Research Note: Responses of broiler chickens to in ovo feeding with clove and cinnamon extract under hot-humid environments

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ABSTRACT An experiment was carried out to evaluate the responses of broiler chickens to in ovo injection of aqueous extracts of clove and cinnamon under a hot-humid environment. The study involved the use of seven hundred hatching eggs from broilers (Ross 308) which were incubated with the use of standard protocol (37.8°C). The incubating eggs (100 each) were randomly selected and assigned to 7 treatments on day 17.5 of incubation, viz.: un-injected eggs (**UE**), eggs injected with 0.5 mL distilled water (**DW**), 2 mg clove (**CL2**), 4 mg clove (**CL4**), 2 mg cinnamon (**CN2**), 4 mg cinnamon (**CN4**), and 3 mg ascorbic acid (**AA**).Data on physiological parameters, hatchability, chick quality, and anatomical characteristics of the chicks were collected and analyzed using one-way analysis of variance. The results obtained revealed that the hatchability of eggs of AA and CN2 was higher compared to DW and UE. However, the hatchability of DW and UE was higher than those of CN4. The total chick quality scores of the control were similar to the other groups. Chick weights at hatch were similar in CL2, CN2, and AA but heavier than CN4, CL4, UE, and DW. The chick-to-egg ratio in AA was comparable to CL2 and CN2 but higher than UE, DW, CN4, and CL4. Total scores for chick quality of AA birds were similar to those of UE, CL2, and CL4 birds but higher than DW, CN2, and CN4 birds.

Key words: thermotolerance, early feeding, antioxidant, broilers

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INTRODUCTION

Due to global warming, the Earth's average surface temperature is rising, causing passive heat stress on the health and performance of animals (Oke et al., 2021a). Commercial broilers are especially vulnerable to heat stress due to enhanced metabolic heat generation and reduced heat dissipation capability due to their rapid growth rate (Sandercock et al., 1995). Heat stress is caused by the relationship between temperature, relative humidity, radiant heat, and air speed. Temperature is the main factor in heat stress causing changes in physiological and metabolic pathways in birds (Oke et al., 2021a). However, due to the energy expended to cope with environmental stressors, chickens raised in the tropics cannot reach their genetic capacity during elevated air temperature or high humidity. As a result, there is a need to investigate effective ways to boost broiler chickens' thermotolerance in hot climates to increase their productivity.

Several attempts have been made to mitigate the impact of thermal stress on birds, ranging from genetic improvements and housing system modification, including ventilation, the installation of evaporative cooling systems, stocking density reduction (West, 2003; Oke et al., 2020), among other measures. Many of these efforts have been effective in reducing heat-related shifts. However, these strategies are complicated and expensive for farmers in developing countries. As a result, a dietary intervention that is affordable and available within the reach of farmers could be a viable option for mitigating the impact of elevated ambient temperature in poultry production in tropical environments. In this regard, there has been a growing interest in the use of phytogenic feed additives that are high in antioxidants (Oke et al., 2017; He et al., 2018; Oke, 2018; Oke et al., 2021b). He et al. (2018) recently found that phytochemical additives reduced the effects of heat stress and increased broiler growth efficiency. The phenolic compounds found in cinnamon

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(Cinnamaldehyde, trans cinnamide (cin), carvacrol and other biological activities) have been reported to be beneficial (Castillo-López et al., 2017).

In ovo technique is the direct injection of substances into the developing embryo to establish a long-lasting effect on the physiology of the chicken embryo. Recent studies have demonstrated that in ovo feeding plays a vital role in maintaining physiological balance and providing oxidative stress protection (Oke et al., 2021b). In poultry studies, egg antioxidants have been shown to positively impact egg hatchability, body mass, osteometric development, immunity, behavior, and chick efficiency, in the early post-hatch period, which is crucial for chicks' survival (Yang et al., 2021). As a result, techniques that increase antioxidants which contribute to the improvement in birds' tolerance against oxidative stress are necessary. Earlier studies have shown promising results on the use of in ovo phytogenic feed additives (Oke et al., 2020). However, there is a scarcity of data on the in ovo administration of cinnamon and clove extracts in broiler chickens despite the extensive use of additives such as vitamin C. Hence, this study aimed to evaluate the effects of the injection of clove and cinnamon extracts at different concentrations in fertile broiler eggs in humid tropical environments.

MATERIALS AND METHODS

Extraction and Administration of Phytogenic Extracts

Cinnamon (Cinnamomum zeylanicum) and clove (Syzygium aromaticum) seeds were purchased from a local farmer. The extracts were obtained using the method of N'nanle et al. (2017).

Experimental Procedure

A total of 1,000 hatching eggs (collected and stored over a 3-d period) from broiler breeder Ross 308 flock at 38 wk of age were sourced from a reputable breeder farm, and the eggs were fumigated, numbered, and weighed. Before setting of the eggs, they were stored in an air-conditioned room (24 °C). The eggs were randomly arranged in a setting tray of a single-stage incubator (SY-7680 incubator, Sanyuan Incubation Ltd, Bengbu, China). The incubator was programmed to maintain temperature and relative humidity at 37.8°C and 60%, respectively. On day 17.5 of incubation, eggs were candled for viability using an ultraviolent hand lamp and a total of 700 fertile eggs were assigned to 7 treatments and 5 replicates, making it a total of 100 eggs per treatment and 20 eggs per replicate in each treatment: un-injected eggs (**UE**), eggs injected with only 0.5 mL distilled water (**DW**), clove injected at 2 mg/egg (**CL2**), and 4 mg/egg (**CL4**), cinnamon injected at 2 mg/egg (**CN2**), and 4 mg/egg (**CN4**) of the extracts (from the sievate) dissolved in distilled water, and 3 mg/egg ascorbic acid (**AA**) was dissolved in 0.5 mL distilled water. The in ovo procedure was done as described by Oke et al. (2021).

Data Collection

Evaluation of Hatchling Parameters Immediately after hatching, 3 chicks per replicate were used to carry out the chick quality evaluation using the Tona scoring system (Tona et al., 2003).

Anatomical Characteristics

At hatch, two chicks in each replicate were euthanized, and the relative weights of the liver, heart, yolk sac, gizzard, hatching muscles, intestinal weight, and yolk-free body weight were recorded.

Statistical Analysis

Data obtained in this study were subjected to analysis of variance for a Completely Randomized Design using SAS (2008) statistical package. Significant main effects means were separated using Tukey's HSD at $P < 0.05$.

RESULTS AND DISCUSSION

Hatchability and Chick Quality

The study was designed to evaluate the in ovo injection of different levels of clove and cinnamon extracts on broiler chickens' perinatal and posthatch performance in a hot-humid environment. Hatchability of eggs, chick weight, chick-to-egg ratio, remaining yolk, yolk absorption and total scores were significantly ($P< 0.05$) affected by in ovo treatment (Table 1). The hatchability of eggs injected with CL2 (92.00%) was higher ($P< 0.05$) compared to the other treatments. The hatchability of AA (89.00%) was similar to CN2 (89.00%) but higher than that of DW (85.00%) and UE (87.00%). In ovo injection could enhance the antioxidant status and performance of birds (Oke et al., 2021b). This study indicates that the lower dose of in ovo extract (clove and cinnamon) enhanced hatchability. The improved hatchability observed in AA birds is in conformity with the observation of Zhu et al. (2019), who reported that vitamin C was effective in combating heat stress in the later phase of incubation, decreasing mortality and enhancing hatchability. Our findings suggest that the bioactive constituent of clove (eugenol) was beneficial in combating the impact of oxidative stress in CL2 treated eggs, thereby increasing hatchability.

Anatomical Characteristics

Eggs administered low levels of the extract (CL2 and CN2) had higher hatchling weight than eggs injected with high doses (CL4 and CN4) (Table 2). These

Table 1. Responses of broiler chicken to in ovo injection of aqueous extract of clove and cinnamon on the hatchability and quality of day-old chicks.

Parameters	UE	DW	AA	CL2	CL4	CN2	CN4	SEM	*P* value
Egg weight (g)	71.75	70.50	71.25	71.25	71.00	71.50	71.75	4.23	0.993
Hatchability (%)	87.0^{c}	85.0^{c}	89.0^{b}	92.0^{a}	74.0^{e}	89.0^{b}	81.0^{d}	0.02	<.001
Chick weight (g)	38.75^{b}	37.25^{b}	42.25^{a}	41.57^{a}	34.0^{b}	41.75^{a}	36.75^{b}	1.78	0.00 1
Chick/egg Ratio	0.54^{b}	0.52^{bc}	0.59^{a}	0.58^{ab}	0.47^{c}	0.58^{ab}	0.51^{bc}	0.02	<.001
Eye	14.0	10.0	14.0	14.0	12.0	12.0	12.0	4.27	0.801
Appearance	10.0	9.0	10.0	9.5	9.0	9.0	8.5	0.92	0.225
Activity	5.75	6.00	5.50	5.5	6.0	6.0	5.7	0.40	0.338
Navel area	7.50	10.50	12.0	9.0	10.5	9.0	9.0	3.00	0.480
Remaining membrane	11.0	11.0	11.0	10.0	10.0	8.0	9.0	1.95	0.260
Leg	16.0	12.0	16.0	16.0	16.0	12.0	14.0	2.89	0.163
Remaining yolk	16.0^{a}	13.0^{b}	16.0^{a}	16.0^{a}	15.0^{ab}	11.0^{c}	11.0^{c}	2.30	0.008
Yolk absorption	12.0^{a}	9.0^{b}	12.0^{a}	12.0^{a}	12.0^{a}	11.0^{ab}	12.0^{a}	1.06	0.004
Total scores(100)	92.25^{ab}	80.5^{b}	96.5^{a}	92.0^{ab}	90.5^{ab}	78.0^{b}	81.25^{b}	6.28	0.001

^{abc}Means within the row bearing different letters differ significantly ($P < 0.05$).AA, 3 mg ascorbic acid; CL2, 2 mg clove; CL4, 4 mg clove; CN2, 2 mg cinnamon; CN4, 4 mg cinnamon; DW, eggs injected with Distilled water; UE, Uninjected eggs.

Table 2. Effect of in ovo injection of broiler eggs with aqueous extracts of clove and cinnamon on anatomical characteristics (relative weight) at hatch.

Parameters	Liver (%)	HM (%)	Gizzard (%)	YFBW (%)	IW (%)	Heart (%)	RY (%)
UE	0.95^{bc}	0.17^{b}	2.41^{bc}	31.47^{b}	2.01^{bc}	0.28	1.24^{ab}
DW	0.90^{c}	0.207^{b}	1.80^{c}	34.76^{b}	1.62^{c}	0.23	2.33^{a}
AA	1.41^{a}	0.13^{b}	2.71^{abc}	43.01^{a}	1.95^{bc}	0.31	2.75^{a}
CL2	1.28^{ab}	0.34^{a}	3.26^{ab}	40.37^{a}	2.43^{ab}	0.3	2.56^{a}
CL4	1.36^{a}	0.20^{b}	3.26^{ab}	40.22^{a}	2.68^{a}	0.32	2.37^{a}
CN2	1.28^{ab}	0.17^{b}	3.46^{ab}	40.42^{a}	2.43^{ab}	0.3	1.01^{b}
CN4	1.06^{abc}	0.19^{b}	3.26^{ab}	33.84^{b}	2.22^{abc}	0.28	1.29^{ab}
SEM	0.157	0.057	0.415	2.034	0.274	0.047	1.853
P value	0.0005	0.0011	0.0001	0.0001	0.0004	0.263	0.0165

^{abc}Means within the row bearing different letters differ ($P < 0.05$).AA, 3 mg ascorbic acid; CL2, 2 mg clove; CL4, 4 mg clove; CN2, 2 mg cinnamon; CN4, 4 mg cinnamon; DW, eggs injected with Distilled water; IW, intestinal weight; HM, hatching muscle; UE, Uninjected eggs; YFBW, yolk free body weight.

suggests that the bioactive constituents of the additives were beneficial to the chicks at the low doses. The increase in weight at hatch of the chicks from the eggs injected with low doses of the extract in this study is in agreement with the result of El-Kholy et al. (2021), who indicated that cobb fertile eggs injected with 0.5 mL of cinnamon increased the weights of hatchlings over the control group. However, the results of this study are in contrast with the findings of Oliveira et al. (2021), who indicated that spraying hatching eggs with clove essential oil did not influence hatching weight. The discrepancies in the findings may be due to the difference in the routes of administration.

Injection of antioxidants has been shown to confer oxidative protection from excessive free radicals that could adversely affect hatching on developing embryos (Oke et al., 2021b). In ovo feeding of clove and cinnamon extracts improved the relative weights of the liver and gizzard at hatch in this study. This suggests that in ovo injection of clove and cinnamon extracts stimulated the digestive system of the chicks, which could result in improved liver function to increase the pancreatic digestive enzymes. In contrast, Oliveira et al. (2021) reported that clove essential oil sprayed on hatching eggs did not affect the relative internal organs of broiler chickens at one day old.

ETHICAL APPROVAL

The experiment was conducted in accordance with the Institutional Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta, Nigeria. During the experiment the chicks and hatching eggs were provided with the proper care and management without creating unnecessary discomfort.

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DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Castillo-López, R. I., E. P. Gutiérrez-Grijalva, N. Leyva-López, L. X. López-Martínez, and J. B. Heredia. 2017. Natural alternatives to growth-promoting antibiotics (GPA) in animal production. J. Anim. Plant Sci. 27:349–359.

El-Kholy, K. H., D. M. Sarhan, and E. A. El-Said. 2021. Effect of in-ovo injection of herbal extracts on post-hatch performance, immunological, and physiological responses of broiler chickens. World's Poult. Sci. J. 11:183–192.

He, S. P., M. A. Arowolo, R. F. Medrano, S. Li, Q. F. Yu, J. Y. Chen, and J. H. He. 2018. Impact of heat stress and nutritional interventions on poultry production. World Poultry Sci. J. 74:647–664.

N'nanle, O., A. Tété-Bénissan, K. Tona, A. Teteh, K. Voemesse, E. Decuypere, and M. Gbeassor. 2017. Effect of in ovo inoculation of Moringa oleifera leaves extract on hatchability and chicken growth performance. Eur. Poult. Sci. 81:1–9.

Oke, O. E. 2018. Evaluation of physiological response and performance by supplementation of Curcuma longa in broiler feed under hot humid tropical climate. Trop. Anim. Health Pro. 50:1071–1077.

Oke, O. E, U. K. Emeshili, O. S. Iyasere, M. O. Abioja, J. O. Daramola, A. O. Ladokun, J. A. Abiona, T. J. Williams, S. A. Rahman, S. O. Rotimi, S. I. Balogun, and A. E. Adejuyigbe. 2017. Physiological responses and performance of broiler chickens offered olive leaf extract under hot humid tropical climate. J. Appl. Poult. Res. 26:376–382.

Oke, O. E., A. Oso, O. S. Iyasere, T. Adebowale, T. Akanji, O. Odusami, S. Udehi, and J. O. Daramola. 2020. Growth performance and physiological responses of helmeted guinea fowl (*Numida meleagris*) to different stocking densities in humid tropical environment. Agric. Trop. Subtr. 53:5–12.

Oke, O. E., O. B. Oyelola, O. S. Iyasere, C. P. Njoku, A. O. Oso, O. M. Oso, and J. O. Daramola. 2021b. *In ovo* injection of black cumin (Nigella sativa) extract on hatching and post hatch performance of thermally challenged broiler chickens during incubation. Poult. Sci. 100:100831.

Oke, O. E, V. A. Uyanga, O. S. Iyasere, F. O. Oke, B. C. Majekdunmi, M. O. Logunleko, J. A. Abiona, E. U. Nwosu, M. O. Abioja, J. A. Daramola, and O. M. Onagbesan. 2021a. Environmental stress and livestock productivity under hot-humid tropics: alleviation and future perspectives. Therm. Biol. 100:103077.

Oliveira, G. D. S., S. T. Nascimento, V. M. Dos Santos, and B. S. Lima Dallago. 2021. Spraying hatching eggs with clove essential oil does not compromise the quality of embryos and one-day-old chicks or broiler performance. Animals 11:2045.

Sandercock, D. A., M. A. Mitchell, and M. G. MacLeod. 1995. Metabolic heat production in fast and slow growing broiler chickens during acute heat stress. Brit. Poult. Sci. 36:868.

SAS. 2008. User's Guide. Version 9.2. SAS Institute Inc., Cary, NC.

Tona, K., F. Bamelis, B. De Ketelaere, V. Bruggeman, V. M. B. Moraes, J. Buyse, O. Onagbesan, and E. Decuypere. 2003. Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poult. Sci. 82:736–741.

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2131–2144.

Yang, S.-B., Q. Yan-jun, X. Ma, W.-M. Luan, P. Sun, A.-Q. Ju, A.-Y. Duan, Y.-N. Zhang, and D.-H. Zhao. 2021. Effects of in ovo injection of astragalus polysaccharide on the intestinal development and mucosal immunity in broiler chickens. Frontiers Vet. Sci. 8:1004.

Zhu, Y. F., S. Z. Li, Q. Z. Sun, and X. J. Yang. 2019. Effect of *in ovo* feeding of vitamin C on antioxidation and immune function of broiler chickens. Animal 13:1927–1933.