

Original Research Article

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Effect of Ascorbic Acid on Some Biochemical Parameters during Heat Stress in Commercial Broilers

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ABSTRACT

Heat stress is one of the most challenging environmental conditions affecting commercial poultry. Compared to other species of domestic animals, broiler chickens are more sensitive to high ambient temperatures. They have no sweat glands, a rapid metabolism and high body temperature. Supplementation of ascorbic acid (vitamin C) has been found to improve feed intake, body weight gain, feed efficiency, nutrient digestibility, immune response and antioxidant status in poultry. The present work was conducted to investigate the effect of ascorbic acid on biochemical parameters during heat stress in commercial broilers. A total number of 96 birds were randomly divided into 4 groups and each group consists of 12 birds in two replicates. Work was done in two conditions, heat and comfort. Heat stressed groups were maintained at $37\pm 5.0^{\circ}\text{C}$ whereas Comfort groups were maintained at $26\pm 1.0^{\circ}\text{C}$. G1 was taken as control whereas G2, G3 and G4 were supplemented with 100 mg, 200 mg and 300 mg of vitamin C respectively. The maximum response of vitamin C was found in G3 group maintained at $37\pm 5.0^{\circ}\text{C}$ supplemented with 200 mg vitamin C followed by G4, G2 group supplemented with 300 mg, 100 mg vitamin C in feed as compared to G1 *i.e.* control group.

Keywords

Ascorbic acid,
Commercial
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Introduction

Heat stress is one of the most challenging environmental conditions affecting commercial poultry. Compared to other species of domestic animals, broiler chickens are more sensitive to high ambient temperatures. They have no sweat glands, a rapid metabolism and high body temperature. Furthermore, fast-growing lean broilers generate more heat than their free-living counterparts living in the wild (Geraert *et al.*, 1993). As environmental temperature rises,

food consumption, growth rate, feeding efficiency and survivability all decline (Mashaly *et al.*, 2004). High stress also causes oxidative stress and thus weakens the *in vivo* antioxidant defense system, pale, soft, exudative-like changes in meat quality have been observed in broilers exposed to acute or short-term heat stress immediately pre-slaughter (Sandercock *et al.*, 2001). Meteorological factors such as high ambient temperature and high relative humidity exert

adverse effects on poultry production (Chen *et al.*, 2013). They also cause heat stress in poultry during the hot dry season (Ayo *et al.*, 2014). Heat shock proteins (HSP) are a group of highly conserved proteins that are constitutively expressed in most cells under normal physiological conditions in every organism from bacteria to man (Alexandrov, 1994).

Chickens also have other physiological mechanisms to improve thermal resistance (Yahav *et al.*, 1997). When living organisms are exposed to thermal and non-thermal stressors, the synthesis of most proteins is retarded; however, a group of highly conserved proteins known as HSPs are rapidly synthesized.

These proteins are essential for organisms living at the edge of their thermal range. It is well documented that one of the most important functions of HSPs is to protect organisms from the toxic effects of heating (Arrigo *et al.*, 2002). HSPs may play important roles in protein assembly and disassembly (Hartl and Hartl, 2002), protein folding and unfolding protein translocation and the refolding of damaged proteins. Substantial attention has been paid to the role of nutritional additives to minimize the effects of heat stress. The withholding of feed as well as the manipulation of dietary protein content, energy density, calcium, use of carbonated water and usage of vitamin C and E are believed to alleviate the effects of heat stress (Lee, 1992). The most significant increase in ascorbic acid demand take place during acute environmental stress such as excessive hot or cold weather and stress conditions increases the metabolic need for this vitamin or that decrease the innate capacity of biosynthesis. Under such conditions, supplementing the poultry diet with vitamin C may have a beneficial effect on performance (Yigit *et al.*, 2002).

Materials and Methods

The experiment was conducted at college of veterinary science and animal husbandry, N.D.V.S.U., Jabalpur, Madhya Pradesh. Total ninety six (96) day old chicks of commercial broiler birds were procured from private hatcheries of Jabalpur. The birds were maintained in the battery cage system in a well-ventilated room in the poultry experimental unit at college with prior permission from Institutional Animal Ethics Committee. Artificial heat was provided to chicks during early period (brooding period) of growth using thermostatically controlled electric brooders.

The battery brooders were cleaned, washed and disinfected by blow lamping and complete house was fumigated using formaldehyde and potassium permanganate four days prior to start of the experiment. Feeders and waterers were carefully cleaned with detergent. Duration of experiment was six months. The feed was offered *ad-libitum* in linear chicks. Aluminum plates of appropriate size and small tin boxes were used in each cage to offer water during early weeks. Due care was taken so that the chicks reach the feeder and waterer in the first week of age. Later on, large size feeders and waterers were attached to each cage in opposite direction. All-mash system of feeding was practiced during the experiment. Fresh and clean drinking water was made available to birds all the time. Thus, during entire period of study uniform conditions of housing, brooding, feeding and watering was maintained for all the groups of the experiment. Broiler birds randomly divided into eight groups. Four groups of birds were maintained in summer conditions (May to June) maintained in heat stress ($37\pm 5.0^{\circ}\text{C}$) ambience, whereas other four groups of birds was maintained at $26\pm 1.0^{\circ}\text{C}$ (comfort temperature) using an air conditioner. Broilers

were kept in closed ventilated system for 45 days during the experimental period. Temperature and humidity of the experimental poultry unit was recorded using a digital thermo-hygrometer.

Diets were formulated as per NRC (1994) specifications presented in Table 1. For analysis of biochemical parameters, blood was collected from individual birds on specified day of experiment i.e. on 15th, 30th and 45st day. The blood samples were collected by cleaning the area by plucking the feather and wiping the area by ethanol swab. A 22 gauge needle was used for collection of blood. The blood samples were collected in heparinized polypropylene tubes (20 IU heparin/ml of blood) were kept in the ice bucket and carried back to the laboratory immediately. In the laboratory, all the blood samples were centrifuged at 3000 rpm for 30 min and plasma was separated. Plasma obtained was kept in the labeled storage vials of 2 ml capacity and stored at -20°C till analysis for biochemical parameters.

The plasma ascorbic acid was estimated using DCIP method described by (Omaye *et al.*, 1979). Plasma glucose concentration was estimated by Trinder's method (Pileggi and Szuskeiweiz, 1974) using diagnostic kits procured from Erba Diagnostics, Mannheim GmbH, Germany. The concentration was expressed in mg/dl of blood glucose. Plasma albumin concentration was estimated as per method described by Dumas *et al.*, (1972) using diagnostic kits procured from Erba Diagnostics, Mannheim GmbH, Germany. The concentration was expressed in g/dl of albumin. Automatic biochemistry analyzer was used for the determination of plasma ascorbic acid and albumin concentration.

The pH was determined using a modification of the iodoacetate method described by Petracci *et al.*, (2004). Lipid peroxidation was

determined by a micro method for TBA-reactive substances (TBARS). Approximately 100 mg of liver was incubated in 0.5 mL 50% trichloroacetic acid containing 1.3% (wt/ vol) thiobarbituric acid (dissolved at 60°C) and heated at 60°C for 1 h, followed by determination of absorbance of supernatant at 532 nm. Tetraethoxypropane, which spontaneously decomposes in an aqueous environment to form malondialdehyde (MDA), was used as a standard and absorbance was expressed as MDA equivalents. MDA equivalents were calculated after subtraction of blank (water), correction for turbidity measured at 650 nm and dilution of the TBA reagent from water contained in the meat. The recorded data was statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1994). Various conditions and treatment groups were compared by using Duncan Multiple Range test (DMRT).

Results and Discussion

The mean plasma ascorbic acid concentration of broilers has been presented in Table 2. The overall mean concentration of AA showed non-significant difference between comfort and heat stressed birds in all the groups. In comfort condition, maximum (6.75±0.07 mg/dl) and minimum (4.57±0.06 mg/dl) concentration of AA was observed in G4 and control group respectively which differ significantly ($p<0.01$). However, in G4 and G3 non-significant difference was observed and similar trend was observed between G3 and G2. In heat stressed condition, maximum (6.76±0.09 mg/dl) and minimum (5.25±0.09 mg/dl) concentration of AA was observed in G4 and control group respectively which differ significantly ($p<0.05$). Further, non-significant difference was observed in G2, G3 and G4 whereas, similar trend was observed between G1 and G2. A significant linear positive trend in AA was noticed with the

levels supplied and their levels in plasma during the investigation. Because AA is actively transported into tissues and its use increases during period of stress such as heat and the bird's synthesizing capacity may become inefficient thus reducing plasma AA concentrations, which might be the case in the unsupplemented group in present investigation. The efficacy of AA supplementing in birds under stressful conditions, depends upon its ability to elevate plasma AA concentrations, thereby preventing tissue depletion. The AA either decreases heat load by lowering heat production or increases heat loss by influencing avenues of thermal exchange between the body and the environment.

On supplementation of varying concentration of AA, the overall mean concentration of plasma AA, showed non-significant difference between comfort and heat stressed broilers in all the groups. Mahmoud *et al.*, (2004) reported that dietary AA supplementation elevated plasma AA and maintained it at high levels after heating but in non-ascorbic acid (N-AA) broiler birds, only heat elevated plasma AA, though the increase was non-significant, which is in corroboration to present findings. Supplementation with AA caused a significant increase in the plasma AA (18.8 to 26.6 µg/ml) when compared to N-AA group (8.7 to 16.4±0.93 µg/ml) which does not match to our findings. As per our reports, in comfort condition, maximum (6.75 mg/dl) and minimum (4.57 mg/dl) concentration of AA was observed in 300 mg AA supplemented group and control group respectively which differ significantly. In the present research investigation the plasma AA content revealed significantly higher levels in birds fed with AA supplemented diets as compared with the non-supplemented one. Similar findings were also reported by Lohakare *et al.*, (2005).

The mean plasma glucose concentration of broilers has been presented in Table 3. The overall mean concentration of glucose showed non-significant difference between comfort and heat stressed birds in all the groups. In comfort condition, G3 and G4 group differ significantly ($p < 0.01$) from G1 but non-significant difference was observed between G2, G3 and G4 group. Also, G1 and G2 group differed non-significantly. In heat stressed condition non-significant difference was observed between G3 and G4. Similar trend was also observed between G2 and G4. Group G1 differ significantly ($p < 0.01$) from all the treatment groups of heat stressed condition showing minimum concentration (217.94±04.96 mg/dl) of glucose. On supplementation of 100 mg, 200 mg and 300 mg AA in feed, it was found that the overall mean concentration of glucose showed non-significant difference between comfort and heat stressed birds in all the groups. Similar to present investigation, various workers reported blood glucose levels in broilers under different thermal stress conditions (Zulkifli *et al.*, 1999; Aksit *et al.*, 2006; Olanrewaju *et al.*, 2010). In present study, significantly higher levels of plasma glucose were observed in 200 mg AA supplemented birds as compared to heat stressed chickens. In comfort condition, present means of plasma glucose at 45th day, on supplementation of AA (287.50±05.50 mg/dl), were comparable to those reported by Borges *et al.*, (2004) in 6 weeks old Cobb broiler (283.4 mg/dl) at 41°C. Higher glucose levels have been reported by supplementation of AA during heat stress condition (Zulkifli *et al.*, 1999; Aksit *et al.*, 2006; Olanrewaju *et al.*, 2010; Borges *et al.*, 2004). Sujatha *et al.*, (2010) reported that the glucose concentration in the synthetic (100 g/tonne of feed) AA supplemented birds was significantly lower as compared to control group after 3rd and 5th week in broiler birds which is not similar to present findings.

Table.1 Formula and chemical composition of broiler ration

| Ingredients | Starter % | Finisher % |
|---------------------------------------|-----------|------------|
| Maize | 58.805 | 59.50 |
| Soybean | 28 | 26 |
| Sunflower meal | 5 | 2.5 |
| Fish meal | 5 | 3 |
| Limestone | 1.0 | 0.8 |
| Di-calcium phosphate | 1.5 | 1.1 |
| Salt | 0.2 | 0.2 |
| DL- Methionine | 0.06 | 0.04 |
| Trace mineral Premix | 0.1 | 0.1 |
| Vitamin premix* | 0.15 | 0.15 |
| Vitamin B complex** | 0.015 | 0.015 |
| Choline chloride | 0.05 | 0.05 |
| Toxin binder | 0.05 | 0.05 |
| Protexim | 0.02 | - |
| Cocciostat | 0.05 | 0.05 |
| De-oiled rise bran | - | 1.42 |
| Rape seed meal | - | 5 |
| Lysine | - | 0.02 |
| Total | 100 | 100 |
| Nutrient Composition | | |
| Crude protein (%) | 21.66 | 18.98 |
| Metabolizable energy (Kcal. ME/Kg)*** | 2843 | 2850 |
| Calcium (%) | 1.17 | 1.17 |
| Available phosphorus (%) | 0.496 | 0.5 |
| Lysine (%) | 1.24 | 1.22 |

*Trace mineral Premix: Mg-300, mn-55, I-0.4, fe-56, Zn-30 and Cu-4kg-1

** Vitamin premix: Vitamin A-8250 IU, Vitamin D₃- 1200 IU, Vitamin k-1mg, Vitamin E-40 IU, Vitamin B1 2mg, Vitamin B₂-4mg, Vitamin B₁₂-10mg, Percent of values specified by NRC, 1994, *** Calculated.

Table.2 Mean plasma ascorbic acid concentration (mg/dl) of broilers at different intervals

| Period | Condition | G1 | G2 | G3 | G4 |
|----------------------|-----------|----------------------------------|-----------------------------------|-----------------------------------|----------------------------------|
| 15 th day | Comfort | 4.80 ^B ± 0.02 (12) | 5.57 ^B ± 0.03 (12) | 6.80 ^A ± 0.03 (12) | 7.16 ^A ± 0.06 (12) |
| | Heat | 5.26 ^B ± 0.03 (12) | 6.01 ^{AB} ± 0.04 (12) | 6.61 ^A ± 0.08 (12) | 6.48 ^A ± 0.08 (12) |
| 30 th day | Comfort | 4.07 ^B ± 0.03 (12) | 6.58 ^A ± 0.03 (12) | 6.62 ^A ± 0.04 (12) | 6.52 ^A ± 0.08 (12) |
| | Heat | 5.90 ± 0.03 (12) | 6.73 ± 0.04 (12) | 6.48 ± 0.05 (12) | 6.50 ± 0.07 (12) |
| 45 th day | Comfort | 4.83 ^B ± 0.03 (12) | 6.70 ^A ± 0.03 (12) | 6.70 ^A ± 0.03 (12) | 6.57 ^A ± 0.09 (12) |
| | Heat | 4.97 ^B ± 1.03 (12) | 6.23 ^{AB} ± 0.02 (12) | 7.20 ^A ± 0.05 (12) | 7.30 ^A ± 0.07 (12) |
| Overall mean | Comfort | 4.57 ^C ± 0.06 (36) | 6.28 ^B ± 0.098 (36) | 6.70 ^{AB} ± 0.02 (36) | 6.75 ^A ± 0.07 (36) |
| | Heat | 5.25 ^b ± 0.09 (36) | 6.33 ^{ab} ± 0.05 (36) | 6.66 ^a ± 0.08 (36) | 6.76 ^a ± 0.09 (36) |

Means bearing different superscripts within same row differ significantly (^{ABC}; p<0.01, ^{ab}; p<0.05).

Comfort (26±1° C), Heat (37±5° C) G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

Table.3 Mean plasma glucose (mg/dl) level of broilers at different intervals

| Period | Condition | G1 | G2 | G3 | G4 |
|----------------------|-----------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| 15 th day | Comfort | 265.00 ^B ±01.44 (12) | 285.67 ^{AB} ±08.01 (12) | 321.75 ^A ± 06.17 (12) | 303.33 ^{AB} ±04.84 (12) |
| | Heat | 239.58 ^B ± 02.93 (12) | 277.83 ^{AB} ±02.51 (12) | 311.25 ^A ± 05.12 (12) | 291.17 ^A ±05.92 (12) |
| 30 th day | Comfort | 246.92 ^B ± 02.71 (12) | 286.33 ^{AB} ±03.41 (12) | 309.75 ^A ± 04.76 (12) | 296.67 ^{AB} ±05.58 (12) |
| | Heat | 233.17 ^C ± 05.20 (12) | 268.00 ^B ±03.21 (12) | 307.33 ^A ± 05.00 (12) | 291.25 ^{AB} ±04.90 (12) |
| 45 th day | Comfort | 242.92 ^B ± 02.33 (12) | 269.58 ^{AB} ±02.74 (12) | 306.92 ^A ± 06.0 (12) | 305.67 ^A ±03.93 (12) |
| | Heat | 181.08 ^B ± 03.44 (12) | 271.75 ^A ± 02.83 (12) | 299.25 ^A ± 05.54 (12) | 280.08 ^A ±05.89 (12) |
| Overall mean | Comfort | 251.61 ^B ±02.05 (36) | 280.53 ^{AB} ±02.16 (36) | 312.81 ^A ± 03.44 (36) | 301.89 ^A ±02.78 (36) |
| | Heat | 217.94 ^C ±04.96 (36) | 272.53 ^B ± 01.75 (36) | 305.94 ^A ± 03.05 (36) | 287.50 ^{AB} ±03.26 (36) |

Means bearing different superscripts within same row differ significantly (^{ABC}; p<0.01).

Comfort (26±1°C), Heat (37±5°C)

G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

Table.4 Mean plasma albumin concentration (g/dl) of broilers at different intervals

| Period | Condition | G1 | G2 | G3 | G4 |
|----------------------|-----------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|
| 15 th day | Comfort | 1.66 ^B ±0.02 (12) | 2.39 ^{AB} ±0.03 (12) | 3.48 ^A ±0.04 (12) | 3.06 ^A ±0.04 (12) |
| | Heat | 1.35 ^b ±0.02 (12) | 1.92 ^{ab} ±0.03 (12) | 3.28 ^a ±0.04 (12) | 3.07 ^{ab} ±0.06 (12) |
| 30 th day | Comfort | 1.75 ^b ±0.02 (12) | 2.91 ^{ab} ±0.04 (12) | 4.19 ^a ±0.05 (12) | 3.87 ^a ±0.06 (12) |
| | Heat | 1.61 ^B ±0.02 (12) | 2.62 ^{AB} ±0.04 (12) | 3.89 ^A ±0.05 (12) | 3.42 ^A ±0.10 (12) |
| 45 th day | Comfort | 1.82 ^b ±0.03 (12) | 3.59 ^{ab} ±0.03 (12) | 4.88 ^a ±0.09 (12) | 4.37 ^{ab} ±0.11 (12) |
| | Heat | 1.62 ^C ±0.02 (12) | 2.61 ^{BC} ±0.09 (12) | 4.76 ^A ±0.08 (12) | 3.68 ^{AB} ±0.10 (12) |
| Overall mean | Comfort | 1.74 ^B ± 0.02 (36) | 2.96 ^{AB} ±0.08 (36) | 4.18 ^A ±0.10 (36) | 3.77 ^A ±0.10 (36) |
| | Heat | 1.52 ^C ± 0.02 (36) | 2.38 ^{BC} ± 0.06 (36) | 3.98 ^A ± 0.11 (36) | 3.39 ^B ±0.06 (36) |

Means bearing different superscripts within same row differ significantly (^{ABC}; p<0.01, ^{abc}; p<0.05).

Comfort (26±1°C), Heat (37±5°C)

G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

Table.5 Breast muscle pH of sacrificed broilers

| Birds | Condition | G1 | G2 | G3 | G4 |
|----------|-----------|-----|-----|-----|-----|
| Broilers | Comfort | 6.9 | 6.4 | 6.2 | 6.5 |
| | Heat | 6.8 | 6.6 | 6.4 | 6.7 |

Comfort (26±1°C), Heat (37±5°C)

G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

Table.6 Thiobarbituric acid value (nmol MDA equivalent/mg wet tissue) in liver homogenates of broilers

| Birds | Condition | G1 | G2 | G3 | G4 |
|----------|-----------|------|------|------|------|
| Broilers | Comfort | 5.13 | 2.85 | 2.34 | 3.43 |
| | Heat | 5.47 | 3.59 | 2.32 | 2.76 |

Comfort (26±1°C), Heat (37±5°C)

G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

The mean plasma albumin concentration of broilers has been presented in Table 4. The overall mean concentration of albumin showed non-significant difference between comfort and heat stressed birds in all the groups. In comfort condition, G3 and G4 group differ significantly ($p < 0.01$) from G1, but non-significant difference was observed between G2, G3 and G4 group. Also, G1 and G2 group differ non-significantly. In heat stressed condition significant ($p < 0.01$) difference was observed between the G1, G3 and G4 groups. Similar trend was also observed between G2 and G3. However, non-significant difference was observed between G1 and G2. Similar trend was also observed between G2 and G4 groups. In the present findings, the AA supplemented birds had higher serum albumin level as compared to control group of broilers. Sabah *et al.*, (2008) reported that the dose of 750 mg/Kg AA gave the highest value of serum albumin followed by 500, 250 mg/Kg AA supplementation respectively, which is in disagreement to our findings in which 200 mg AA resulted in the highest concentration of albumin followed by 300 mg and 100 mg supplemented group. Results obtained from the present investigation also indicate that dietary AA has beneficial effects on commercial broilers.

Kutlu and Forbes (1993) reported that vitamin C supplementation increases serum albumin concentrations.

The breast muscle pH of broilers has been presented in Table 5. On day 45, in the comfort condition, the higher breast muscle pH of 6.9 was observed in control and G1 group of sacrificed broilers, whereas, the lower pH of 6.2 was recorded in G3, supplemented with 200 mg AA. On day 45, in heat stressed condition, the higher breast muscle pH of 6.8 was observed in control group of sacrificed broilers, whereas, the lower pH of 6.4 was recorded in G3, supplemented with 200 mg AA. The pH of the sacrificed birds is comparatively higher as compared to the ultimate pH obtained after few hours of sacrifice, which suggests the beneficial effect of AA supplementation in broilers. Present findings are in disagreement with findings of Kadim *et al.*, (2009), who reported that ascorbic acid supplementation in drinking water affects meat quality and *Pectoralis* muscles collected during the hot season group of birds had significantly higher pH than those collected during cool season group. The possible justification for present findings might be the fact that high ambient temperatures reduce the bird's feed intake and

impose physiological stresses, which activate glycogenolysis in skeletal muscle (Kreikemeier *et al.*, 1998).

The thiobarbituric acid value of broilers has been presented in Table 6. In the comfort condition, the higher TBA value of sacrificed broilers on day 45 was 5.13 nmol MDA equivalent / mg wet tissue in control group whereas, the lower TBA value was recorded 2.34 nmol MDA equivalent / mg wet tissue in G3, which was supplemented with 200 mg AA.

In the heat stressed condition, the higher TBA value of sacrificed broilers on day 45 was 5.47 nmol MDA equivalent / mg wet tissue in control group whereas, the lower TBA value was recorded 2.32 nmol MDA equivalent / mg wet tissue in G3, which was supplemented with 200 mg AA. The probable reason for such reports might be due to the fact that rearing broiler chickens for 5-6 weeks may not be long enough for the ascorbic acid to have an effect on muscle metabolism, which could also be the reason for present findings. Jang *et al.*, (2014) reported that dietary supplementation with vitamin C subjected to summer HS in broilers markedly decreased (TBA value) lipid peroxidation in liver compared with the control group, is in agreement to our findings. In the present study, dietary vitamin C significantly decreased hepatic lipid peroxidation. In particular, several studies demonstrated that dietary vitamin C showed a significant decrease in MDA value, as an indicator of lipid peroxidation (Cherian *et al.*, 1996 and Sahin *et al.*, 2002).

Significant increase in plasma glucose concentration is indicative of glucogenic effect of ascorbic acid in chickens. Reduction in thiobarbituric acid value indicates amelioration in oxidative stress via reduced lipid peroxidation in chickens.

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