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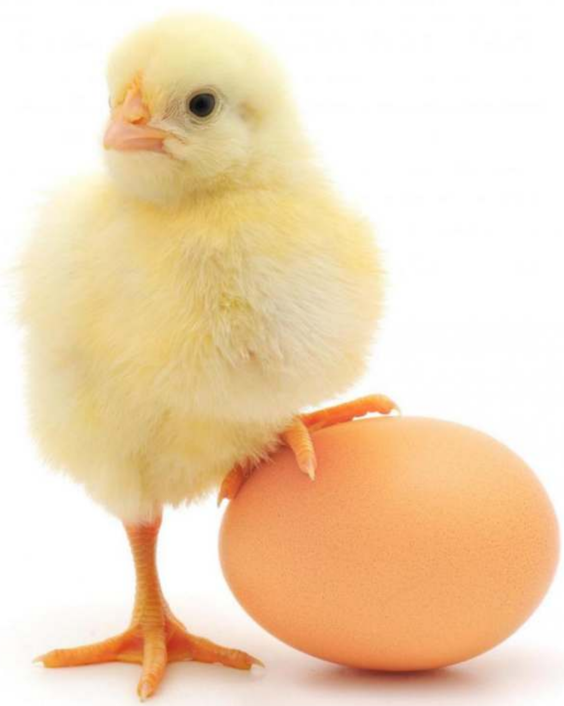

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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Research Article
Effect of Gallotannoids on the Productive Performance and Stress Index of Broiler Chickens under Stress Conditions

[1]Kanokporn Poungpong, [2]Eisuke Sumiyoshi and [1]Chaiyapoom Bunchasak

[1]Department of Animal Science, Faculty of Agriculture, Kasetsart University, 50 Ladyaow, Chatuchak, Bangkok 10900, Thailand
[2]echoes of sciences, 4-20-1 Shukugawara, Tama-ku, Kawasaki 2140021, Japan

Abstract
Background and Objective: This study was designed to investigate the effects of hydrolyzable tannoids (gallotannoids) on the productive performance and stress hormone levels (cortisol) of broilers, from hatching to 42 days of age, under high environmental stresses (i.e. heat stress, high stocking density, dirty litter). **Materials and Methods:** Two hundred and eighty-eight male broiler chickens (Ross 308 strain) were fed a corn-soybean meal-based diet under high stress conditions. The birds were devided into three groups: (1) control group, (2) group that received synthetic vitamin C at 286 ppm and (3) group that received gallotanoid at 100 ppm. Feed and water were offered *ad libitum*. **Results:** Gallotannoids significantly improved body weight gain (BWG) and feed conversion ratio (FCR) compared to the control and synthetic vitamin C groups ($p<0.05$) during the finisher period (days 35-42). During the overall period (days 0-42), gallotannoids significantly improved the European production efficiency factor (EPEF) and FCR compared to the control group ($p<0.05$), whereas the synthetic vitamin C did not significantly improve these measures ($p>0.05$). Blood cortisol hormone levels were decreased by the synthetic vitamin C, as well as by the gallotanoids ($p>0.05$). **Conclusion:** Results indicate that, at the tested doses and under high stress conditions, gallotannoid supplementation can improve broiler production performance more efficiently than synthetic vitamin C.

Key words: Broiler production, gallotannins, hydrolyzable tannoids, oxidative stress, synthetic vitamin C

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Corresponding Author: Kanokporn Poungpong, Department of Animal Science, Faculty of Agriculture, Kasetsart University, 50 Ladyaow, Chatuchak, Bangkok 10900, Thailand

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

The productive performance of intensively raised commercial poultry may be suppressed by raised oxidative stress levels[1-2] related to environmental stresses such as high temperatures, high stocking density and poor sanitation[3-5]. Consequently, antioxidants have become widely used to maintain the physiological functions and production of the animals[6]. The antioxidant, L-ascorbic acid (vitamin C), is widely used in poultry production to reduce the negative effects of oxidative stress on animal production. Despite all poultry species having the ability to synthesize vitamin C in the kidney or liver[7], exogenous vitamin C is reported to improve the productive performance of broilers[8-9]. In addition, a reduction in blood cortisol levels and improvement of the immune response or lymphocyte proliferation has been demonstrated in laying hens raised under heat stress after vitamin C supplementation[10-11].

Recently, gallotannoids, which have a high antioxidant activity, have appeared as an alternative feed additive[12]. Gallotannoids are low molecular weight hydrolyzable tannoids, with a structure similar to that of the vitamin C, that are found in natural plants, such as the *Phyllanthus emblica* fruit (Amla). Whilst the active pharmacophore of synthetic vitamin C and gallotannoids is identical, the antioxidative activity of gallotannoids is nearly six fold higher than synthetic ethyl cellulose-coated vitamin C.[13] Importantly, this high antioxidative activity has been shown to reduce lipid peroxidation in the brain[14-15] and heart[16] and may therefore reduce associated cellular damage and promote tissue regeneration in these organs[17-18]. Under high temperature conditions, gallotannoid supplementation has also been shown to increase egg shell thickness of laying hens compared to synthetic vitamin C[19], however this was not associated with the growth performance of broiler chickens[20]. Chickens reared under commercial settings are exposed to multiple stressors such as high heat, high stocking density and poor management. To date, no report has shown a significant advantage of gallotannoid over synthetic vitamin C supplementation for the rearing of broiler chickens with multi-stressors. Thus, the objective of this study was to investigate the comparative effects of natural hydrolyzable tannoids and synthetic vitamin C on the production of broilers, from hatching to 42 days of age, under multi-stressors.

MATERIALS AND METHODS

Experimental design, animals and diets: This experiment was carried out using a randomized complete block design (RCBD). Two hundred and eighty-eight (288) newly hatched male commercial strain (Ross 308) broiler chickens were randomly allocated to three experimental groups with eight replications of 12 birds each. A practical corn-soybean meal diet was formulated for each growing phase. Three experimental diets were given as follows:

- Control (corn-soybean meal diet)
- Control+286 ppm L-ascorbic acid-2-phosphate (equivalent to 100 ppm vitamin C)
- Control+100 ppm herbal product (hydrolyzable tannoids 12%) (Commercial Name: Herbal C)

The composition and nutrient content of the experimental diets are shown in Table 1. All experimental diets were formulated without antibiotics and mycotoxin binder products, except for coccidiostat. Synthetic vitamin C or gallotannoids were added to the feed during mash mixing (before pelleting). All diets were pelleted under conditioning temperatures of 82°C.

Feeding program: Birds were fed crumble pellet feed from hatching to day 10, thereafter the birds were fed pellet feeds until the end of the 42-day test period. Feed was changed from starter to grower at day 10 and from grower to finisher at day 24. Birds in all groups were allowed to freely consume feed and water.

Animal management: The experiment was conducted on a semi-commercial farm in a closed house with tunnel ventilation and an evaporative cooling system. Birds were raised on solid concrete floor pens with rice hull as bedding material. The size of each pen was 1.0×1.0 m and each pen was equipped with a tubular feeder and two nipple water drinkers. All birds were vaccinated for Newcastle disease and infectious bronchitis at 7 days of age and for Gumboro disease at 14 days of age. All other management activities were in accordance with the Ross 308 management manual.

Stress conditions: A high stocking density, high temperature and dirty litter conditions were employed as multi-stressors. To this end, 12 birds were raised to a pen instead of the normal stocking density of 10 birds/pen and the maximum house temperature was set at 31°C during weeks 2-5 (days 8-35) and 33°C during week 6 (days 36-42). Dirty litter was provided by the use of 50% used and 50% new litter.

Sampling and measurement: On a pen basis, feed consumption, body weight and feed intake were measured

Table 1: Composition and calculated nutrient contents of diets

Ingredients	Starter (0-10 days)	Grower (10-24 days)	Finisher (24-42 days)
Corn 7.9%	57.110	59.650	61.730
SBM dh 49.5%	25.110	21.410	18.060
Full fat soy 37.0%	7.000	6.000	5.000
Canola meal 37.8%	4.000	5.000	6.000
Rice bran SE (palm oil: 7300)	1.740	3.290	4.930
MDCP 21.8% P/16.3%Ca	2.310	2.120	1.940
Limestone 40.4% Ca	1.050	0.920	0.820
V/M premix	0.200	0.200	0.200
Salt	0.250	0.250	0.250
Sodium bicarbonate	0.200	0.200	0.200
L-Lysine HCl	0.250	0.240	0.220
DL-Methionine	0.270	0.240	0.210
L-Threonine	0.100	0.080	0.060
Choline chloride 60%	0.060	0.050	0.030
Pellet binder (Pelex dry)	0.300	0.300	0.300
Coccidiostat (Salino 12%)	0.050	0.050	0.050
	100.000	100.000	100.000
Dry matter	89.220	89.300	89.400
ME for poultry	3000.000	3100.000	3200.000
Crude protein	21.500	19.800	18.300
Crude fat	5.670	7.070	8.540
Linoleic acid	1.970	2.080	2.190
Crude fiber	3.090	3.030	2.980
Lysine	1.340	1.220	1.120
Arginine	1.420	1.290	1.170
Methionine	0.610	0.560	0.510
Met+Cys	0.980	0.910	0.850
Cystine	0.370	0.360	0.340
Threonine	0.920	0.830	0.760
Tryptophan	0.240	0.220	0.200
Gly+Ser	0.770	0.790	0.810
Histidine	0.570	0.530	0.490
Isoleucine	0.920	0.840	0.770
Leucine	1.780	1.670	1.570
Valine	1.030	0.950	0.880
Phenylalanin	0.410	0.420	0.420
Calcium	0.960	0.870	0.790
Phosphorus-total	0.890	0.840	0.790
Phosphorus-avail	0.510	0.470	0.430
Non phytate	0.480	0.450	0.420
Potassium	0.890	0.820	0.750
Choline	1700.000	1600.000	1500.000
Sodium	0.160	0.160	0.160
Chloride	0.180	0.180	0.180
Salt	0.290	0.290	0.300

for body weight gain (BWG) and feed conversion ratio (FCR), (ages 0-10, 10-24, 24-35 and 35-42 days). At 35 and 42 days of age, blood samples were collected from the jugular veins of two birds per pen. Blood serum samples at 35 days of age were prepared and analyzed for immune titers of antibodies against avian infectious bronchitis virus (IBV) using an enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions. Serum samples at 42 days of age were analyzed for cortisol (the stress hormone) using an AIA®-360 benchtop analyzer by ST AIA-PACK CORT (TOSOH Bioscience, Malaysia) and a competitive enzyme immunoassay.

Statistical analysis: All experimental data were analyzed using an analysis of variance procedure with a randomized complete block design using statistical analysis system (SAS Inst. Inc., Cary, NC, USA). Differences among treatment means were determined via Duncan's multiple range test and a probability level of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The comparative effects of synthetic vitamin C or gallotannoids on the productive performance of broilers under stress conditions from 1-42 days of age are shown in Table 2. During the starter-grower period (days 0-24), there was no difference in productive performance between the groups. Body weight gain of chicks fed gallotannoids was significantly higher than that of the control and synthetic vitamin C groups (p<0.01), whilst the FCR of the control was the lowest (p<0.05) at 24-35 days of age. At 35-42 days of age, gallotannoids supplementation led to a better growth rate and FCR than in the other groups (p<0.01).

During the starter-grower phase (days 0-24), body weight and FCR met the strain's recommendations (ROSS 308, 2017). This indicated that the chicks were not seriously affected by the temperature, stocking density and litter quality stressors. Furthermore, supplementing antioxidants in the diet did not affect productive performance during this phase. These findings are consistent with a previous report which demonstrated that adding synthetic vitamin C or gallotannoids as antioxidant sources did not improve the dressing yield of birds until the third week under normal ambient conditions[21]. However, an improvement in broiler production performance under less stressful environments has previously been reported with vitamin C supplementation[9-10,22].

At 35 and 42 days of age, compared to strain standards, the body weight of the control group was suppressed by 11% and 20%, respectively. Accordingly, during the finisher period (days 35-42), the environmental temperature of the present study was within the range of heat stress conditions (30.1-35.2°C) for broiler chickens[20]. During the later period (days 24-35 and days 35-42), whilst stressors negatively affected the growth performance of the chickens, antioxidant supplementation significantly improved FCR in both periods. Interestingly, during these periods, BWG was significantly higher in the gallotannoid supplemented group compared to the synthetic vitamin C supplemented and control groups.

During the overall study period, it was clear that gallotannoid supplementation improved BWG and the FCR of the chickens (Table 3). The body weight of chickens fed

Table 2: Effects of synthetic vitamin C and gallotannoids on production performance of broilers under stress conditions during 1-10, 10-24, 24-35 and 35-42 days of age

Period (days)	Items	Control	Synthetic vitamin C	Gallotannoids	p-value	Pooled SEM
	Initial weight (g)	43.000	43.000	43.000		
	BW (10 days, g)	293.000	292.000	293.000	0.98	0.002
1-10	BWG (g)	250.000	249.000	250.000	0.98	0.010
	FI (g)	313.000	312.000	312.000	0.89	0.010
	FCR	1.250	1.250	1.250	0.87	0.010
	Livability (%)	100.000	100.000	100.000		
	BW (24 days, g)	1,164.000	1,166.000	1,169.000	0.96	0.010
	BWG (g)	871.000	873.000	876.000	0.95	0.010
10-24	FI (g)	1,205.000	1,200.000	1,195.000	0.93	0.020
	FCR	1.380	1.370	1.360	0.89	0.030
	Livability (%)	100.000	100.000	100.000		
	BW (35 days, g)	2,026.000	2,049.000	2,088.000	0.15	0.020
	BWG (g)	862.000[b]	883.000[b]	919.000[a]	<0.01	0.010
24-35	FI (g)	1,581.000	1,521.000	1,556.000	0.13	0.020
	FCR	1.830[a]	1.72[b]	1.690[b]	0.01	0.030
	Livability (%)	93.750	96.880	95.830	0.39	1.590
	BW (42 days, g)	2,415.000[b]	2,449.000[b]	2,530.000[a]	<0.05	0.020
	BWG (g)	389.000[b]	400.000[b]	442.000[a]	<0.01	0.010
35-42	FI (g)	1,015.000	1,008.000	1,029.000	0.59	0.010
	FCR	2.610[a]	2.520[a]	2.330[b]	<0.01	0.030
	Livability (%)	98.960	98.960	98.960	1.00	0.910

[a,b]Means within column with no common superscript differ significantly (p<0.05). BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio corrected for mortality and culls, Livability: 100-% dead and culled birds

Table 3: Effects of synthetic vitamin C and gallotannoids on production performance and EPEF value of broilers under stress conditions during the overall period (1-42 days of age)

Period (days)	Items	Control	Synthetic vitamin C	Gallotannoids	p-value	Pooled SEM
	Final body weight (g)	2,415.000[b]	2,449.000[b]	2,530.000[a]	<0.05	0.03
	BWG (g)	2,372.000[b]	2,406.000[b]	2,487.000[a]	0.02	0.03
1-42	FI (g)	4,086.000	4,020.000	4,066.000	0.43	0.04
	FCR	1.720[b]	1.670[ab]	1.640[a]	0.03	0.02
	Livability (%)	92.710	95.830	94.790	0.53	1.94
	EPEF[1]	309.700[b]	335.700[ab]	349.900[a]	0.04	10.29

[a,b]Means within column with no common superscript differ significantly (p<0.05). [1]EPEF (European production efficiency factor) = $\frac{\text{Livability (\%)}\times\text{Final body weight (g)}}{\text{Age (d)}\times\text{FCR}}\times 100$

Table 4: Effect of synthetic vitamin C and gallotannoids on blood cortisol (42 days of age) and IBV titer (35 days of age) of broilers under stress condition

Items	Control	Synthetic vitamin C	Gallotannoids	p-value	Pooled SEM
Cortisol (ng dL^{-1}) at 42 days	14.86	12.38	11.67	0.30	1.36
Decrement from control (%)	NA	16.60	21.40		
IBV titer at 35 days	536.00	380.71	624.83	0.22	59.32

gallotannoids was significantly heavier than those of the other groups and the FCR was significantly higher than that of the control group (p<0.05). Because BWG and the FCR were improved with antioxidant supplementation but without affecting the feed intake of the chickens, we speculate that the antioxidants (particularly gallotannoids) directly improved the physiological functions of these broiler chickens under stress, leading to the improvement of their productive performance. The effects of synthetic vitamin C and gallotannoids on the productive performance and European production efficiency factor (EPEF) values for broilers under stress conditions, over the whole study period (1-42 days of age), are shown in Table 3. The EPEF value of chickens fed gallotannoids was significantly higher than the control group (p<0.05). EPEF is commonly used to evaluate the overall economic performance of poultry production. Under a normal environment, Chatterjee *et al.*[23] reported that supplemental gallotannoids had better EPEF than the synthetic vitamin C supplementation, although their report did not show a statistically significant improvement. The present study showed that gallotannoid supplementation resulted in a significantly higher EPEF values compared to the control group, with synthetic vitamin C supplementation not showing any effect, in the presence of environmental stresses. This indicates an economic advantage of using gallotannoids over synthetic vitamin C under stress conditions.

Table 4 shows the effect of synthetic vitamin C and gallotannoids on blood cortisol and the IBV titer of broilers at 42 and 35 days of age, respectively. There was no significant difference in blood cortisol and IBV titers among experimental groups. Despite not statistically significant, blood cortisol levels decreased by 16.6 and 21.4% when supplemented with synthetic vitamin C and gallotannoids, respectively. Accordingly, an improvement of immune titers and a reduction of blood cortisol levels with 500 ppm vitamin C under high stress conditions have been reported [10,22].

Due to environmental stress, poor immune titers against IBV was indicated by high variation of the titer level of IBV at 35 days of age. Lohakare[24] has previously reported that supplemental vitamin C at 200 ppm improved the performance and immunity of commercial broiler chickens. Therefore, the low levels of antioxidant supplementation (100 ppm) used in the present study may not be sufficient to promote an immune response, nevertheless productive performance was improved.

CONCLUSION

The supplementation of antioxidants (synthetic vitamin C and gallotannoids) may prevent the negative effects of multi-stresses in commercially-raised broiler chickens. Gallotannoids appeared to have a more beneficial effect than synthetic vitamin C (at the equivalent to 100 ppm).

SIGNIFICANCE STATEMENT

The current study found that a gallotannoids supplement can be used as an alternative to antioxidants in broiler chicken feed. This study will help researchers better understand the use of gallotannoids as a natural antioxidant to improve the production of broiler chickens under high stress conditions.

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