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Research Article
Effects of a Mixture of Wood Charcoal Powder and Wood Vinegar Solution on *Escherichia coli*, Ammonia Nitrogen, Vitamin C and Productive Performance of Laying Hens

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Abstract

Background and Objective: This study was conducted to investigate the effects of a mixture of wood charcoal powder and wood vinegar solution (WCV) on *Escherichia coli* (*E. coli*), ammonia nitrogen and vitamin C on the production performance of laying hens. **Methodology:** At 64 weeks of age, 96 laying hens were divided into four groups on an equal-weight basis. Each group of 24 birds had four replicates. The basal mash diet (2,600 kcal kg^{-1} metabolizable energy and 170 g kg^{-1} crude protein) was formulated to meet the nutritional requirements for layers. The basal diet was supplemented with wood charcoal powder and wood vinegar solution (WCV) at 0 (control group), 1, 2 or 3%. **Results:** At 72 weeks of age, all parameters of productive performance showed the highest values in the 2% dietary WCV group. The yolk color and Haugh unit, however, were the highest in the 3% dietary WCV group ($p<0.05$). The fecal *E. coli* concentration increased at the early stage of 66 weeks of age in the 1% dietary WCV group, with no change in ammonia nitrogen. **Conclusion:** These results may be attributed to the reducing power of WCV itself and to the vitamin C increase through WCV. These factors, by amplifying the anaerobic environment of the intestinal lumen, increased *E. coli* concentrations and thereby improved production performance by effectively using ammonia nitrogen. This outcome was due to the 2-3% WCV supplementation, which is most suitable for tropical climates.

Key words: Productive performance, wood charcoal, vinegar, *Escherichia coli*, ammonia nitrogen, vitamin C

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chicken breeding under tropical conditions often subjects chickens to heat stress. Such heat stress causes panting to eliminate excess body temperature, thereby inducing respiratory alkalosis[1,2]. Heat stress also elevates reactive oxygen species (active oxygen) due to disruption of the electron transport assemblies of the cell membrane[3]. Although reactive oxygen species affect the body's responses to infection, heavy metals and ethanol toxicity, excessive reactive oxygen species accelerate oxidative stress, which in turn induces diabetes, aging, kidney disease and cancer[4,5]. Human diseases, such as atherosclerosis and cancer, might be caused, in part, by oxidant damage to tissues. The reduction (deoxidation) of accumulated active oxygen, however, is known to be induced by the reductase vitamin C. The antioxidant effects of vitamin C have been demonstrated in many experiments *in vitro*[6]. Increased production performance may be achieved by using vitamin C supplements to mitigate the excessive reactive oxygen species caused by heat stress.

The large intestines of hens are an anaerobic environment. Intestinal bacteria living in the upper part of the intestine consume oxygen but the oxygen concentration in the gastrointestinal tract is relatively low. The oxygen concentration in the intestinal tract decreases caudally. In the large intestine, anaerobic bacteria such as *Escherichia coli* (*E. coli*), which is the predominant facultative anaerobe in the gastrointestinal tract, are inhibited[7]. Large intestinal bacteria are known to promote a reduction reaction (deoxidation). Bilirubin excreted into the intestine, for example, is known to be decomposed and reduced by bacterial deoxidation in the large intestine, resulting in urobilinogen. When urobilinogen is abundant, *E. coli* also increases[8]. Fortunately, *E. coli* can biosynthesize vitamin 1 and the presence of each enzyme involved in this biosynthesis has been demonstrated[9]. The abovementioned facts suggest the need to develop additives to increase *E. coli* via the anaerobicization of the intestinal luminal environment by reduction.

The continued use of artificial hormones to promote poultry growth performance has spurred the development of drug-resistant bacteria and environmental contamination. As a result, the European Union has banned the use of antibiotics as growth promoters since January 2006. Since then, natural alternatives have been developed to improve poultry product safety.

A mixture composed of wood charcoal powder and wood vinegar solution (WCV) has been reported to improve feed efficiency by promoting hypertrophied intestinal villi in chickens and pigs[10,11]. When 130-day-old male layer chickens were fed 0, 1, 3 or 5% dietary WCV, body weight gain tended to be higher in the 1 and 3% groups versus the 0% control group[10]. A more recent study showed that collagen in semimembranosus muscles increased, versus the control group, in chickens as young as 21 days old that were fed 0.8, 1.0, or 1.2% dietary WCV[12]. As such, veterinarians in Germany, France, Denmark and other European countries have imported WCV from Japan to serve as a natural substance for improving animal production.

The removal of nitrogen dioxide by activated carbons and the reduction of nitrogen monoxide by charcoal have long been established practices in Japan[13,14]. It is possibility that the wood charcoal content of WCV has the power to reduce accumulated active oxygen, such as through the reductase of vitamin C. The present study investigated the possibility that WCV mitigates the incidence of *E. coli* by amplifying anaerobicization of the intestinal luminal environment. As a first step, testing was conducted to determine the optimal percentage addition rate for conventional feeding systems in Thailand, which possesses a tropical climate, as opposed to Japan's temperate climate. Accordingly, this study aimed to investigate the effect of 0, 1, 2 and 3% dietary WCV diets on the productive performance of laying hens aged 64-72 weeks in Thailand. To measure vitamin C in blood serum, 0, 0.8 and 1% dietary levels were fed to male layer chickens in Japan.

MATERIALS AND METHODS

Diets and birds: Lohmann Brown laying hens were obtained from a local commercial company and fed a commercial diet (2,600 kcal kg^{-1} metabolizable energy and 170 g kg^{-1} crude protein) *ad libitum.* At 64 weeks of age, 96 laying hens were divided into four groups on an equal-weight basis. Each group of 24 birds had four replicates. Each replicate was further divided into two large cages (40×40×36 cm), with three birds in each cage. The birds were housed under natural conditions with a daily lighting regimen of 16 h of light (at a mean temperature of approximately 33°C).

The basal mash diet (2,600 kcal kg^{-1} metabolizable energy and 170 g kg^{-1} crude protein) was formulated to meet the nutritional requirements for layers[15]. The basal diet was supplemented with WCV at 0 for the control group and 1, 2 or 3% for the test groups. The commercial WCV was produced by Miyazaki Midori Seiyaku Co. Ltd. (Miyazaki, Japan). Briefly, wood vinegar was obtained after cooling smoke during the production of charcoal from the bark (without the woody part) of broad leaf trees by dry distillation at 350°C. The wood vinegar solution was then absorbed into the charcoal powder (1 L:4 kg) (Table 1). Feed and water were provided *ad libitum* during the nine-week testing period.

Table 1: Composition of wood charcoal powder and wood vinegar solution (WCV)

Items	Amount (%)
Moisture	30.6±1
Crude protein	4.9±1
Crude lipid	0.3±0.2
Crude fiber	40.6±10
Crude ash	13.0±3
Calcium	2.7±1
Phosphorus	0.1±0.009
Salmonella	Negative

Demonstration of the reduction power of WCV: Ten grams of WCV was added by +375.1 mV of distilled water to a final volume of 100 mL. Then, this solution was measured at 23 (room temperature) using an oxidation-reduction potential meter (YUSB-010R type, DKK-TOA YAMAGATA CORPORATION, Yamagata, Japan). Similarly, each composition of wood vinegar (10 mL) and wood charcoal (10 g) in WCV was measured after adding 100 mL of distilled water.

Performance and egg quality: Testing was conducted over nine consecutive weeks. Egg production was recorded daily and feed consumption was measured weekly throughout the experiment. Eggs from each group were collected biweekly to measure the following egg qualities: egg weight, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color and Haugh unit. Egg weight was measured using an electronic digital balance. Individual eggs were broken on a metal plate. Haugh unit and yolk color were measured using the TSS QCD system (Technical Services and Supplies, Dunnington, York, UK). The weights of the albumen, egg yolk and shell were measured using an electronic digital balance. Shell thickness was measured using a caliper at three locations on the egg (air cell, equator and sharp end) after removal of the shell membrane from the shell. The mean of the three values was recorded as the shell thickness per egg. The shell ratio, albumen ratio and yolk ratio were calculated for each individual egg as follows:

$$\text{Shell ratio} = \frac{\text{Shell weight}}{\text{Egg weight}} \times 100$$

$$\text{Albumen ratio} = \frac{\text{Albumen weight}}{\text{Egg weight}} \times 100$$

$$\text{Yolk ratio} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times 100$$

Fecal microflora ammonia nitrogen analyses: The counts of pathogenic bacteria were measured during the last week of three periods (during weeks 3, 6 and 9 of the study). Plastic plates were placed under the cage and fresh feces were collected from three birds per group. The collected feces were transferred to a vinyl bag and blended to obtain a homogeneous mass of feces. A 1 g sample was then transferred to a test tube. Samples were assayed within one h of collection. Each sample was serially diluted with a buffer of peptone water from 10^{-2} to 10^{-5} mL. Bacterial counts were performed for the appropriate dilutions. *Escherichia coli* colony counts were assessed using chromID™ Coli agar (bioMérieux SA, Marcy l'Etoile, France). The colonies on each plate were counted after incubation in an aerobic chamber at 37°C for 24 h. Colony-forming units (CFUs) were defined as distinct colonies measuring at least 1 mm in diameter. The results are expressed as the log 10 CFU g^{-1} of fresh feces. The ammonia nitrogen (NH_3-N) of the fresh feces was analyzed using the Kjeldahl technique in accordance with the guidelines of the Association of Analytical Chemists.

The experiment was conducted in accordance with the guidelines and rules for animal experiments of the Faculty of Animal Science and Agricultural Technology, Silpakorn University, Thailand.

Statistical analysis: Data from both experiments (production performance and egg quality) were statistically analyzed using one-way analysis of variance (ANOVA) in the SPSS statistical software package (version 19.0; IBM Corp. Armonk, NY, US). Significant differences among the treatments were determined with Tukey's studentized range tests. Statistical significance was set at $p<0.05$.

Feeding experiment using male layers for vitamin C measurements in blood serum in Japan: At Kagawa University in Japan, male layer chickens were raised according to the conventional feeding treatment in a windowed room under natural daylight and fluorescent illumination (12 h light and 12 h darkness per day) for7-200 days in February (at a mean temperature of approximately 5°C). Groups were fed a basal diet (CP, 15%; ME, 2800 kcal kg^{-1}) with WCV added at 0 (Cont-0), 0.8 (WCV-0.8) or 1 (WCV-1) percent for the measurement of total vitamin C in blood serum.

Testing was performed in accordance with the guidelines and rules of care and use of laboratory animal experimentation established by Kagawa University in Japan. The experimental procedure was approved by the Animal Research Committee of Kagawa University.

For the measurement of vitamin C in blood serum, 2 mL blood was collected at 20 weeks of age from the vena basilica of male layer chickens and total vitamin C in the serum was measured according to the instructions provided with the vitamin C measuring kit used (Shima Laboratories Co. Ltd., Tokyo, Japan), which were modified according to the colorimetric determination method described by Bradley *et al.*[16]. Statistical analysis of vitamin C was conducted using an independent samples t-test.

RESULTS

The present WCV clearly showed reduction power, as+375.1 mV of distilled water was reduced to +162.4 mV after adding WCV to the distilled water. Similarly, +375.1 mV of distilled water was reduced to +247.2 mV in wood vinegar and to +184.6 mV in charcoal powder.

The productive performance of laying hens, measured at three-week intervals from 64-72 weeks of age, did not vary among groups (Table 2). Overall, the 2% dietary WCV group showed the highest values for all parameters after 72 weeks of age. Regarding egg quality of laying hens at each week of age from 64-72 weeks of age (Table 3), shell thickness tended to decrease with increasing levels of WCV supplementation and shell thickness significantly decreased in the 2 and 3% dietary WCV groups at 66 weeks of age and from 64-72 weeks of age ($p<0.05$). The shell thickness in the 3% dietary WCV group was significantly decreased at 72 weeks of age ($p<0.05$). The shell ratio, albumen ratio and yolk ratio of laying hens at three-week intervals from 64-72 weeks of age did not vary among the groups. The yolk color among the experimental groups became darker with increasing levels of WCV supplementation and the yolk color of the 3% dietary WCV group showed greater improvement than did the control and 1% dietary WCV groups ($p<0.05$) at 72 weeks of age. The 3% dietary WCV group showed greater improvement than did the 1% group ($p< 0.05$). Haugh unit at 72 weeks of age increased with increasing WCV supplementation and the value of the 3% dietary WCV group was higher than that of the control ($p<0.05$).

Compared with the control group, the fecal *E. coli* count was significantly higher in the 1% WCV group at 66 weeks of age ($p<0.05$) (Table 4). The total average *E. coli* count from 64-72 weeks of age was greater in the WCV groups than in the control and the 1% WCV group had the highest incidence of *E. coli*.

Regarding fecal ammonia nitrogen, there was no significant difference among the groups from 64-72 weeks of age. Ammonia nitrogen, however, tended to decrease with hen age (Table 5). The total average ammonia nitrogen from 64-72 weeks of age tended to increase with increasing levels of WCV supplementation.

Table 2: Effect of dietary wood charcoal powder and wood vinegar solution (WCV) on productive performance of laying hens during 64-72 weeks of age (n = 4)

	WCV (%)					
Items	0	1	2	3	SEM	p-value
66 weeks of age						
Feed consumption, g/(hen*day)	106.73	109.00	105.61	105.73	2.35	0.96
Hen-day egg production (%)	87.50	84.89	83.69	71.79	3.04	0.28
Egg mass, g egg/(hen*day)	53.76	50.81	50.61	47.51	1.40	0.52
Feed efficiency	0.50	0.47	0.48	0.45	0.01	0.32
68 weeks of age						
Feed consumption, g/(hen*day)	106.57	108.70	107.14	105.10	3.54	0.99
Hen-day egg production (%)	89.53	75.60	83.09	76.67	3.21	0.42
Egg mass, g egg/(hen*day)	52.55	44.82	45.11	45.53	1.94	0.47
Feed efficiency	0.49	0.40	0.42	0.45	0.02	0.38
70 weeks of age						
Feed consumption, g/(hen*day)	113.94	118.08	108.24	136.40	8.30	0.70
Hen-day egg production (%)	85.00	80.00	87.98	82.02	2.72	0.78
Egg mass, g egg/(hen*day)	50.90	49.50	54.23	49.71	1.44	0.67
Feed efficiency	0.45	0.42	0.50	0.46	0.03	0.86
72 weeks of age						
Feed consumption, g/(hen*day)	108.04	108.46	110.05	101.53	2.61	0.71
Hen-day egg production (%)	83.01	76.79	93.93	80.90	2.96	0.20
Egg mass, g egg/(hen*day)	49.13	46.38	55.72	48.71	1.73	0.27
Feed efficiency	0.45	0.43	0.50	0.49	0.01	0.44
64-72 weeks of age						
Feed consumption, g/(hen*day)	111.71	115.52	105.04	115.70	3.40	0.70
Hen-day egg production (%)	87.03	80.04	91.35	80.49	2.49	0.33
Egg mass, g egg/(hen*day)	52.12	48.52	54.50	48.55	3.26	0.42
Feed efficiency	0.47	0.42	0.52	0.47	0.06	0.36

There are no significant differences between the groups ($p>0.05$), WCV: Wood charcoal powder and wood vinegar solution

Table 3: Effect of dietary wood charcoal powder and wood vinegar solution (WCV) on egg quality of laying hens during 64-72 weeks of age (n = 12)

	WCV (%)					
Items	0	1	2	3	SEM	p-value
66 weeks of age						
Shell thickness (mm)	0.36[a]	0.34[a]	0.29[b]	0.27[b]	0.01	0.001
Shell ratio (%)	10.38	9.86	10.39	10.08	0.26	0.340
Albumen ratio (%)	63.89	65.42	65.20	65.41	0.33	0.300
Yolk ratio (%)	25.72	24.71	24.40	24.49	0.31	0.420
Yolk color	8.75	8.67	8.67	8.90	0.07	0.670
Haugh unit	87.68	83.75	80.64	85.32	1.04	0.110
68 weeks of age						
Shell thickness (mm)	0.37	0.36	0.34	0.35	0.01	0.870
Shell ratio (%)	10.58	10.39	9.78	10.64	0.17	0.260
Albumen ratio (%)	64.05	63.57	64.41	64.10	0.40	0.910
Yolk ratio (%)	25.35	26.03	25.79	25.25	0.35	0.850
Yolk color	10.50	10.41	11.25	11.18	0.16	0.130
Haugh unit	84.07	82.65	82.65	87.51	1.38	0.550
70 weeks of age						
Shell thickness (mm)	0.36	0.35	0.33	0.35	0.01	0.170
Shell ratio (%)	10.60	10.32	10.07	10.96	0.13	0.080
Albumen ratio (%)	63.40	63.28	64.63	63.45	0.34	0.470
Yolk ratio (%)	25.98	26.39	25.28	25.57	0.30	0.620
Yolk color	11.33	11.45	11.58	11.41	0.09	0.830
Haugh unit	82.15	83.31	85.14	82.35	1.19	0.810
72 weeks of age						
Shell thickness (mm)	0.33[ab]	0.35[a]	0.33[ab]	0.31[b]	0.01	0.030
Shell ratio (%)	10.39	10.46	10.31	10.32	0.13	0.970
Albumen ratio (%)	63.02	62.03	64.04	63.86	0.42	0.310
Yolk ratio (%)	26.58	27.50	25.63	25.81	0.40	0.350
Yolk color	10.91[b]	10.75[b]	11.58[ab]	11.83[a]	0.13	0.006
Haugh unit	81.22[b]	87.10[ab]	88.62[ab]	93.93[a]	1.44	0.020
64-72 weeks of age						
Shell thickness (mm)	0.36[a]	0.35[a]	0.32[b]	0.32[b]	0.01	0.001
Shell ratio (%)	10.49	10.27	10.14	10.49	0.07	0.220
Albumen ratio (%)	63.59	63.56	64.57	64.29	0.17	0.090
Yolk ratio (%)	25.91	26.16	25.28	25.21	0.15	0.070
Yolk color	10.37[ab]	10.25[b]	10.77[ab]	10.86[a]	0.07	0.006
Haugh unit	82.63	84.25	84.27	86.57	0.86	0.460

[ab]Values with different superscripts in the same row are significantly different ($p<0.05$), WCV: Wood charcoal powder and wood vinegar solution

Table 4: Effect of dietary wood charcoal powder and wood vinegar solution (WCV) on fecal *Escherichia coli* counts (log 10 CFU g^{-1}) of laying hens during 64-72 weeks of age (n = 3)

	WCV (%)					
Escherichia coli	0	1	2	3	SEM	p-value
Weeks of age						
66	5.07[b]	5.82[a]	4.56[c]	5.17[b]	0.13	0.01
69	5.55	5.52	6.38	6.02	0.16	0.21
72	5.78	6.68	5.61	6.11	0.22	0.41
64-72	5.47	6.01	5.51	5.77	0.10	0.29

[abc]Values with different superscripts in the same row are significantly different ($p<0.05$), WCV: Wood charcoal powder and wood vinegar solution

Table 5: Effect of dietary wood charcoal powder and wood vinegar solution (WCV) on fecal ammonia nitrogen (mg g^{-1} wet feces) of laying hens during 64-72 weeks of age (n = 4)

	WCV (%)					
Weeks of age	0	1	2	3	SEM	p-value
66	0.33	0.32	0.36	0.34	0.02	0.94
69	0.24	0.25	0.27	0.25	0.01	0.95
72	0.21	0.22	0.22	0.29	0.01	0.25
64-72	0.26	0.27	0.28	0.29	0.01	0.71

There are no significant differences between the groups ($p>0.05$), WCV: Wood charcoal powder and wood vinegar solution

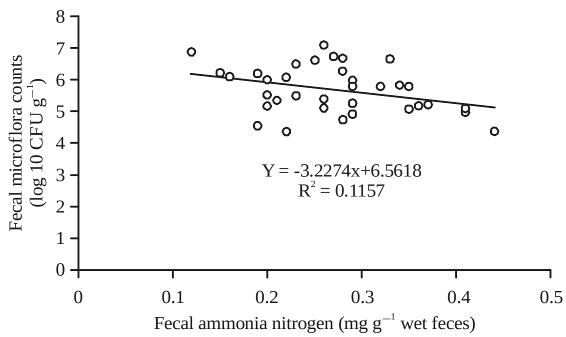

Fig. 1: Linear regression analysis between *Escherichia coli* values and ammonia nitrogen (n = 36, p<0.05)

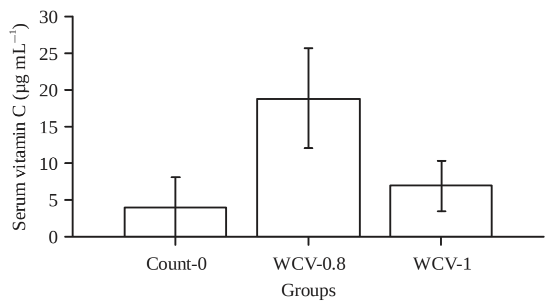

Fig. 2: Serum vitamin C of male chickens fed the basal diet supplemented with wood charcoal powder and wood vinegar solution (WCV) at 0% (Cont-0), 0.8% (WCV-0.8) and 1% (WCV-1) levels (n = 4)

In the linear regression analysis between *E. coli* values and ammonia nitrogen, a negative correlation was found (y = -3.2274x+6.5618, R^2 = 0.1157) ($p<0.05$) (Fig. 1). The confidence coefficients of the control, 1, 2 and 3% WCV groups were 0.39, 0.09, 0.07 and 0.07, respectively. Compared with that in the control group, the increase in *E. coli* in the WCV groups did not reduce ammonia nitrogen.

In roosters fed the 0, 0.8, or 1% WCV diets, vitamin C was richer in the WCV groups than in the Cont-0 group (Fig. 2). The WCV- 0.8 group had higher vitamin C than WCV-1, although the difference was not significant.

DISCUSSION

In the intestine, ingested protein is digested into amino acids and absorbed by intestinal absorptive epithelial cells before the disruption of ingested protein during passage through the small bowel, where unabsorbed amino acids are converted to ammonia nitrogen. Residual undigested matter degraded by large intestinal bacteria is used as a source of energy[17]. Ammonia nitrogen is reported to be utilized by *Bacteroides ruminicola* and by a significant proportion of the bacterial population in the colon of pigs[18,19]. A portion of the non-absorbed intestinal content is excreted as ammonia nitrogen. The present findings, namely, that *E. coli* tended to increase while ammonia nitrogen tended to decrease with increasing age in all groups, suggest that the residual undigested matter might be decreased due to effective absorption of undigested protein by colonization of *E. coli* in the rectum with aging.

Compared with that in the control, the *E. coli* count was significantly higher at 66 weeks of age in the 1% WCV group. The like lyreason that *E. coli* counts were higher in the WCV groups than in the control group is related to the pore sizes of wood charcoal and the substances in wood vinegar. The charcoal of WCV can adsorb *Salmonella enteritidis* (pathogenic bacteria) more effectively than it can *Enterococcus faecium* (normal intestinal ora) because the pores are of a suf cient diameter to adsorb *S. enteritidis* but not *E. faecium*[20]. The removal of large substances requires large pores in activated charcoal, whereas small substances require small pores[21]. However, wood vinegar in WCV can inhibit the growth of *S. enteritidis* but stimulate the growth of *E. faecium*[21]. Therefore, the *E. coli* treated with WCV in the present study may not be a harmful large intestinal bacterium.

The WCV formula used in the present experiment clearly demonstrated reduction power. Although, carbons have reduction power[13] and the metabolic reaction of large intestinal bacteria is known to be a reduction reaction (deoxidation), the present increase in *E. coli* was induced by amplifying the anaerobic environment of the intestinal lumen due to the reduction power of WCV. However, the *E. coli* failed to lower ammonia nitrogen. It appears that the increased *E. coli* counts due to the WCV reduction power digested residual undigested matter, which was absorbed into the intestinal blood. The reason may be the relatively lower ammonia nitrogen levels in the rectum. As ammonia nitrogen was secreted into the ceca with an increasing injection of glutamic acid into chicken blood[22], extra nitrogen in the blood was again secreted into the ceca, resulting in higher ammonia nitrogen levels with an increase in the dietary WCV level.

It is generally accepted that chickens can synthesize vitamin C and *E. coli* can also synthesize vitamins by digesting cellulose in the insoluble dietary fiber[9,23]. *Escherichia coli* has been reported to uptake vitamin B12[24]. Vitamin C has antioxidant effects[6]. Dietary fiber decreases oxygen tension and oxidation-reduction potential and the increase in oxygen tension and oxidation-reduction potential due to fiber shortage can induce a decrease in anaerobic bacterial counts[25]. Based on these reports, the increased *E. coli* in the WCV groups is thought to more effectively remove reactive oxygen species by a direct reduction action (deoxidation) and by indirect reduction due to vitamin C synthesis by *E. coli*. Increased vitamin C levels compared with those of the control were found in the WCV groups after feeding 0.8 and 1% WCV diets. In our previous study, WCV supplementation increased collagen in egg yolks and egg whites[26] and elevated collagen in the musculus semimembranosus[12]. In this study, we did not determine why the vitamin C concentration was greater in the 0.8% vs. the 1% WCV group. Perhaps the 1% WCV group needs more vitamin C to produce collagen than does the 0.8% WCV group, resulting in decreased stored vitamin C in the 1% WCV group, because vitamin C is required for collagen formation[27] and the musculus semimembranosus of male broilers had much more total collagen in the 1% WCV group than that in 0, 0.8 and 1.2% WCV groups at 21 days of age[12]. These results suggest that the present WCV formula can stimulate vitamin C production. The antioxidant effects of vitamin C have been demonstrated in many experiments *in vitro*[6]. Accumulated active oxygen is known to be reduced by reductases such as vitamin C. WCV, with its ability to stimulate vitamin C production (resulting in an indirect reduction effect due to vitamin C) and the direct reduction power of wood carbons[13], might reduce reactive oxygen species during heat stress. The present productive performance of laying hens under heat stress improved at the 2% WCV level, while the 1% WCV level was suitable for egg production under the temperate conditions of Japan[26]. This discrepancy might be caused by the ability of WCV to exclude reactive oxygen species and thus improve productive performance in tropical areas. After 72 weeks of age, the Haugh unit also improved with increasing dietary WCV levels. These results indicate that a 2% WCV supplementation is best able to improve productive performance in tropical regions.

CONCLUSION

After 72 weeks of age, all parameters of productive performance were at their highest values in the 2% dietary WCV group (not significantly different) but the yolk color and Haugh unit were highest in the 3% dietary WCV group ($p<0.05$). The fecal *E. coli* count increased at 66 weeks of age in the 1% dietary WCV group, with no change in ammonia nitrogen.

SIGNIFICANCE STATEMENT

This study determined that WCV can be beneficial for improved productive performance in laying hens. This study will also help researchers understand the reducing power of WCV and vitamin C.

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