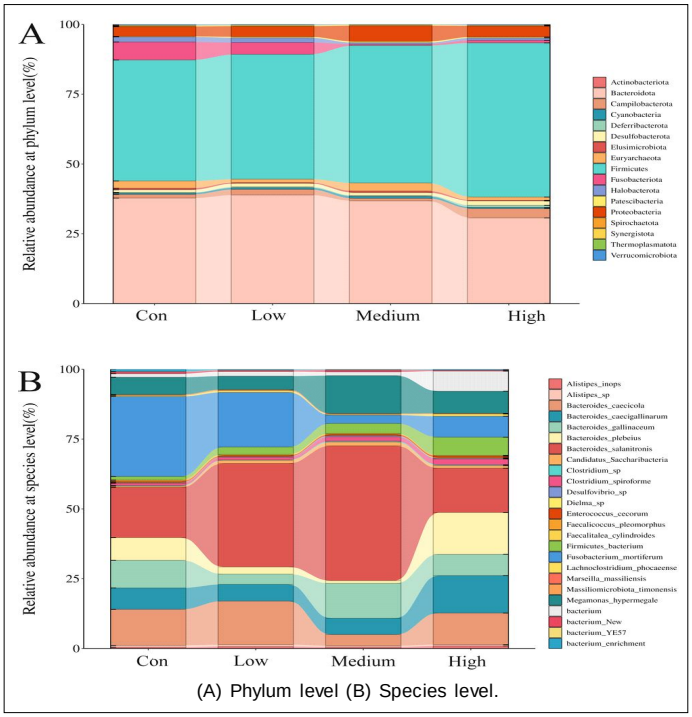
As an essential defense organ of the organism, the liver is resistant to exogenous additives, which may produce liver damage in animals (Ye *et al.,* 2018). Liver damage releases large amounts of metabolites, such as ALT, into the bloodstream (Shao *et al.,* 2022). Changes in metabolites such as ALT and AST in the blood can directly reflect liver damage. A significant decrease in ALT levels observed in three PPPs groups compared with the CON group. This suggests that PPPs have the ability to protect the liver.

Reproductive hormones (E2, FSH and LH) in laying hens directly impact egg production and F/E ratio (He *et al.,* 2022). The significant improvement in F/E ratio and egg production rate of the PPPs group might be attributed to



(A) Venn diagram (B) PCA analysis.

Fig 10: Venn diagram with microbial β-diversity analysis.

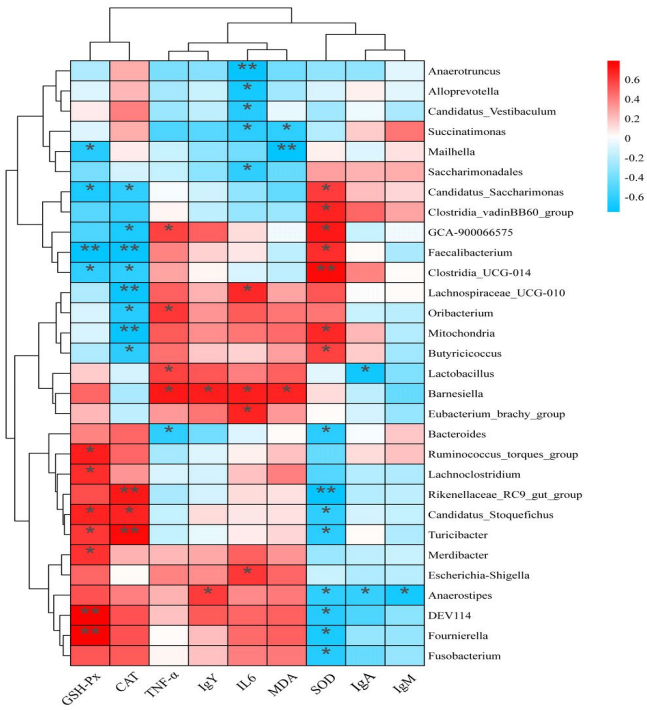


(A) Phylum level (B) Species level.

Fig 11: Structural composition of microorganisms at phylum level and species level.

increased E2, FSH and LH levels of serum in the PPP groups, indicating improved reproductive performance of the laying hens. However, studies have shown that excessive polyphenol concentrations can instead impair reproductive performance (Fraser, 2008). Similarly, in present study also levels of reproduction hormones and corresponding egg production performance, decreased at higher supplementation rate in HP group above medium level of supplementation in MP groups. Thyroid hormones are mainly involved in the body's material metabolism processes (Rafique *et al.,* 2020). T4 is the main secretion product of the thyroid gland, which is relatively inactive and is converted into the highly active T3 by 52 deiodinase (Mancini *et al.,* 2005). Studies have shown that serum T3 decreases under stress. (Meng *et al.,* 2022). In contrast, the addition of thyroxine to the diet improves egg production in laying hens, which is attributed to the fact that thyroid hormones enhance the body's metabolism and promote the absorption and utilization of glucose by the cells. (Yehuda-Shnaidman *et al.,* 2014; Rastogi *et al.,* 2015). The organism's enhanced nutrient utilization efficiency leads to an increase in production performance. In the present study, we found that PPPs increased serum T3 and T4 levels, which helped to improve nutrient utilization in laying hens, in line with our previous findings of increased apparent digestibility of CP and DM.

The most crucial function of plant polyphenols is improving the body's antioxidant capacity and immunity. (Taher *et al.,* 2022; Liao *et al.,* 2019; Khan *et al.,* 2019). Several studies have confirmed that plant polyphenols exhibit good antioxidant capacity in vivo and *in vitro*. Immunity and antioxidant capacity are closely related (Liu *et al.,* 2019). Plant polyphenols indirectly enhance immunity by increasing antioxidant capacity. PPPs showed similar effects in the present study. PPPs increased serum SOD activity and decreased MDA levels. However, CAT activity was significantly lower in the HP group than in the CON group, which suggests that high concentration of PPPs may have side effects on the organism. Changes in serum immunocompetence in the PPPs group were similar to antioxidant capacity. Low and medium concentrations of PPPs reduced the levels of TNF-α, but high concentration of PPPs did not show similar results. Meanwhile, the LP and MP groups reduced the levels of pro-inflammatoryfactor IL-6. The HP group were not significantly different from the CON group, however. The high concentration PPPs group had significantly higher levels of lg A than the CON group, but the lg M level did not differ significantly. The above results indicate that PPPs can improve antioxidant capacity and immunity in laying hens. There was a significant correlation with the concentration of PPPs.



Note: The color bar indicates the correlation of the indicators (positive correlation, red; negative correlation, blue). **P*<0.05; ***P* < 0.01; ****P*<0.001 using Spearman correlation in Fig 12.

Fig 12: Correlation analysis of microorganisms with immune and antioxidant indicators.

The colonic microorganisms of laying hens have significant effects on nutrient absorption and immune performance (Xie *et al.,* 2020). Many studies have shown that plant polyphenols can significantly affect the structure of intestinal microbial communities (Scarsella *et al.,* 2020; Sweeney *et al.,* 2022). Polyphenols are only partially degraded in the stomach; the vast majority can enter the intestinal tract to work. (Jakesevic *et al.,* 2011; Han *et al.,* 2020). Therefore, polyphenols can indirectly alter laying hens' nutrient uptake and immunity by influencing the structure of the colonic microbial community. α-diversity structure showed that PPPs influenced the colonic microbial community structure of laying hens and that low of PPPs reduced the microbial community abundance. β-diversity structure also showed that PPPs influenced the microbial community structure and there was a difference between the groups of different PPPs concentrations. *Firmicutes* play a crucial role in nutrition and metabolism through short chain fatty acid synthesis (Jin *et al.,* 2024). PPPs significantly up-regulated the relative abundance of *Firmicutes*. PPPs also inhibit the growth of harmful bacteria, including *Fusobacteria*, *Campylobacter* and *Fusobacteria mortiferum*. Meanwhile, PPPs also inhibit the growth of some bacteria that utilize nutrients, including *Halobacterota*, to achieve a high efficiency of nutrient utilization in the intestinal tract, especially the utilization of amino acids. While inhibiting harmful bacteria, PPPs promoted the growth of some probiotics, especially at MP group, where the most significant performance was observed. *Megamonas hypermegale* can ferment various carbohydrates (Shang *et al.,* 2020), with the end products being acetic, propionic and lactic acids. Intestinal epithelial cells will eventually absorb short chain fatty acids, enhancing the body's immunity (Ren *et al.,* 2022). Medium concentration of PPPs promoted the growth of this bacterium, but high concentration of PPPs, on the contrary, did not show promotive results. The heat map also showed that the structure of the colonic microbial community is closely related to antioxidant properties and immunity. This is because various metabolites of microorganisms can enter the bloodstream through the intestinal barrier, directly affecting the organism's antioxidant and immune properties. Therefore, PPPs can inhibit the growth of harmful bacteria and promote the growth of intestinal probiotics. The change is closely related to the effect of PPPs addition.

CONCLUSION

The addition of PPPs to the diets of laying hens improved gut health by regulating the gut microbial community. Additionally, PPPs increased antioxidant capacity and immune function by promoting probiotics and inhibiting harmful bacteria. This, in turn, enhanced nutrient utilization, particularly the absorption of dry matter (DM), crude protein (CP) and calcium, which positively impacted metabolic and immune status. The improved nutrient absorption contributed to better reproductive hormone levels, promoting increased egg production and improved egg quality. However, the effect of PPPs did not increase with the rise of concentration and the high concentration of PPPs did not perform significantly more potent than the medium concentration of PPPs in all aspects, especially the levels of the LH and E2, which were instead reduced. Specifically, PPPs enhanced eggshell strength and thickness through better calcium utilization. Therefore, the optimal concentration of PPPs in this study was medium. PPPs have good prospects for application as natural extract additives.

ACKNOWLEDGEMENT

This present study was supported by the National Key Research and Development Program of China (2018YFD 0501103).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The experiment was conducted in accordance with the Guide for Care and Use of Laboratory Animals of the National Research Council, China (GB/T 35892-2018). The experiment was approved and supervised by the Experimental Animal Ethics Committee of Henan University of Animal Husbandry and Economy.

Authors contribution

Hongrui Xu: Conceptualization, Methodology, Writing, review and editing, Supervision. Zengxin Lv, Mengyan Dang: Writing original draft, Experimental operation. Anqun Huang, Liangxin Guo, Furong Nie, Jianan Hou: Methodology, Writing, review and editing. All authors have read, improved and approved the manuscript.

Data availability

Data will be made available on request.

Conflict of interest

The authors declare that there are no conflicts of interest.

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