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The Effect of Vitamin C on Oxidative Stress, Nitric Oxide Formation and Viability of Broiler Finisher Blood Cells Subjected to High Ambient Temperature

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ABSTRACT

Background: Vitamin C (VitC) is a widely used antioxidant in poultry production. However, there is some ambiguity about the effects of VitC in reducing oxidative stress in poultry when they are under heat stress. Therefore, the objective of this study was to investigate the effects of VitC on reducing oxidative stress and nitric oxide (NO) formation which affects the viability of the broiler finisher blood cells (BFBC).

Methods: BFBC were maintained at temperature at 41°C and the temperature increasing from 41°C to 45°C. At 41°C to 45°C, BFBC supplemented with VitC solution at concentrations of 0, 25, 50 and 75 µmol. Then, total antioxidant power (FRAP), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), NO in supernatant and the viability of BFBC were measured.

Result: It was found that MDA and NO levels were increased at high ambient temperature ($P<0.05$). At the same time, the viability of BFBC was decreased ($P<0.05$). Vitamin C at the concentration of 50 µmol could reduce MDA and NO better than at other concentrations ($P<0.05$) and increased the viability of BFBC ($P<0.05$). This phenomenon indicated that VitC reduced oxidative stress and NO levels, which were important factors for BFBC viability at high ambient temperature.

Key words: Broiler finisher blood cell (BFBC), Heat stress, Nitric oxide, Oxidative stress, Vitamin C.

INTRODUCTION

Global warming is causing anomalies of climatic and natural conditions such as suboptimal temperatures, droughts, wildfires, floods and storms *etc.* (Zhao *et al.*, 2022). In addition, Lee *et al.* (2021) hae reported that a continuous increase in environmental temperature adversely affects the performance and health of animals. High ambient temperature is the major stressor in the poultry production system (Goel, 2021). In the tropics, environmental temperatures and high relative humidity exert a significant influence on poultry production resulting from heat stress (Shakeri *et al*., 2020; Nawaz *et al.*, 2021). Generally, heat stress can disturb the balance between the generation of reactive oxygen species (ROS) and the antioxidant system (Jang *et al.*, 2014).

Vitamin C or ascorbic acid is a water-soluble vitamin, which is required for a range of metabolic reactions in animals, such as inhibition of active oxygen species (Rajabi and Torki, 2021). Generally, VitC is synthesized from glucose (Ahmadu *et al.*, 2016) in the kidney of poultry (Adenkola and Angani, 2017). However, dietary supplementation of VitC is not widely practiced in poultry production, because it is believed that poultry can synthesize sufficient amounts of VitC (Rajabi and Torki, 2021; Yu *et al.*, 2021). This vitamin can effectively protect the body from various deleterious effects of free radicals, both reactive oxygen and nitrogen species (Righi *et al.*, 2020). Vitamin C plays major role as a cellular antioxidant and also participates in the regeneration of reduced glutathione from the oxidised form in the cytoplasm (Ahmadu *et al.*, 2016). Therefore, VitC plays a crucial role in alleviating the cellular damage of tissues and preventing heat stress (Yu *et al.*, 2021). However, VitC concentration was decreased in poultry that experienced heat stress (Horváth and Babinszky, 2018). Even if poultry have the ability to synthesis VitC, the amount of this vitamin was still insufficient for chickens experiencing heat stress (Rajabi and Torki, 2021). In heat-stressed broilers, dietary VitC supplementations have improved performance and humoral immunity (Saracila *et al.*, 2021). In addition, VitC could ameliorate other heat stress induced problems such as oxidative stress (Abadin and Kathoon, 2013). On the other hand, Mosleha *et al.* (2018) found that VitC could not reduce malondialdehyde (MDA), a parameter of oxidative stress of heat stressed broilers.

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Previous studies, have found that VitC could reduce the effects of oxidative stress in heat stressed poultry. In contrast, some studies reported that VitC could not decrease oxidative stress. This discrepancy might be caused by many factors. Therefore, the objective of this study was to investigate the effects of VitC on total antioxidant power (FRAP), hydrogen peroxide (H_2O_2), MDA and nitric oxide (NO) production; and viability of BFBC when subjected to a high environmental temperature. The knowledge resulting from this study will help confirm the efficacy of VitC a suitable level for reducing the effects of environmental temperature on broilers by using the BFBC as an *in vitro* model.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Experimentation of Mahasarakham University (license number: IACUC-MSU-01/2021).

Animal

The present study was an *in vitro* study and was adapted from the method developed by Aengwanich and Wandee (2022). The experiment was performed during February-April 2022 at the laboratory, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham, Thailand. One healthy male broiler, 28 days of age, was obtained from a poultry farm in Mahasarakham province. The male broiler was reared in housing for 28 days. A broiler finisher was fed *ad libitum* with commercial feed and continuous water supplies. In this study, a broiler finisher was used as a blood donor.

Experimental design and treatments

The experimental design and treatments are shown in Table 1. The details of the experiment were as follows.

1 ml of blood sample was collected from the jugular vein of the broiler finisher and placed in a tube containing heparin. The heparinised broiler blood was then added to phosphate buffered saline (PBS), pH 7.4 and centrifuged at 2500 rpm (769 × g) for 5 min. The supernatant was discarded. This process was performed twice. TCG was diluted in 1:200 PBS, pH 7.4. In the other 4 groups, BFBC at the ambient temperature increasing from 41°C to 45°C was supplemented with VitC at concentrations of 0 (TPCG), 25, 50, 75 µmol in PBS, pH 7.4, each concentration with the same ratio of dilution as TCG.

The diluted blood samples were placed in 2 water baths. In the first water bath, the temperature was maintained at 41°C throughout the experimental period. In the second water bath, TPCG and BFBC were diluted with different levels of VitC and were conditioned at 41°C for 30 min before starting the experiment and then continuously increasing temperature from 41°C to 45°C. BFBC were maintained for 30 min at defined temperatures in the ambient range 41°C to 45°C. At the end of the incubation period, all test tubes were removed from the water bath. Then FRAP, MDA, NO and H_2O_2 in the supernatant and viability of blood cells were investigated from sedimented blood.

Determination of indicators

FRAP and MDA and NO were measured by the method described by Aengwanich and Wandee (2021, 2022), respectively. Measurement of H_2O_2 was performed according to the method of Orprayoon *et al.* (2020). Blood cell viability was investigated by a method adapted from the report of Vajrabhaya and Korsuwannawong (2018).

Statistical analysis

The normal distribution of data was tested. Data were analysed using one-way analysis of variance. Means were separated by Duncan's multiple range tests. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Results are shown in the Fig 1-5. Vit C was used as an external antioxidant for studying the efficacy of this vitamin to reduce the effect of high ambient temperature on BFBC. It was found that FRAP of BFBC that had been diluted with VitC increased when VitC concentration was increased. However, at ambient temperatures increasing from 41°C to 45°C, FRAP of TPCG was not different from BFBC diluted with VitC at the concentration of 25 µmol. It was possible that as the ambient temperature increased from 41°C to

Table 1: Experimental design and treatments of the present study.

List	Detail of experimental design and treatments
1. Design	Completely randomized design (CRD)
2. Treatments	1. The first group, BFBC, was maintained at 41°C throughout the experimental period (the control group, TCG).
	2. The second group, BFBC, was maintained at ambient temperatures increasing from 41°C to 45°C (the positive control group, TPCG).
	3. The third group, BFBC was maintained at ambient temperatures increasing from 41°C to 45°C and was supplemented with VitC at concentrations of 25 µmol.
	4. The fourth group, BFBC, was maintained at ambient temperatures increasing from 41°C to 45°C and was supplemented with VitC at concentrations of 50 µmol.
	5. The fifth group, BFBC, was maintained at ambient temperatures increasing from 41°C to 45°C and was supplemented with VitC at concentrations of 75 µmol.

Each treatment consisted of 3 replications.

45°C, cell membranes of BFBC might have been damaged, resulting in the intracellular antioxidants leaking outward to the supernatant. This assumption was in line with the report of Aengwanich and Wandee (2021). Thus, the FRAP of these two groups was not different.

MDA is a lipid peroxidation product (Gaschler and Stockwell, 2017) that is used to indicate the occurrence of the oxidative stress (Aengwanich and Wandee, 2022). In this study, it was found that MDA of TPCG was higher than TCG. This result was similar to the report of Akbarian *et al.* (2016) and Bai *et al.* (2019), who found that after broilers had been subjected to high environmental temperature, MDA in serum increased. When considering the MDA level of the BFBC at ambient temperatures increasing from 41°C to 45°C, it was found that the group that had been diluted with VitC at higher concentrations resulted in decreased MDA in the supernatant. The results of this study showed that the increased VitC concentration could effectively reduce the occurrence of oxidative stress and are consonant with the report of Khan *et al.* (2012), who found that VitC could reduce MDA in heat stressed chicken. In contrast, Mosleha *et al.* (2018) found that VitC could not reduce MDA in heat stressed broilers. Therefore, the utilization of VitC to reduce the occurrence of oxidative stress might give both results. - 1.)

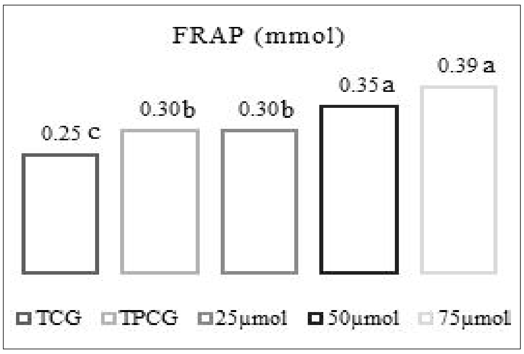

Fig 1: FRAP in the supernatant of TCG and BFBC at the ambient temperature increasing from 41°C to 45°C that diluted with VitC at concentrations of 0 (TPCG), 25, 50 and 75 µmol, respectively. Means with different letters are significantly different among treatments (P<0.05).

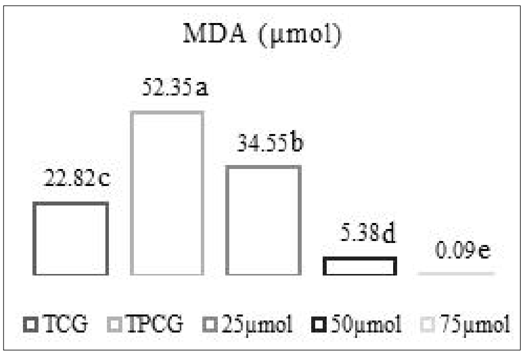

Fig 2: MDA in the supernatant of TCG and BFBC at the ambient temperature increasing from 41°C to 45°C that diluted with VitC at concentrations of 0 (TPCG), 25, 50 and 75 µmol, respectively. Means with different letters are significantly different among treatments (P<0.05).

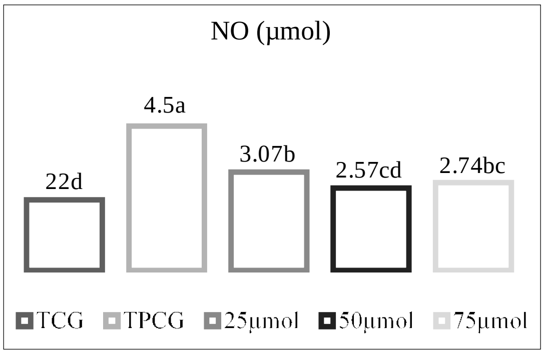

Fig 3: NO in the supernatant of TCG and BFBC at the ambient temperature increasing from 41°C to 45°C that diluted with VitC at concentrations of 0 (TPCG), 25, 50 and 75 µmol, respectively. Means with different letters are significantly different among treatments (P<0.05).

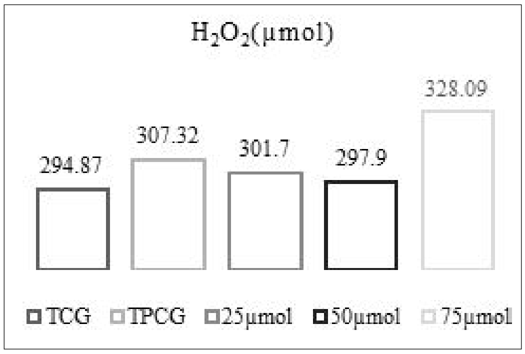

Fig 4: H_2O_2 in the supernatant of TCG and BFBC at the ambient temperature increasing from 41°C to 45°C that diluted with VitC at concentrations of 0 (TPCG), 25, 50 and 75 µmol, respectively.

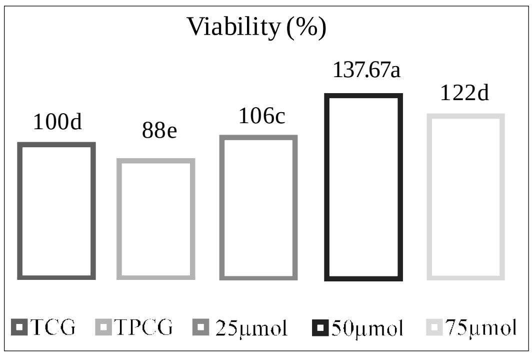

Fig 5: BFBC viability of TCG and BFBC at the ambient temperature increasing from 41°C to 45°C that diluted with VitC at concentrations of 0 (TPCG), 25, 50 and 75 µmol, respectively. Means with different letters are significantly different among treatments (P<0.05).

it could reduce the occurrence of oxidative stress or 2.) have no effect, for which there may be other factors that need to be considered together, even if in the present study, It was found that VitC had high efficacy to reduce MDA levels. However, this study was performed *in vitro* and BFBC were exposed to VitC directly and this might represent the real efficacy of this vitamin to reduce oxidative stress during BFBC exposed to high ambient temperature.

NO is a free radical which is produced in cells through catalysis by nitrous oxide synthase. Heat stress can upregulate the NOS gene and cause overproduction of NO (Hu *et al*., 2019), finally resulting in cell death (Wang *et al.*, 2010). In the present study, NO of TPCG was higher than TCG. This result showed that when the BFBC are at a high ambient temperature, they will increase the production of NO. This finding was consistent with the report of Vinoth *et al.* (2016) who found that when chicks were exposed to high environmental temperature, there was over production of NO. As the ambient temperature increased from 41°C to 45°C, we found that the NO levels of BFBC diluted with vitC were lower than TPCG. Also, the NO of BFBC that had been diluted with VitC at the concentration of 50 µmol was lower than at the concentration of 25 µmol. This result indicated that vitC could decrease NO production when BFBC were exposed to high ambient temperature. The efficacy of VitC to reduce NO found in this study was in agreement with the report of Akolkar *et al.* (2017) who found that VitC could reduce the production of NO *via* the modulation of NO synthases.

In the present study, H_2O_2 of TCG and other groups at ambient temperatures increasing from 41°C to 45°C were not significantly different. This result is different from the report of Ohno *et al.* (2009), who found that VitC could stimulate the production of extracellular H_2O_2. Aengwanich and Wandee (2022) found that when broiler blood cells were maintained at 44-45°C or higher, these high ambient temperatures resulted in apoptotic broiler blood cells. In this study, the viability of TCG was higher than that of TPCG. This phenomenon is consistent with the increase in MDA and NO when BFBC were subjected to ambient temperatures increasing from 41°C to 45°C. Therefore, the viability of BFBC at ambient temperatures increasing from 41°C to 45°C was lower than at 41°C and this might result from the increase of MDA and NO as the ambient temperature was increased. However, at ambient temperatures increasing from 41°C to 45°C, the viability of the BFBC that had been diluted with VitC at the concentration of 50 µmol was higher than TPCG and BFBC that was diluted with VitC at concentrations of 25 and 75 µmol. This result was similar to the report of Wu *et al*. (2015), who found that optimal VitC concentration could increase viability of periodontal ligament cells. However, although VitC at the concentration of 50 µmol could increase viability of BFBC more than at other concentrations, when the VitC concentration was increased to 75 µmol, the viability of BFBC was decreased due to the increased H_2O_2 of BFBC when diluted with VitC at this concentration.

CONCLUSION

Increasing ambient temperature induced BFBC to experience oxidative and nitrosative stress. The supplementation of VitC in the diluent of BFBC at the high ambient temperature resulted in decreasing occurrence of oxidative and nitrosative stress. In addition, VitC also increased BFBC viability at high ambient temperature. Overall, it can be concluded that VitC increased the viability of BFBC when subjected to high ambient temperatures because this vitamin could decrease oxidative and nitrosative stress. Finally, the suitable level of VitC for reducing the effect of oxidative and nitrosative stress of BFBC when exposed to high ambient temperature was 50 µmol.

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Conflict of interest: None.

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