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Original Article

Effects of Bamboo Vinegar on Production Performance, Egg Quality, Antioxidant Status, and Serum Biochemistry of Laying Hens in Summer

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■Keywords

Bamboo vinegar, egg quality, antioxidant, laying hen.

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ABSTRACT

This study investigated the effects of bamboo vinegar (BV) on production performance, egg quality, antioxidant status, and serum biochemical parameters of laying hens during summer. One hundred twenty 36-week-old Hy-Line Brown laying hens were randomly assigned to 4 groups, with 30 hens per group. The control group was fed a basal diet and 1 mL/kg, 5 mL/kg, and 10 mL/kg BV were added to the basal diet of the 0.1% BV, 0.5% BV and 1.0% BV groups, respectively. The experiment lasted 30 days and showed that the production performance was not significantly affected by the addition of BV (p>0.05). Egg shape index and glutathione peroxidase activity in serum in BV-supplemented groups were significantly increased (p<0.05). The total protein and the albumin content in the 0.1% BV, 0.5% BV, and 1.0% BV groups were significantly increased compared to the control group (p<0.05). These results suggested that dietary BV could improve the antioxidant capacity and the egg shape index of laying hens in summer.

INTRODUCTION

Increasing summer temperatures under the influence of the global greenhouse effect have been an important factor interfering with the production of traditional farming industries (Zaboli *et al.,* 2019; Rubio *et al.,* 2021). The avian egg industry was being severely affected because of birds' unique physiological structure. Birds regulate body temperature through feathers rather than sweat glands, and they cannot regulate body temperature at high temperatures, especially during the period of higher metabolism of egg production (Emami *et al.*, 2020). Once temperature and humidity exceed the tolerance of laying hens, they enter into a state of heat stress. In heat stress conditions, laying hens develop symptoms of lethargy, reduced feed intake, gastrointestinal disorders, and reduced egg production. Notably, the efficiency of calcium absorption by hens is substantially reduced under gastrointestinal stress, leading to poor eggshell quality and declining egg production rates.

Moreover, studies have proven that increasing the calcium content of the feed cannot improve eggshell quality and even negatively affects absorption (Wasti *et al.,* 2020). Among the strategies to alleviate heat stress, nutrition regulation has been the most commonly used one in productive contexts. Many researchers reported the positive effects of feeding acidifiers on chickens: their research found that acidifiers could improve intestinal digestion and absorption by decreasing intestinal pH, intestinal digestive enzyme activity, and intestinal villi, thereby reducing the damage caused by high temperatures in laying hens (McWhorter *et al.,* 2020; Nielsen & Knochel, 2020; Gao *et al.,* 2021).

Bamboo vinegar (BV) acetic acid is an organic acid containing various components (Singhal *et al.,* 2013). Due to its complex composition and synergy between various components, BV has a variety of biological functions such as sterilization, deodorization, anti-oxidation, anti-inflammatory, and growth promotion. The main organic component of BV is acetic acid, a commonly used organic acidifying agent. Organic acids have been used in poultry diets for decades, positively impacting broilers' physiology, production status, and laying hens (Beier *et al.,* 2019; Adewole *et al.,* 2021; Chen *et al.,* 2021). BV has also been widely applied in agriculture, health care, environmental sanitation, and medicine (Wang *et al.,* 2018; Guo *et al.* 2020).

Our previous studies found that adding BV to broiler diets could improve growth performance and meat quality (Pu S *et al.,* 2015). Prior studies have focused on the effects of BV on tissue morphology in the chicken intestinal tract and changes of microbial populations. However, research on applying BV in laying hens has been minimal. There is also no report on the effects of BV on the antioxidant status and serum biochemistry of laying hens in summer. Therefore, the present study employed Hy-Line Brown laying hens to evaluate the effects of BV on production performance, egg quality, antioxidant status, and serum biochemistry of laying hens in summer.

MATERIALS AND METHODS

All animal experiments were approved by The Guide for Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (permit number SYXK (Su) 2011-0036).

Birds, experimental design, and diets (n=30)

The experiment was carried out at the Nanjing Panchu Mechanized Chicken Farm (Jiangsu province, located at 32.24° north latitude and 188.68° east longitude) from the 2nd of June to the 11th of July, 2018. The laying hens were fed a basal diet for 10 days to adapt to the environment, and egg production was recorded. At 36 weeks old, 120 Hy-Line brown hens with similar body weights (1.9 ± 0.15 kg) were randomly assigned to four groups, with 30 hens per group. Table 1 shows the ingredients and nutrient composition of the basal diet. The control (CON) group was fed a basal diet, and the 0.1% BV group, 0.5% BV group, and 1.0% BV group received an added 1 mL/kg, 5 mL/kg, and 10 mL/kg BV (at feed grade), respectively. Feeding lasted 30 days. Table 2 presents the chemical compositions of BV (Jiangyin Zhongju Biotechnology Co., Ltd., Jiangsu, China). Hens were fed twice daily (6:30 am and 4:30 pm) and given access to water and feed *ad libitum*.

Table 1 – Ingredients and nutrient composition of the basal diet (on a dry matter basis).

Variable	Content (%)	Variable	Content (%)
Ingredients		Nutrient levels	
Corn	63.00	ME (kcal/kg)	2,663
Soybean meal	24.00	Crude protein	16.71
Limestone	8.00	Calcium	3.52
Premix[1)]	3.00	Available phosphorus	0.38
Salt	0.40	Lysine	0.72
Stone meal	1.60	Methionine	0.35
Total	100.00	Methionine + Cysteine	0.62

[1)] Content in each kilogram of premix: fish meal, phytase, vitamin A, 100 KIU; vitamin D_3, 50 KIU; vitamin B_1 58 mg; vitamin B_2 154 mg; vitamin B_6 78 mg; vitamin B_{12} 0.6 mg; biotin, 3.41 mg; Mn, 1400 mg; Fe, 1400 mg; Zn, 1400 mg; Cu, 170 mg; Se, 5 mg.

Abbreviations: ME, metabolizable energy

Table 2 – Chemical composition of the bamboo vinegar.

Main component	Content (%)
Water	80.000~90.000
Acetic acid	0.320
Methanol	0.023
Propionic	0.020
Amino acid	0.015~0.028
Phenol	0.006
O-Methoxy-phenol	0.004
Furfural	0.002
Others	0.500~1.000

The Hy-Line Brown hens were raised in 3-layer stepped metal cages (length×width×height: 50 cm×45 cm×45 cm) at ambient temperature. Each cage was equipped with a feeding trough and a nipple water drinker. Light control throughout the experiment consisted of 16 hours of lighting (from 6:00 am to 10:00 pm) and 8 hours of darkness daily. The sheds were open ventilated, without wet curtains or fans. Chicken manure was treated with blisters and cleaned once a day. The temperature and relative humidity in the poultry house were recorded daily for one hour (RC-4HC, Jingchuang Electric Co., Ltd., Jiangsu, China). The recorders were placed two meters from the floor and distributed along both sides of the house. The temperature-humidity index (THI) was calculated as follows: (1.8×AT+32)-(0.55×RH/100)×[(1.8×AT+32)-58], AT (°C): ambient temperature, RH (%): relative humidity.

Production performance and egg quality (n=15)

Calculated production performance includes daily feed intake, egg production, weight, mass, and feed conversion ratio (FCR). The remaining feed, egg number, and egg weight daily was recorded daily in the experimental units to calculate average daily feed intake, production, and weight. Calculated Egg mass was calculated by multiplying egg weight by egg production, while FCR = feed intake/egg mass output. Only 15 eggs were collected in each group (5 eggs/replicate). Furthermore, egg quality traits were measured every 10 days, including egg weight, relative yolk weight, egg shape index, eggshell strength, albumen height, yolk color, Haugh unit, and eggshell thickness. The egg and yolk weights were measured using an electronic balance (Fuzhou Huazhi Scientific Instrument Co., Ltd., Fujian, China). A multifunction egg tester was used to analyze albumen height, yolk color, and Haugh unit measured using (Robotmation Co., Ltd., Japan). Egg width, length, and eggshell thickness were determined using a digital caliper (Shanghai MNT Industrial Co., Ltd., China), and eggshell strength with an eggshell force gauge (Robotmation Co., Ltd., Japan). Additionally, the relative yolk weight was calculated as the ratio of egg yolk weight to egg weight. Egg shape index resulted from the length divided by the width of the eggs, and eggshell thickness was the sum of the external shell and its internal membrane.

Serum biochemistry parameters and antioxidant status in serum (n=6)

After the 30 days of experimental feeding, 6 chickens were randomly selected from each group for venous blood collection. The antioxidant status of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) levels in the serum were analyzed using kits according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). Serum-related biochemical parameters, such as total protein (TP), albumin (ALB), uric acid (UA), urea nitrogen (BUN), glucose (GLU), and calcium (Ca) levels were determined with purchased kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The serum globulin (GLB) content differed between TP and ALB content.

Statistical analysis

All the data from the fully randomized experiment were statistically analyzed using the one-way ANOVA function of the Statistical Product Service Solution (SPSS) software (Version 20.0; SPSS, Inc., Chicago, USA). Differences among means were assessed using Tukey's HSD test. Results were expressed as means ± standard error of the mean (SEM), and $p<0.05$ indicates a significant difference.

RESULTS

Temperature and relative humidity in the laying hens' house

The temperature, relative humidity, and maximum (Max) THI of the laying hens house during the experimental period are shown in Fig 1. According to Fig 1C, the Max THI for the 17 experiment days was close to or higher than 72, which is the threshold for the Max THI. This means that laying hens were under heat and humidity stress for 17 days of this experiment.

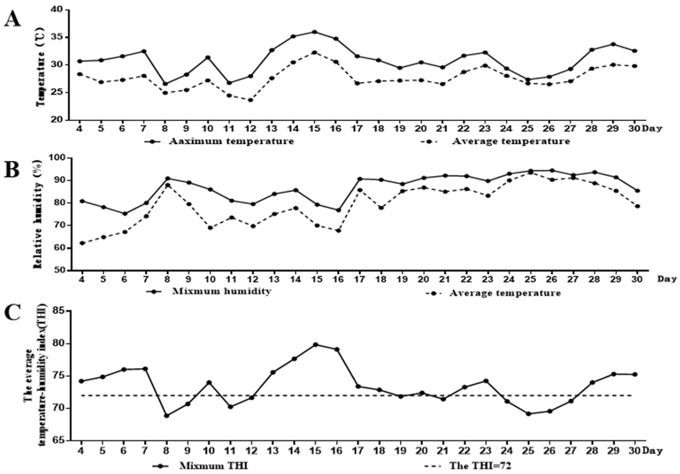

Figure 1 – Changes in temperature and humidity during the test.

Temperature(A), relative humidity (B), and maximum temperature-humidity index (C) in the laying hens' house during the experimental period. The room temperature and the relative humidity were auto-measured every hour from the 4th day. Figures above the dotted line indicate laying hens in a hot, humid condition.

Production performance

The effects of BV on egg production, egg weight, egg mass, and FCR were not significantly different between the four groups ($p>0.05$) (Table 3).

Egg quality

Table 4 shows the BV effect on laying hens' egg quality after feeding for 10, 20, and 30 days. After feeding BV for 10 days, the Haugh unit of the 0.1% BV group significantly increased ($p<0.05$). After feeding BV for 20 days, the egg shape index of 0.5% BV group significantly increased ($p<0.05$), and the 0.5% BV and 0.1% BV groups had considerably improved eggshell thickness. The egg shape index of three BV groups was significantly increased ($p<0.05$), and the eggshell

Table 3 – Effect of bamboo vinegar on production performance of laying hens for 30 days.

Variable	CON	0.1% BV	0.5% BV	1.0% BV	*p*-value		
					Treatment	Linear	Quadratic
Feed intake (g/hen/d)	103.00 ± 1.31	101.75 ± 0.54	103.04 ± 0.76	103.63 ± 1.51	0.686	0.538	0.428
Egg production (%)	88.26 ± 0.90	89.13 ± 2.30	88.55 ± 2.58	89.13 ± 1.09	0.824	0.499	0.684
Egg weight (g)	57.86 ± 0.33	58.02 ± 1.05	56.96 ± 2.02	57.40 ± 0.33	0.709	0.456	0.845
Egg mass (g/hen/d)	53.33 ± 0.47	54.01 ± 1.82	52.71 ± 1.02	53.58 ± 0.73	0.872	0.917	0.936
Egg/Feed (%)	1.78 ± 0.01	1.78 ± 0.02	1.80 ± 0.01	1.80 ± 0.00	0.315	0.166	0.750

Note: CON, control.

n = 30

Table 4 – Effect of bamboo vinegar (BV) on egg quality of laying hens for 10, 20 and 30 days.

Variable	CON	0.1% BV	0.5% BV	1.0% BV	*p*-value		
					Treatment	Linear	Quadratic
10th d							
Egg weight (g)	57.97 ± 0.78	59.15 ± 0.89	58.13 ± 1.11	60.03 ± 1.18	0.457	0.272	0.725
Yolk weight (g)	14.13 ± 0.31	14.57 ± 0.30	14.61 ± 0.22	14.67 ± 0.22	0.4708	0.166	0.478
The relative yolk weight	0.24 ± 0.00	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.00	0.783	0.666	0.289
Egg shape index	1.26 ± 0.26	1.30 ± 0.01	1.30 ± 0.01	1.29 ± 0.01	0.236	0.252	0.094
Albumen height (mm)	6.75 ± 0.66	7.98 ± 0.31	7.72± 0.33	7.22 ± 0.30	0.197	0.555	0.045
Yolk color (score)	9.92 ± 0.20	10.06 ± 0.19	10.16 ± 0.17	9.95 ± 0.21	0.793	0.825	0.360
Haugh unit	80.68 ± 4.71[b]	89.46 ± 1.63[a]	88.10 ± 1.88[ab]	84.67 ± 1.98[ab]	0.13	0.397	0.031
Eggshell thickness (mm)	0.41 ± 0.02[a]	0.44 ± 0.01[b]	0.45 ± 0.01[b]	0.45 ± 0.01[b]	0.163	0.046	0.325
20th d							
Egg weight (g)	59.87 ± 0.45[a]	59.03 ± 0.54[ab]	56.92 ± 0.62[c]	57.68 ± 0.37[bc]	0.001	<0.001	0.122
Yolk weight (g)	14.98 ± 0.28	14.89 ± 0.30	14.47 ± 0.22	14.16 ± 0.41	0.244	0.053	0.737
The relative yolk weight	0.25 ± 0.00	0.25 ± 0.01	0.25 ± 0.00	0.25 ± 0.01	0.631	0.591	0.298
Egg shape index	1.28 ± 0.01[b]	1.30 ± 0.01[ab]	1.31 ± 0.01[a]	1.29 ± 0.01[ab]	0.138	0.171	0.060
Albumen height (mm)	7.16 ± 0.42	7.39 ± 0.30	7.38 ± 0.33	7.51 ± 0.27	0.902	0.481	0.883
Yolk color (score)	9.31 ± 0.22	9.71 ± 0.15	9.71± 0.12	9.71 ± 0.21	0.373	0.153	0.291
Haugh unit	83.69 ± 2.68	85.95 ± 1.79	86.35 ± 1.81	87.06 ± 1.60	0.673	0.239	0.693
Eggshell thickness (mm)	0.40 ± 0.01[c]	0.44 ± 0.01[ab]	0.46 ± 0.01[a]	0.42 ± 0.01[bc]	< 0.001	0.064	<0.001
30th d							
Egg weight (g)	57.79 ± 0.82	58.73 ± 0.69	57.14 ± 0.67	57.32 ± 0.76	0.421	0.364	0.608
Yolk weight (g)	14.56± 0.25	14.77 ± 0.24	14.37 ± 0.21	14.37 ± 0.22	0.569	0.355	0.6550.
The relative yolk weight	0.25 ± 0.01	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.996	0.807	0.978
Egg shape index	1.27 ± 0.01[b]	1.30 ± 0.01[a]	1.31 ± 0.01[a]	1.30 ± 0.01[a]	0.023	0.016	0.042
Albumen height (mm)	7.64 ± 0.29	7.30 ± 0.22	7.20 ± 0.22	7.52 ± 0.24	0.573	0.664	0.181
Yolk color (score)	10.05 ± 0.17	10.22 ± 0.14	10.01 ± 0.17	9.80 ± 0.19	0.352	0.205	0.194
Haugh unit	87.85 ± 1.52	85.74 ± 1.37	85.53 ± 1.34	87.32 ± 1.32	0.568	0.773	0.165
Eggshell thickness (mm)	0.36 ± 0.01[a]	0.32 ± 0.01[b]	0.32 ± 0.01[b]	0.31 ± 0.01[b]	< 0.001	< 0.001	0.008

Note: CON, control.

Different lowercase letters indicate statistically significant differences ($p<0.05$).

n = 15

Table 5 – Effect of bamboo vinegar (BV) on serum antioxidant status of laying hens for 30 days.

Variable	CON	0.1% BV	0.5% BV	1.0% BV	*p*-value		
					Treatment	Linear	Quadratic
GSH-PX (U/mL)	1608.21 ± 247.34[b]	2026.67 ± 42.09[a]	2215.38 ± 111.73[a]	2450.46 ± 197.39[a]	0.040	0.006	0.625
T-SOD (U/mL)	217.06 ± 14.47	233.58 ± 14.95	251.14 ± 2.85	240.14 ± 9.83	0.290	0.117	0.262
MDA (nmol/mL)	4.13 ± 0.82	4.23 ± 0.68	3.82 ± 0.98	3.41 ± 0.14	0.856	0.455	0.735

Note: CON, control; GSH-PX, glutathione peroxidase; T-SOD, Total superoxide dismutase; MDA, malondialdehyde.

Different letters indicate statistically significant differences ($p<0.05$).

n = 6

thickness of three BV groups decreased ($p<0.05$) after 30 days.

Serum antioxidant status

Compared with the control group, the GSH-PX activity of 0.1% BV, 0.5% BV, and 1.0% BV groups significantly increased ($p<0.05$). The BV-supplemented level did not affect T-SOD and MDA serum contents.

Serum biochemical indexes

As shown in Table 6, compared with the control group, the TP content of the 0.5% BV group was significantly increased ($p<0.05$), and the ALB content in the 0.1% BV and 1.0% BV group was significantly increased ($p<0.05$). However, the effect of BV on the GLB, ALB/GLB, UA, BUN, GLU, and Ga serum levels were similar between the four groups ($p>0.05$).

DISCUSSION

This study indicated that dietary BV supplementation did not increase egg weight, but tended to increase egg shape index. Since there was no obvious difference in mean daily feed intake, BV may increase the egg shape index by increasing feed utilization rather than feed intake. Our previous study found that adding bamboo vinegar to laying hens' diets could improve egg production performance (YAN *et al.,* 2020). However, improvements in egg production performance were not seen in this trial, possibly due to differences in treatment times and fluctuating temperature differences between the poultry houses. This trial is linked to our previous study (YAN Menghe *et al.,* 2020), which shortened the test cycle and explored the effect of BV on laying hens' laying performance in shorter egg production cycles.

Table 6 – Effect of bamboo vinegar (BV) on serum biochemistry parameters of laying hens for 30 days.

Variable	CON	0.1% BV	0.5% BV	1.0% BV	*p*-value		
					Treatment	Linear	Quadratic
TP (g/L)	51.56 ± 2.31^{b}	56.58 ± 1.78^{ab}	61.49 ± 2.23^{a}	56.36 ± 1.43^{ab}	0.034	0.047	0.025
ALB (g/L)	14.50 ± 1.56^{b}	17.62 ± 0.97^{a}	16.95 ± 0.59^{ab}	19.07 ± 0.19^{a}	0.056	0.017	0.619
GLB (g/L)	37.06 ± 3.78	38.96 ± 2.16	43.5 ± 2.34	37.42 ± 1.09	0.183	0.579	0.103
ALB/GLB	0.41 ± 0.08	0.46 ± 0.04	0.42 ± 0.04	0.51 ± 0.01	0.292	0.187	0.206
UA (mg/L)	46.91 ± 3.17	52.81 ± 0.65	52.06 ± 1.35	51.40 ± 1.69	0.215	0.181	0.131
BUN (mmol/L)	2.13 ± 0.01	1.67 ± 0.13	1.77 ± 0.16	1.93 ± 0.18	0.926	0.976	0.922
GLU (mmol/L)	16.67 ± 1.00	16.09 ± 1.49	17.55 ± 2.01	18.22 ± 0.89	0.661	0.315	0.654
Ca (mmol/L)	3.32 ± 0.22	3.42 ± 0.13	3.50 ± 0.09	3.65 ± 0.09	0.466	0.135	0.857

Note: CON, control; TP, total protein; ALB, albumin; GLB, globulin; ALB/GLB, albumin/globulin. UA, uric acid; BUN, urea nitrogen; GLU, glucose; Ca, calcium.
Different letters indicate statistically significant differences ($p<0.05$).
n = 6

Egg Haugh unit increased in the BV group only at 10 days. However, there was no significant difference in the Haugh unit of the eggs at 20 and 30 days. However, at 30 days, egg albumin levels were significantly higher. This might be attributed to the antioxidant property of the Acetic acid present in BV, which probably minimized albumen quality deterioration through lower lipid and protein oxidation. Acetic acid significantly increased Haugh units of Japanese quails, probably due to decreased bacterial pollution of eggs (Attia *et al.,* 2013). In vitro studies reported that BV had more robust antifungal efficiency than acetic and formic acid. BV inhibits the growth of bacteria and fungi with a dose-dependent pattern (Chu *et al.,* 2013, Rattanawut *et al.,* 2018). Therefore, the increased Haugh unit and albumin of laying hens in this study may be due to the antibacterial function of BV.

Sengor & Soltan *et al.* reported that adding organic acids to the diet could significantly improve eggshell thickness. A strong correlation was observed between the increase in eggshell thickness and the increase in Ca serum concentration (Sengor *et al.*, 2007; Soltan *et al.*, 2008). In the first 20 days, an increase in eggshell thickness may be due to the main components in BV being organic acids. Intestinal pH decreases after BV feeding, causing changes to intestinal flora, calcium solubility, and the calcium absorption capacity of the small intestine. Studies have reported that calcium improves eggshell thickness (Xin *et al.*, 2021). However, at 30 days, the eggshell thickness was significantly reduced, probably due to the BV content and the different treatment times. Besides, the intestinal tract, being in an acidic environment for a long time, decomposes calcium carbonate in the eggshell, thus decreasing eggshell thickness.

Animal biochemical changes in the serum directly reflect animal health and metabolism. When ambient temperature exceeded the thermoneutral region for poultry, biochemical and hematological blood parameters changed (Khondowe *et al.,* 2021). High temperatures will increase protein degradation in the body and reduce the serum's TP, ALB, and GLB levels (Barrett *et al.*, 2019). Acidifiers have been proven to increase the protein content and decrease the UA level in the serum of chickens under heat stress (Gao *et al.*, 2021). In the present study, TP and ALB serum contents increased with the addition of BV. In recent years, natural plant extracts containing phenolic compounds have been considered potential dietary antioxidants to reduce body peroxidation (Hammad, 2013). An *in vitro* study confirmed that BV has an intense antioxidant activity and could scavange free radicals, which showed good correlations with total phenolic contents (Li *et al.,* 2012; Harikrishnan *et al.,* 2021). The antioxidative enzyme system is the first line of antioxidant defense. SOD and GSH-PX are the main antioxidant enzymes in the body and play an important role in scavenging excess free radicals. When SOD and GSH-PX activities increase, the ability to eliminate free radicals increases (Yahfoufi *et al.*, 2018). Reports found that feeding bamboo vinegar power increased GSH-PX and T-SOD activity. Moreover, BV in the diets of weaned pigs increased the activity of GSH-PX (Chu *et al.,* 2013; Qu *et al.,* 2018). In our study, the GSH-Px activity in serum was higher in laying hens fed BV at 30 days, which indicated that BV could effectively improve the antioxidant status of laying hens and eggs, which may contribute to the increase of albumin contents. BV had an excellent antioxidant activity for laying hens during summer. The antioxidant activity of BV could contribute to maintaining organismal homeostasis in laying hens in summer by scavenging free radicals and improving the immune defense function.

Although summer temperatures are higher, they are not at the level that puts laying hens under heat stress every day. The concern of our study was the effect of BV on production performance, egg quality, antioxidant status, and serum biochemistry of laying hens in this generally high-temperature environment. Therefore, we did not set a specific control group (hens fed a control diet under thermoneutral conditions).

CONCLUSIONS

In summary, adding BV to the diet of laying hens in the summer increased the egg shape index and serum GSH-PX activity, TP, and ALB contents. Current results suggest that BV can improve antioxidant capacity and protein utilization, thereby alleviating the harmful effects of summer conditions on laying hens.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to conceptualization, analysis, and finalization of this manuscript.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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