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Effects of feeding time with betaine diet on growth performance, blood markers, and short chain fatty acids in meat ducks exposed to heat stress

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ABSTRACT

The objective of this study was to determine effect of the diet containing 1200 betaine diet on blood biomarkers, electrolytes, gas and cecum short-chain fatty acid profile in meat ducks exposed to very hot environment (high relative humidity) conditions from d 21–42 (11:00 to 17:00 h; 33–43 °C; 70% relative humidity). On the day of hatching, a total of 320 meat ducks(average, 48.6 g body weight) were randomly assigned to 4 treatments with 4 pens per treatment and 20 ducks per pen for 42 d. Dietary treatments consisted of: 1) the control diet fed ad libitum, 2) the betaine diet fed ad libitum, 3) the betaine diet fed from 05:00 to 10:00 and 17:00 to 20:00 h, and 4) the betaine diet fed from 17:00 to 20:00 h. Ducks fed the betaine diets had greater body weight at d 42 than those fed the control diet ($P < 0.05$). The total red blood cell counts, hematocrit, hemoglobin, mean corpuscular volume, red cell distribution width, platelet count, plateletcrit and mean platelet volume concentration in ducks fed the control diet were lower than those fed the betaine diets ($P < 0.05$). Blood electrolyte concentrations in ducks fed the betaine diet at different feeding times had greater values than those fed the control diet ($P < 0.05$). Partial pressure of carbon dioxide, partial pressure of oxygen, base excess of extracellular fluid, bicarbonate and total carbon dioxide concentrations in ducks fed the control diets were lower than those on the betaine treatments ($P < 0.05$). Respective to ducks fed the betaine diets had greater concentrations of total SCFA, acetic acid, propionic acid in the cecum than those fed the control diet ($P < 0.05$). However, ducks fed the betaine diets had lower levels of butyric acid, isobutyric acid, valeric acid, isovaleric acid than those fed the control diet ($P < 0.05$). The body weight, feed intake, sodium, hematocrit, hemoglobin, base excess of extracelluar fluid, mean corpuscular volume in ducks fed the betaine diet from fed 05:00 to 10:00 and 17:00 to 20:00 h were greater ($P < 0.05$) than those fed the betaine diet ad libitum and from 17:00 to 20:00 h. The results of this study indicated that restricted feeding of the diet with 1200 ppm betaine in morning and afternoon can have beneficial effects on meat ducks exposed to the very hot environment (high relative humidity) condition.

1. Introduction

The extreme heat or working under very hot environment (high relative humidity) can lead to "heat stress". It activates stress mechanisms of the body and induces adverse effects on the production and the health of animals (Patience et al., 2005; Sharma et al., 2013). When ducks are exposed to very hot environment (high relative humidity), concentrations of hematological indices, electrolyte, blood gas and cecum short-chain fatty acid (SCFA) are all decreased (Tamzil et al., 2013). The difference between blood profile and hematological index would be important for evaluating the health and nutritional status of animals. As a general biomarker of immune function, it has sensitive response in poultry to stress (Habibu et al., 2014; Ju et al., 2011).

Betaine is a bipolar compound that has 3 hydrophobic methyl groups, and it acts a hydrophilic carboxyl groups, it acts as a donor of methyl groups like choline and methionine (Mahmoudnia and Madani, 2012). The effect of betaine can reduce damages to animals caused by high-temperature stress, thus improving growth performance of animals (Eklund et al., 2005; Hassan et al., 2011). Betaine is involved in protein synthesis and energy metabolism. It can prevent dehydration and preserve moisture within cells by adjusting osmotic pressure of animals when they are exposed to very hot environment (high relative humidity) conditions. It can also improve nutrient digestibility and growth performance of animals (Eklund et al., 2006). Betaine can also save energy needed for sodium-potassium pump under very hot environment (high relative humidity) conditions for growth (Remus, 2001). It can also increase the absorption and availability of nutrients by expanding intestinal mucosa (Mahmoudnia and Madani, 2012).

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Under normal conditions, addition of 0.5% betaine to the diet of meat ducks can increase their body weights but decrease feed conversion ratio (Wang et al., 2004). In a previous study, Hwangbo et al. (2015) found that when feeding diets containing different levels of betaine were ad libitum fed to ducks exposed to very hot environment (high relative humidity), 1200 ppm level could alleviate the stress and improve the productivity by maintaining homeostasis of blood profile and increasing SCFA.

Diet restriction as a feeding management strategy to alleviate adverse effects of extreme heat or working under very hot environment (high relative humidity) on poultry has been examined previously (Sahraei, 2012). When broiler chickens exposed to very hot environment (high relative humidity), night time restricted feeding along with day time fasting has been reported to be able to improve the body weight by adjusting concentrations of blood lipid and SCFA (Yoon et al., 2013). The hot environment (high relative humidity) conditions can induce poor health and reduce productivity due to health deterioration of animals. Therefore, our major interest was to overcome this problem. The purpose of this study was to determine the effect of feeding time of a diet containing 1200 ppm betaine on blood biomarkers, electrolytes, blood gas, cecum SCFA in meat ducks exposed to very hot environment (high relative humidity) conditions. The results of this study could provide basic knowledge on productivity improvement for meat ducks under very hot environment (high relative humidity).

2. Materials and methods

2.1. Experimental design

A total of 320 meat duck (Cherry Valley, *Anas platyrhynchos*) were obtained from a commercial hatchery (Yangpyung, Kyunggido, Republic of Korea) and randomly assigned to 4 treatments (4 replicate pens per treatment; 20 ducks per replicate pens). They were raised for 42 d. From d 21–42, all ducks were exposed to very hot environment condition (33–43 °C, and 70% relative humidity) from 11:00 to 17:00 h and provided with 15 °C water, and they were maintained at 22–24 °C during the remaining time. The 4 treatments were: 1) control treatment fed ad libitum (CON); 2) the betaine diet (containing 1200 ppm betaine) ad libitum (BTA); 3) the betaine diet fed from 05:00 to 10:00 and 17:00 to 20:00 h(BTT); 4) the betaine diet fed from 17:00 to 10:00 h (BTO).

2.2. Feeding management

Experimental diets were manufactured so that the nutrient requirement of meat ducks suggested by the National Research Council (1994) could be satisfied or slightly exceeded. All nutrient contents were identically adjusted. During the starter period (d 0–21), the ducks were allowed to freely have drinking water and basal diet under normal condition. During the finishing period (d 21–42), the duck diets were fed with basal diet and the experimental diet was manufactured by mixing 1200 ppm betaine with duck finisher diet consisting mostly of corn meal and soybean meal under very hot environment (high relative humidity) conditions. The addition level of betaine was based on the result of a previous study (Hwangbo et al., 2015). The mixing ratio of raw grain materials depending on the amount of betaine (coated and 97%; Beta-key, Excentials, Rotterdam, Netherland) added to the experimental diet was adjusted by decreasing the amount of corn addition. During the entire period, continuous lighting was applied to all treatments. For bedding, chaff was laid with a 10 cm height from the floor of each pen. The temperature of the animal raising room was maintained at 33 °C until 3 d from the initial day of hatching. It was then lowered by 2–3 °C per wk. The feed intake and body weight of animals were recorded every 10 d during the experimental period. Their growth performance such as body weight, feed intake and feed efficiency (body weight/feed intake) were measured. All experiment procedures including animals followed the scientific and ethical regulations suggested by EEC Directive of 1986 (86/609/EEC). They study protocol (KW-141027-1) was approved by Institutional Animal Care and Use Committee of Kangwon National University (Chuncheon, Republic of Korea).

2.3. Blood biomarkers, electrolyte, and blood gas

On the day of the experiment, ducks were fasted for 10 h from midnight and 3 mL of blood was taken into a plain tube (Greine Co Ltd, Adelaide, Australia) by cardiac puncture. Blood were centrifuged at 3,000*g* for 20 min at 4 °C. Serum was rapidly frozen using liquid N and stored at −20 °C until biochemical analysis. Hematological indices, total red blood cell counts (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), red cell distribution width (RDW), platelet count (PLT), plateletcrit (PCT), and mean platelet volume (MPV) were measured using an automated blood cell counter (FORCYTE; Oxford Science, Las Vegas, NV, US). Concentrations of blood electrolyte, blood gas, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), base excess of extracellular fluid (BEecf), bicarbonate (HCO_3) and total carbon dioxide content (TCO_2) were measured using a Handheld blood analyzer (Vetscan i-Stat; Abaxis Inc., Union City, CA, US) or a blood gas analyzer (Rapidchem 700 744/75; SIEMENS, Maryland Heights, MO, US). The analytical and operation units of the automatic analyzer were calibrated for values typical for duck.

2.4. Short chain fatty acid (SCFA) profile

After blood sampling, 3 ducks whose body weights were close to the average body weight were selected from the replicate pen of each treatment. They were sacrificed through cervical vertebrae dislocation following the recommendation of laboratory animal euthanasia (Yoon et al., 2013). The cecum was anaerobically taken by tying both ends of the cecum with a thread and stored in an icebox. SCFA was measured using a gas chromatographic system (model GC-15A, Shimadzu Corp., Kyoto, Japan). A total of 0.5*g* of the cecum content was added into a 20 mL screw cap tube and mixed with 5 mL of distilled water. This was homogenized and centrifuged at 10,000*g* for 10 min at 4 °C. Supernatant (1 mL) was transferred to an ampoule bottle and acidified by adding 0.2 mL of 25% H_3PO_4 solution. After homogenization of the sample, the ampoule bottle was maintained on ice for more than 30 min and centrifuged at 10,000*g* for 10 min at 4 °C. The GC was equipped with a glass column filled with 10% SP-1000/1% H_3PO_4 (180 cm x 4 mm; Supelco, Inc., Belleponte, PA, US) and attached to flame ionization detector. The initial temperatures of oven, injector and flame-ionization-detector were 100, 240 and 250 °C, respectively. The column was operated at 100–150 °C, along with highly purified N_2 (1.8 mL/min) as a carrier gas. The flow rate was 3 mL/min (Park et al., 2013).

2.5. Statistical analysis

Statistical analysis for all data obtained was performed using SAS (Version 9.1; SAS Inst. Inc., Cary, NC, US). The Pen was used as the experimental unit for all data. The differences in mean values among treatments were determined by one-way ANOVA and analyzed by Turkey's multiple range test ($P < 0.05$). Mean values with pooled standard error of mean were presented for each treatment (Table 1).

3. Results and discussion

3.1. Growth performance and blood biomarkers

The effect of feeding time with diet containing 1200 ppm betaine on