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To:

First author/ Corresponding: **Edi Erwan**

Coauthor: **Triani Adelina, Alaidin Koto, Vebera Maslami**

We are pleased to inform you that your manuscript # **JWPR-1173** entitled "**The Potency of Oral Administration of L-Citrulline as Anti Heat Stress Agent in KUB Chickens**" has been accepted for publication in volume 10 issue 1 of **The Journal of World's Poultry Research (JWPR)**.

Thank you for submitting your manuscript to JWPR. Should you have any question, please contact us.

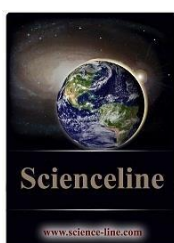
A handwritten signature in black ink, appearing to read 'Daryoush Babazadeh', is shown on a light-colored background.

Sincerely,

Daryoush Babazadeh, DVM, DVSc, PhD of Avian Diseases

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From: editor jwpr <editorjwpr@gmail.com>

To: Edi Erwan <erwan_edi@yahoo.com>

Sent: Sunday, 19 January 2020, 08:41:01 pm GMT+7

Subject: Re: Journal of World's Poultry Research - Review Results 1173

Dear E. Erwan,

Thank you for your email. The paper will be published on March issue after full revisions. The next edition of paper will be sent for continuing the review process.

Best Regards,
Daryoush Babazadeh

On Sun, Jan 19, 2020 at 14:52 Edi Erwan <erwan_edi@yahoo.com> wrote:

Dear Dr. Daryoush Babazadeh,

The Potency of Oral Administration of L-Citrulline as Anti Heat Stress Agent in KUB Chickens

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ABSTRACT

L-citrulline (L-Cit) is considered one of the potential amino acids that are able to reduce body temperature in layer chicks. However, there are no reports on functions of L-Cit in KUB chickens. Therefore, the present study aimed to evaluate the effects of oral administration of L-Cit on feed intake, body temperature and plasma biochemical parameters of KUB chickens over two hours post-administration. Neonatal KUB chicks were orally administered with different doses of L-Cit (0, 3.75, 7.5 and 15 mmol/kg body weight). At 30, 60 and 120 min, feed intake was determined, also rectal temperature of chicks was measured by using a digital thermometer.

At 120 min after administration, the blood samples were immediately collected through the jugular vein, then biochemical parameters of plasma (total cholesterol, triacylglycerol, and total glucose) were analyzed. The obtained results indicated that feed intake, body temperature, and plasma metabolites were not significantly influenced by different doses of L-Cit. In conclusion, L-Cit could not able to improve feed intake, decrease body temperature and change plasma metabolites in KUB chicks.

Keywords: Body temperature, Feed intake, KUB chick, L- citrulline, Plasma metabolites

INTRODUCTION

In general, native chickens play an important role in supporting income generation, especially in many underdeveloped countries (Padhi, 2016). KUB chickens are offspring of

local chickens in several areas of West Java that are obtained after six generations of a systematically-controlled breeding system (Purbarani et al., 2019). The KUB chickens have several advantages compared to their previous generations as well as local chickens. For instance, KUB chickens have high rates of egg production (160-180 eggs/year), high hatchability, and low feed conversion ratio (Sartika, 2016). Besides that, KUB chicken is considered a meat type breed (Hidayah et al., 2019) and the high demand for chicken meat might potentially be fulfilled by increasing the productivity of KUB chicken.

In order to obtain good performance as an expression of the genetically improved breed, some efforts such as optimal management conditions (housing and feeding), is needed to increase biological and economic productivity. Many factors may influence poultry production in tropical and subtropical areas. The climatic environment is one of the important factors affect production efficiency in these regions (Renaudeau et al., 2012). Poultry experiences heat distress when the combination of relative humidity and ambient temperature rise above the comfort zone (Teeter and Belay, 1996). There are concerns about reduced poultry production due to high ambient temperatures not only in tropical regions but also in some countries that occupy the temperate where heat stress is an occasional problem during the summer months.

Several nutritional strategies have been reported to overcome the adverse effects of heat stress in poultry (Teeter and Belay, 1996; Yahav, 2000; Erwan et al., 2013a, 2014, 2017; Chowdhury et al., 2015). Heat stress reduces laying percentage, egg size, eggshell durability, and body weight gain, as well as increases mortality in layers (Sterling et al., 2003; Lin et al., 2004; Franco-Jimenez and Beck, 2007). Also, it appears to have a detrimental impact on poultry meat quality, however, it has been reported that breast meat characteristics remain unaffected by heat stress (Goo et al, 2019).

Nutrients, especially amino acids, are widely used as anti-stress agents with regard to psychological and physiological stress (Yamane et al., 2009; Hamasu et al., 2010; Erwan et al., 2012). Amino acid supplements, particularly essential amino acids, has been used in attempts to overcome problems of heat stress in birds (Mendes et al., 1997; Daghir et al., 2003).

It has been reported that oral administration of L-citrulline (L-Cit) decreased the body temperature in layer chicks (Chowdhury et al., 2015). However, there are no reports on the function of L-Cit in KUB chicks. Therefore, the purpose of this study was to examine the effect of orally administered L- Cit on rectal temperature and some plasma biochemical parameters in KUB chicks.

MATERIALS AND METHODS

This research was conducted at the Poultry Division Field Laboratory, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia in 2018.

Ethical approval

This experiment was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Animal, at the Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Pekanbaru, Indonesia

Animals

Thirty-two, one-day-old, KUB chicks were purchased from a local hatchery and housed in a wire-meshed cage (50 × 35 × 33 cm) at a constant temperature of 30 ± 1°C and with continuous light. Feed (Charoen Phokpand, Indonesia) and water were provided *ad libitum*. Feed composition is presented in table 1. One day before the experiment, chicks (4 days old) were weighed individually and assigned to treatment and control groups based on their body weight to produce uniform groups. The number of animals used in each group was kept to the minimum (n = 8) that would still ensure adequate statistical power.

Experimental design

After a habituation period, chicks were randomly selected and divided into four groups each consisting of eight chicks. The chicks were reared individually in experimental cages and had *ad libitum* access to diet up to the time of the experiment. On the day of the experiment, each chick (5 days old) received L-Cit orally (treatment groups) or distilled water (control group) by the plastic needle on small syringe. Based on our recent report on L- or D-Asp and L-Cit in layer chicks (Erwan et al., 2013b, 2014; Chowdhury et al., 2015), treatment groups received 3.75, 7.5 or 15.0 mmol/ kg body weight as the low, medium and high dose, respectively.

The chicks were fed *ad libitum* diets for 2 h immediately after the treatment. At the end of the experiments (2 h after L-Cit administration), birds were decapitated under chloroform anesthesia. Blood samples were collected in heparinized tubes and centrifuged for 15 min at 5,000 g, and the plasma was collected and stored at -20 °C until analysis took place.

Analysis of plasma biochemical parameters

The biochemical parameters of plasma including total glucose, total cholesterol, and

triacylglycerol were determined with Microlab 300 (Vital Scientific, Netherland) as per the manufacturer's instructions. All the samples were assayed together and in a random sequence for each metabolite.

Measurement of feed intake and rectal temperature

At 30, 60 and 120 min after L-Cit administration, feed intake was calculated by measuring the reduction in the amount of feed consumed from a pre-weighed feeder. Similarly, rectal temperature of chicks was determined at 30, 60 and 120 min after L-Cit administration using a digital thermometer with an accuracy of $\pm 0.1^{\circ}\text{C}$ (Thermalert TH-5, Physitemp Instruments Inc., USA). It took about 5 sec to measure rectal temperature by inserting the probe.

Statistical analysis

Data on feed intake and rectal temperature were analyzed by repeated-measures two-way analysis of variance (ANOVA). Plasma metabolites were statistically analyzed by one-way ANOVA and regression equations. When significant differences were found, the comparison of means was performed using Tukey's test as a post hoc test. Differences were considered significant at $p < 0.05$. Values are presented as means \pm SEM. Statistical analysis was carried out using the commercially available package StatView (Version 5, SAS Institute, Cary, USA). Data were checked by the Thompson rejection test to eliminate outliers ($p < 0.05$), and the remaining data were used for the analysis among groups.

RESULTS AND DISCUSSION

The aim of the current study was to reveal whether the L-Cit amino acid influenced feed intake and have thermoregulatory functions in KUB chicks. Feed intake was not significantly affected by oral administration of several doses of L-Cit (data not shown). This result was consistent with the previous report by [Erwan et al. \(2016\)](#) who revealed that the inclusion of watermelon rind in ration during two weeks did not influence feed intake in broiler chicks. However, the aforementioned authors demonstrated that feed consumption increased when chicks fed for three weeks. The present findings also contrasted with previous findings in layers where L-Cit given for up to 9% caused a significant increase in feed intake in male layer chicks ([Nguyen et al., 2019](#)). These discrepancies in the feed intake in response to L-Cit may be due to variations in the experimental period, differences in doses, or species differences. Further experimentation would be needed to clarify the factors involved in this

disparity.

As shown in diagram 1, rectal temperatures of KUB chicks did not change significantly by the oral administration of L-Cit. This result was not consistent with the previous report that indicated the oral administration of L-Cit decreased the body temperature in layer and broiler chicks (Chowdhury et al., 2017; Erwan and Febriyanti, 2015). The body temperature of KUB chicks ranged from 39.5 to 40.5 °C, which was still in the normal range. The different effects observed in this study can be attributed to the difference in chicken type. It is well known that indigenous chickens are more adaptable to stress than commercial chickens. Duangjinda (2017) revealed that indigenous chickens from a tropical environment had lower heterophil-to-lymphocyte ratio and mortality rate than those of commercial broilers under heat stress conditions. Similarly, Aengwanich (2007) revealed that Thai indigenous chickens and Thai crossbreed chickens can tolerate higher temperatures than broiler chickens.

Table 2 shows the effect of oral administration of different doses of L-Cit on certain biochemical parameters of plasma, indicating no significant changes were observed. The effect of oral administration of L-Cit on total cholesterol, triacylglycerol, and glucose in the plasma of indigenous chicks is unclear. Watermelon rind contains a natural source of citrulline (Rimando and Perkins-Veazie, 2005). A previous study found that oral administration of red watermelon rind juice dose-dependently increased glucose and triacylglycerol in broiler chickens (Erwan et al., 2016). In contrast, Chowdhury et al. (2017) revealed that oral administration of L-Cit decreased glucose in layer male chicks. It is thought the differences in results may be due to different strains of chicken used.

Heat stress affects plasma metabolites in poultry (Xie et al., 2015). An increase in plasma cholesterol indicates that the animal has undergone heat stress in chicks (Olanrewaju et al., 2010). According to Altan et al. (2000), cholesterol in the body can come from *de novo* biosynthesis. *De novo* cholesterol biosynthesis is largely influenced by the broiler stress level (Setyadi et al., 2013). Blood cholesterol levels in this study ranged from 150 to 175 mg/dl, which was higher than the normal range (52-148 mg/dl) (Basmacioglu and Ergul, 2005).

The results obtained in the present study indicated that triacylglycerol ranged from 49-91 mg/dl. The highest level of triacylglycerol in this study was still in the ranges of previous report (Olanrewaju et al., 2010). When chickens are in a state of heat stress, plasma triacylglycerol levels drop (Sun et al., 2015). Environmental stress increases adrenocorticotrophic hormone which stimulates the adrenal cortex to increase glucocorticoid secretion (von Borell and Ladewig, 1989). Blood glucose has a relationship with heat ambient temperature in poultry (Olanrewaju et al., 2010). Chowdhury et al. (2017) suggested

that lower plasma glucose after oral administration of L-Cit may decrease the substrate for heat production and result in lower body temperature in chicks. Glucose in the blood serves as a buffer molecule for osmotic pressure thus normal osmotic pressure can be maintained even in a state of dehydration and heat stress (Guay et al., 2007) to maintain blood pressure and cardiovascular rhythms (Tan et al., 2010). In this study glucose concentration in chickens' blood ranged from 230-250 mg/dl. This range is included in the normal glucose levels, which indicates the chickens did not experience heat stress. Normal range glucose concentration in broiler blood is 230 - 370 mg/dl (Sulistyoningsih, 2004).

CONCLUSION

L-citrulline could not able to improve feed intake and decrease the body temperature in KUB chicks. No significant changes in plasma glucose, total cholesterol and triacylglycerol were detected in the current study, indicating the oral administration of L-citrulline may not affect the metabolism of these components in KUB chicks.

DECLARATIONS

Acknowledgments

All authors are very grateful to the Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia for supporting.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

Edi Erwan conducted the research, prepared data and performed the statistical analysis. Vebera Maslami, Triani Adelina and Alaidin Koto wrote the article. All authors checked and approved the final version of the manuscript.

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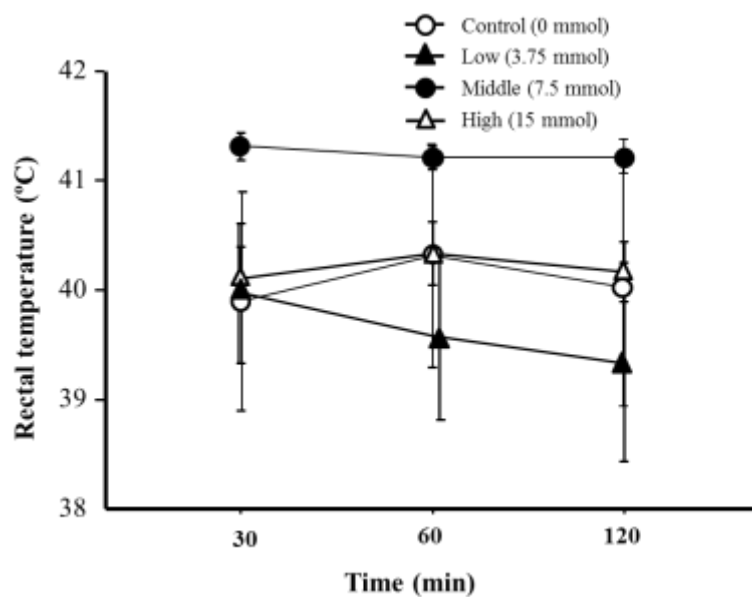


Diagram 1. The effect of different doses of L-citrulline on body temperature of KUB chicks over two hours after oral administration.

Table 1. The percentage of nutrient content in ration

Nutrient	Value
Crude Protein (%)	23.50
Crude Fiber (%)	1.88
Crude Fat (%)	5.87
Ca (%)	0.29
P (%)	0.15
ME (Kcal/kg)	3,050

Ca: Calcium, P: Phosphor, ME: Metabolizable Energy; *Commercial feed: CP511 PT, Charoen Pokphand, Indonesia; **Mineral Premix: Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; biotin, 0.1 mg; choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu, 12 mg; I, 1 mg; Se, 0.2 mg, and adequate antioxidant.

Table 2. Effects of oral administration of different doses of L-citrulline on plasma metabolites in KUB chicks

Values are expressed as means \pm SEM.