DOI : <http://doi.org/10.22438/jeb/41/2/MRN-1258>

JEBTM p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

Effect of dietary metabolic energy on growth performance, blood homeostasis in ducks under heat stress-related climate change

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Paper received: 14.08.2019

Revised received: 31.12.2019

Accepted: 08.01.2020

Abstract

Aim: This study was carried out to investigate the effect of dietary metabolic energy on growth performance, homeostasis of blood biochemical parameters, immunoglobulin G (IgG), and corticosterone in meat duck exposed to heat stress.

Methodology: A total of 320 meat ducks (Cherry valley, *Anas platyrhynchos*) were randomly allotted into five groups. In all experimental treatment groups, dietary crude protein content was fixed at 18%. metabolic energy level for the control group (thermononeutral temperature, 26°C) was set at 3000 kcal kg⁻¹, while metabolic energy levels for heat stress groups were set at 2900, 3000, 3100 or 3200 kcal kg⁻¹. Heat stress conditions were artificially induced at 36°C with relative humidity of 70% once daily (from 11:00 to 16:00 hr) during finisher periods (day 22 to 42).

Results: Compared to control group, heat stress groups with different metabolic energy showed significant decrease in body weight gain, feed intake, red blood cell, and platelets. Heat stress groups with different metabolic energies showed significant increase in feed conversion ratio, blood lipid profiles, aspartate aminotransferase, alanine transferase, electrolytes, pH, gas concentration, immunoglobulin G, and corticosterone compared to the control group. Among heat stress groups, red blood cells and platelets were significantly higher in the heat stress group with metabolic energy of 3000 compared to those in other groups. Blood IgG and corticosterone levels were significantly lower in 2900 and 3000 groups than those in heat stress groups with metabolic energy of 3100 and 3200.

Interpretation: The results of this study suggest that dietary metabolic energy 3000 kcal kg⁻¹ level can improve the growth performance of meat duck exposed to heat stress by modulating homeostasis of blood biochemical parameters.

Key words: Blood homeostasis, Corticosterone, Ducks, Growth performance, Heat stress, Immunoglobulin G

How to cite : Shin, J. S. , K.H. Um and B.S. Park: Effect of dietary metabolic energy on growth performance, blood homeostasis in ducks under heat stress-related climate change. *J. Environ. Biol.*, **41**, 171-177 (2020).

Introduction

Climate change is threatening the survival of livestock and the sustainability of livestock production systems. Heat stress associated with climate change can lead to imbalance in blood homeostasis of animals and adversely affect duck mortality and farm income (Das *et al.*, 2016). Blood biochemical parameters are important biomarkers to evaluate the health and nutritional conditions as well as metabolic reactions in animals against heat stress (Habibu *et al.*, 2014). It has been reported that heat stressed ducks and other birds have lower red blood cell and platelet counts and lower blood gas concentration, except Po_2 (Park *et al.*, 2015). Especially, the amount of CO_2 discharged out of their body through respiration and blood pH is increased, leading to panting (Park and Park, 2017). Decreased levels of red blood cells in birds including ducks exposed to heat stress, during heat stress can trigger anemia and increase their mortality rates (Bang *et al.*, 2015).

Electrolytes play important role in maintaining acid-base balance, osmotic pressure, and cell membrane potential. When ducks and other birds are heat-stressed, the excretion of potassium (K^+) and sodium (Na^+) through their feces and urine is increased (Sayed and Downing, 2011). Dietary metabolic energy and physiological state of birds, including ducks can determine their ability to produce optimal immune responses (Yang *et al.*, 2015). In birds under heat stress, immune function is known to be important. However, there is increasing evidence showing that immunity is susceptible to metabolic energy. The immune system in birds under heat stress can increase metabolic energy consumption and negatively affect energy metabolism (Honda *et al.*, 2015; Yang *et al.*, 2015).

Unlike mammals, birds have no sweat gland on their skin, making it difficult for them to maintain appropriate body temperature as they are covered with feathers. Therefore, birds including ducks are vulnerable to heat stress (Park *et al.*, 2016). It is generally accepted that depression of immunological criteria such as immune cells is the result of increased corticosterone. Thus, changes in the immune cells could cause changes in immunoglobulin G (IgG) and corticosterone levels. Heat stress can decrease the growth of immune cells and change blood concentration of adrenocorticotrophic hormone, thus affecting lymphatic tissues and reducing immunity while increasing secretion of corticosterone to mobilize energy so that the body can confront heat stress in broiler chickens (Park *et al.*, 2015). However, birds under heat stress may have problems to dissipate metabolic heat, leading to increased metabolic heat production that can further increase heat stress.

Poultry including ducks do not have bladder, and also excrete feces and urine together through cloaca. Therefore, metabolic energy is used in poultry rather than digestible energy (Alvarenga *et al.*, 2013). Some studies have concluded that when energy level is low in diet, gain in body weight of broiler chickens at thermo-neutral temperature is decreased (Ghaffari *et al.*,

2007). At the same time, when energy levels are high body weight gain is increased (Ghaffari *et al.*, 2007). It has been reported that increasing energy level in laying hens can improve their body weight gain (Philippe, 2009). Fan *et al.* (2008) reported that daily body weight gain of ducks at thermo-neutral temperature is increased while average daily feed intake and feed conversion ratio are decreased when dietary energy is increased. Increasing metabolic energy in diets can increase body temperature through metabolic heat generation due to excessive energy.

Nutritional and physiological research studies related to metabolic energy have been conducted to improve the growth ability of livestock, including ducks during heat stress (Johnson, 2018). Adverse effect of heat stress on the growth performance of animals, including broiler chickens has been extensively investigated (Habibu *et al.*, 2014). However, studies on the dietary metabolic energy under heat stress for ducks are meagre as those for broilers (Zeng *et al.*, 2015). Metabolic energy is very important as it limits the growth performance of poultry under heat stress (Lin *et al.*, 2006). Several nutritional strategies including dietary levels of metabolic energy have been suggested in poultry to relieve the adverse effect of heat stress (Attia *et al.*, 2010). However, few research studies have examined the effect of metabolic energy levels on blood biochemical parameters related to growth performance of meat ducks exposed to heat stress. Therefore, the objective of this study was to investigate the effect of different dietary metabolic energy levels on blood biochemical parameters, IgG levels and corticosterone levels in meat ducks exposed to heat stress.

Materials and Methods

Animals and experimental design: A total of 150 one-day-old male Cherry valley meat ducks (*Anas platy rhynchos*) were obtained from a duck hatchery (Yusung Hyungjae Hatchery, Daejeon, South Korea). Animals were completely randomized in repetition of 10 heads with 3 replicates per group for 42 days. Five treatments included one control group (3000 kcal kg^{-1} , thermoneutral temperature, 26°C) and four heat stress groups with metabolic energy of 2900, 3000, 3100, and 3200 kcal kg^{-1} in diet containing 18% of crude protein. Under normal condition, nutrient requirements of duck were 18% crude protein and 3000 kcal kg^{-1} metabolic energy (NRC, 1994). The growth performance of birds under heat stress is influenced by metabolic energy level when crude proteins are immobilized (Attia *et al.*, 2010; Zeng *et al.*, 2015; Shin *et al.*, 2017). Therefore, different metabolic energy levels were used in the present study. Animal experimental procedures followed the scientific and ethical rules in accordance with EU - directive 2013/63/EU.

Experimental diet and feeding management: Nutrient contents of experimental diets (Table 1) were analyzed according to Association of Official Analytical Chemists (2007). During the experimental period, continuous lighting and drinking water were provided *ad libitum* to ducks without limitation. During the starter period (day 1 to 21), chow diet was

provided without limitation. During the finisher period (day to 42), artificial heat at 36°C was provided for 5 hr (from 11:00 to 16:00) per day. Relative humidity was adjusted to 70% to induce heat stress. During the experimental period, anthropogenic heat stress to ducks was carried out in a systematized housing room with controlled temperature and humidity. Body weight gain (BW) and feed intake were recorded at day 21 and day 42. Data of feed intake and BW were used to determine the feed conversion ratio (FCR). Mortality included animals with leg disabilities and dead individuals.

Estimation of blood lipids, glucose and liver functional enzymes: On day 42, animals from each pen (eight birds from each treatment group) were randomly selected for blood collection after fasting for 7 hr. A total of 3 ml of blood was obtained from each duck by cardiac puncture using a plain tube (Greiner, Co Ltd, Australia). Serum was obtained after centrifuging the blood sample at 3,000 rpm for 20 min at 4°C. The concentrations of triacylglycerol (TAG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), glucose, aspartate aminotransferase (AST), and alanine transferase (ALT) were measured with an automatic analyzer (Hitachi 917, Japan) using biochemical kits (Sigma Co., St. Louis, MO, USA).

Estimation of blood hematological parameters: To examine blood hematological parameters, an automated blood cell counter (Forcyte Hematology Analyzer, Oxford Science Inc., Oxford, CT, USA) was used to measure total red blood cell counts (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), plateletcrit (PCT), and mean platelet volume (MPV). A VetScan i-STAT 1 handheld analyzer (Abaxis, USA) was employed to measure the levels of blood electrolytes. RAPID Chem 744/754 blood gas analyzer (Siemens, Malvern, PA, USA) was utilized to measure the levels of blood gases.

Estimation of blood electrolytes, pH and gas: The concentrations of blood electrolytes such as chloride (Cl⁻), sodium (Na⁺), potassium (K⁺), and blood gases such as partial pressure carbon dioxide (PCO₂), partial pressure of oxygen (PO₂), bicarbonate (HCO₃⁻), anion gap (AnGap), and total carbon dioxide (TCO₂) were measured using an electrolyte analyzer (RAPIDChem 744/754, Siemens, USA). Blood pH was measured with a desktop blood pH meter (Shaoxing Worner Lab Equipment Co., LTD, USA).

Estimation of blood IgG and corticosterone: The concentrations of IgG and corticosterone were measured using chicken ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) and EIA kit (Enzyme immunoassay kit, IDS, Boldon, UK), respectively. After biochemical treatment of each sample according to the protocol of each manufacturer, the absorbance was measured at 450 nm wavelength on a precision microplate reader (Molecular Devices Inc., Sunnyvale, CA, USA).

Statistical analyses: Data obtained in this study (except data of mortality) were analyzed according to a completely randomized design with one-way analysis of variance (ANOVA) using IBM SPSS statistics 22 followed by Turkey's multiple range test. Statistical significance level was set at $p < 0.05$.

Results and Discussion

The normal control and heat stress group with metabolic energy of 3000 were significantly ($p < 0.05$) increased in BW (day 1 to 42) compared to other HS group. However, the BW (day 1 to 21) tended to decrease in the heat stress group with metabolic energy of 3000 compared to other heat stress groups, while FCR showed the reverse trend. There was no mortality in normal control group. Mortality in heat stress groups with metabolic energy was in the following order: Heat stress 3100 > 3200 > 2900 > 3000 (Table 2). It has been shown that heat stress can lower growth performance in animals, including ducks than normal control (Sugiharto *et al.*, 2017). The present study revealed that ducks reared under normal control had improved body weight gain and FCR than heat stress groups throughout the experimental period.

The results of this study showed that heat stress group with metabolic energy of 3000 had beneficial effects on body weight gain and FCR in meat ducks under heat stress compared to heat stress groups with metabolic energy of 2900, 3100 or 3200. It is well known that heat stress can decrease feed intake, metabolic heat production, and the growth rate of broiler chickens compared to normal control (Temim *et al.*, 2000). It has been reported that feed intake decreases when metabolic energy level is too low or too high in animal feed under heat stress compared to normal control (Park and Park, 2017). It has been reported that feed intake decreases when metabolic energy level is too low or too high in animal feed under heat stress compared to normal control. Poultry usually does not have the chance to move to a comfortable temperature under heat stress (Perween *et al.*, 2016). The decreasing feed intake of animals according to different metabolic energy under heat stress may be mainly due to undesirable distribution of spent metabolic energy by energy metabolism compared to normal control (Shin *et al.*, 2017).

Compared to the normal control group, heat stress groups (Table 3) showed significantly higher blood lipids level (except HDL-C), aspartate aminotransferase, alanine aminotransferase ($p < 0.05$). Among heat stress groups, blood lipids level, TAG, TC, LDL-C (except in 3000 and 3100), AST, and ALT in 2900 and 3000 groups were significantly less than those in other heat stress groups with metabolic energy of 3100 and 3200 ($p < 0.05$). The glucose level in the heat stress group with metabolic energy of 3000 was significantly higher than that in other heat stress groups ($p < 0.05$). The exact mechanism by which dietary metabolic energy increases blood lipid levels in heat stress ducks is currently unknown. In the present study, low level of blood lipids, AST and ALT in heat stress groups with metabolic energy of 2900 or 3000 to ducks might be due to imbalanced nutritional metabolism associated with damage of

Table 1: Composition of the experimental diets (% as-fed basis)

D 1-21		D 22-42, metabolic energy (kcal kg ⁻¹)				
		Control	2900	3000	3100	3200
Yellow corn	51.55	52.30	51.70	52.30	56.10	62.60
Soybean oil meal	33.50	14.63	20.00	14.63	10.23	2.20
Wheat grain	5.00	20.00	20.00	20.00	15.00	10.00
Tallow	4.00	2.33	1.45	2.33	3.00	3.00
Whole soybean	-	-	-	-	2.70	4.00
Fish meal	3.00	-	-	-	-	-
Corn gluten	-	7.00	3.30	7.00	9.00	14.00
Limestone	1.04	1.14	1.14	1.14	1.26	1.25
Dicalcium phosphate	0.83	1.15	1.09	1.15	1.19	1.28
Sodium chloride	0.20	0.25	0.25	0.25	0.25	0.25
Vit.-Min-premix1	0.10	0.1	0.10	0.10	0.10	0.10
D,L-Methionine-50%	0.39	0.28	0.34	0.28	0.26	0.16
Choline chloride-50%	0.08	-	-	-	-	-
Lysine-54%	-	0.82	0.63	0.82	0.91	1.16
Analyzed nutrients,%	-	100	100	100	100	100
Metabolic energy (kcal kg ⁻¹)	3,000	3,000	2,900	3,000	3,100	3,200
Crude protein	23.01	18.23	18.22	18.23	18.22	18.22
Crude fat	6.13	4.86	4.02	4.86	6.04	6.37
Crude fiber	2.56	2.52	2.73	2.52	2.42	2.15

Control (thermoneutral temperature, 26°C), Metabolic energy groups exposed to heat stress (36°C, from 11:00 to 16:00 hrs). ¹Provided per kilogram of diet: Vitamin A: 10,000,000 KIU; Vitamin D3: 5,000,000 KIU; Vitamin E: 20,000 KIU; Vitamin K3: 3,000 mg; Vitamin B1: 2,000 mg; Vitamin B2: 6,000 mg; Vitamin B6: 3,000 mg; Vitamin B12: 16 mg; Niacin: 50,000 mg; Pantothenic acid: 13,000 mg; Folic acid: 1,500 mg; Biotin: 100 mg; Cu: 5,000 mg; I, 1,250 mg; Mn: 100,000 mg; Zn: 100,000 mg; Se: 300 mg; Fe: 40,000 mg; Co: 250 mg

Table 2: Effects of different metabolic energy on growth performance in ducks exposed to heat stress Control (thermoneutral temperature, 26°C); Metabolic energy groups exposed to heat stress (36°C from 11:00 to 16:00 hrs). ¹BW: body weight; FCR: feed conversion ratio

Item ¹	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
BW (g) day 1 to 21	1,108±38.122	1,087±37.67	1,097±37.08	1,103±37.07	1,097±36.88	0.002
BW (g) day 1 to 42	3,854±109.3 ^a	3,080±98.7 ^c	3,250±115.3 ^b	3,019±103.2 ^c	3,008±105.7 ^c	0.015
Feed intake (g) day 1 to 42	4,975±123.4 ^a	4,645±153.6 ^c	4,782±132.2 ^b	4,667±143.1 ^c	4,609±150.5 ^c	0.008
FCR	1.29±0.02 ^c	1.51±0.03 ^a	1.47±0.02 ^b	1.54±0.04 ^a	1.53±0.03 ^a	0.00.1
Mortality (%)	0	3	2	5	5	-

Values are mean±²S.E. ^{a,b,c}Different letters indicate significant level between different groups ($p<0.05$)

duodenal villus and liver cells caused by heat stress compared to normal control (Shin *et al.*, 2017). Thus, it is very important to determine the appropriate level of metabolic energy for meat ducks exposed to heat stress. Heat stress occurs when the body can no longer maintain thermoregulatory homeostasis compared to normal control (Park and Park, 2017). It has been reported that heat stress can increase the levels of blood lipids (Habibian *et al.*, 2014), blood ALT and AST in broiler chickens as compared to normal control (Khan *et al.*, 2002). On the other hand, blood lipids in broiler chickens exposed to heat stress decreased compared to normal control (Shim *et al.*, 2006). At thermoneutral temperature, increasing dietary metabolic energy levels generally tend to increase the levels of blood lipids and glucose in broiler chickens

(Corduk *et al.*, 2007). Compared to control group, heat stress groups (Table 4) showed significant decreases of RBC and platelets ($p<0.05$). Among heat stress groups, RBC and platelets were significantly higher in heat stress group with metabolic energy of 3000 than those in other groups except for MCV and MCHC in the heat stress group with metabolic energy of 2900. The present study showed that maintaining metabolic energy at 3000 kcal kg⁻¹ in a duck diet could alleviate heat stress damage by keeping blood biochemical parameters constant. The lower blood RBC and platelets in other metabolic energy groups compared to those in group with metabolic energy of 3000 in ducks exposed to heat stress might be due to imbalance of nutritional metabolism caused by liver injury and other organ dysfunctions due to too low

Table 3: Effects of different metabolic energy on levels of blood biochemical parameters in ducks exposed to heat stress (mg dl⁻¹)

Item ¹	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
TAG	117.8±4.17 ^{ab}	164.4±6.26 ^c	130.6±5.29 ^d	241.0±9.68 ^b	267.8±9.38 ^a	0.017
TC	248.6±7.41 ^c	287.9±11.04 ^b	282.6±10.34 ^b	348.6±11.41 ^a	331.9±11.97 ^a	0.015
LDL-C	140.5±5.68 ^d	171.1±6.78 ^c	175.8±6.41 ^c	205.7±7.96 ^c	230.5±8.08 ^a	0.008
HDL-C	73.70±2.55 ^b	83.49±3.36 ^a	84.37±3.09 ^a	72.98±3.64 ^c	74.49±2.72 ^b	0.005
Glucose	214.3±8.01 ^a	197.3±6.70 ^b	208.1±7.87 ^a	195.3±6.50 ^b	172.2±7.74 ^c	0.001
ALT (U ⁻¹)	35.67±1.48 ^b	35.87±1.55 ^b	36.33±1.54 ^b	48.15±1.92 ^a	47.78±1.63 ^a	0.001
AST (U ⁻¹)	31.87±1.36 ^b	32.33±1.45 ^b	32.51±1.64 ^b	66.08±2.87 ^a	66.87±2.34 ^a	0.002

Control (thermoneutral temperature- 26°C); Metabolic energy groups exposed to heat stress (36°C from 11:00 to 16:00 hrs). ¹TAG: Triacylglycerol; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. Values are mean of eight replicates ± ²S.E. ^{a,b,c,d} Different letters indicate significant level between different groups ($p < 0.05$)

Table 4: Effects of different metabolic energy on levels of red blood cell and platelet profiles in ducks exposed to heat stress

Items ¹	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
RBC						
Hb (gdl ⁻¹)	12.09±0.46 ^{ab}	9.92±0.47 ^b	11.74±0.39 ^a	10.06±0.35 ^b	8.82±0.34 ^b	0.001
HCT (g100g ⁻¹)	50.53±1.77 ^a	45.48±2.01 ^b	48.75±2.02 ^a	45.31±1.36 ^b	43.48±1.62 ^c	0.016
MCHC (gdl ⁻¹)	40.28±2.08 ^a	39.59±1.87 ^a	40.21±2.01 ^a	35.04±1.26 ^b	35.18±1.01 ^b	0.007
MCV (fl)	170.3±6.20 ^a	155.2±6.09 ^b	159.3±5.88 ^c	159.1±5.43 ^b	139.9±6.09 ^c	0.015
RBC (m μ l ⁻¹)	8.57±0.35 ^a	5.57±0.26 ^c	7.77±0.29 ^b	4.71±0.22 ^d	4.47±0.17 ^d	0.001
RDW (g100g ⁻¹)	9.03±0.34 ^a	8.03±0.29 ^b	9.15±0.38 ^a	7.50±0.31 ^b	5.17±0.23 ^c	0.002
Platelets						
MPV (fl)	19.04±0.72 ^a	16.48±0.68 ^b	18.42±0.82 ^a	15.66±0.86 ^b	15.39±0.58 ^b	0.006
PCT (g100g ⁻¹)	0.07±0.02	0.06±0.02	0.07±0.03	0.05±0.02	0.06±0.01	0.201
PLT (k μ l ⁻¹)	47.10±1.85 ^a	45.06±2.12 ^b	48.46±2.49 ^a	42.67±1.88 ^c	43.13±2.02 ^c	0.001

Control (thermoneutral temperature, 26°C); Metabolic energy groups exposed to heat stress (36°C from 11:00 to 16:00 hrs). ¹RBC: Red blood cell; HCT: Hematocrit; Hb: Hemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell volume distribution width; PLT: Platelet; PCT: Plateletcrit; MPV: Mean plasma volume. Values are mean of eight replicates ± ²S.E. ^{a,b,c,d} Different letters indicate significant level between different groups ($p < 0.05$)

or too high dietary metabolic energy (Shin *et al.*, 2017). However, results on this theory are currently insufficient. It has been reported that HCT is lower due to red cell damage and reduction in the number and size of RBC when an animal is exposed to heat stress compared to normal control (Park and Park, 2017). Heat stress may result in fewer numbers of RBC and platelets with blood dilution due to increased water intake, although there is no change in plasma volume constituting moisture evaporation from outside the cell compared to normal control (Park and Park, 2017).

It has been reported that blood biochemical parameters are decreased in broiler chickens exposed to heat stress compared to normal control (Aengwanich, 2007). Hematologic findings are important general biomarkers for health and nutritional status of animals. They are sensitive indicators of the stress response and immune function in poultry (Habibu *et al.*, 2014). Compared to the control group, (Table 5) heat stress

groups showed significant increase in the for levels of electrolytes ($p < 0.05$). Among heat stress groups, levels of blood electrolytes in heat stress 2900 and 3000 were significantly lower than those in heat stress groups with metabolic energy of 3100 and 3200 ($p < 0.05$). Compared to the control group, (Table 6) heat stress groups showed significant increase in pH and gas concentrations, except for PO₂ ($p < 0.05$). The Po₂ levels were significantly reduced in heat stress groups compared to those in control group ($p < 0.05$). Among heat stress groups, blood HCO₃ and PCO₂ concentrations were significantly lower in heat stress groups with metabolic energies of 2900 and 3000 than those in heat stress groups with metabolic energy of 3100 and 3200 ($p < 0.05$). In the present study, the levels of blood electrolytes such as Na⁺, K⁺, and Cl⁻ were increased by heat stress, especially in 3100 and 3200 groups compared to those in control group, heat stress groups with metabolic energy of 2900, or 3000. This is the same result as the imbalance of blood electrolytes due to

Table 5: Effects of different metabolic energy on levels of blood electrolyte in ducks exposed to heat stress (mmol l⁻¹)

Items	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
Chloride (Cl ⁻)	86.7±3.76 ^{c1}	108.7±4.62 ^b	106.0±3.53 ^b	136.3±5.34 ^a	134.0±4.96 ^a	0.015
Potassium (K ⁺)	4.27±0.18 ^c	6.73±0.78 ^b	4.33±0.73 ^c	8.14±0.38 ^a	7.57±0.29 ^a	0.003
Sodium (Na ⁺)	117.1±5.08 ^d	148.9±6.03 ^b	141.0±5.83 ^c	157.9±6.70 ^a	157.3±7.04 ^a	0.018

Control (thermoneutral temperature-26 °C); Metabolic energy groups exposed to heat stress (36 °C from 11:00 to 16:00 hrs). Values are mean of eight replicates ± S.E. ^{a,b,c,d} Different letters indicate significant level between different groups ($p < 0.05$)

Table 6: Effects of different metabolic energy on blood pH and gas concentration in ducks exposed to heat stress

Items ¹	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
HCO ₃ (mmol l ⁻¹)	20.76±3.73 ^{c2}	24.16±4.36 ^b	21.03±4.43 ^c	26.22±4.13 ^a	26.30±3.58 ^a	0.007
PCO ₂ (mm Hg)	35.52±5.01 ^d	43.15±3.88 ^b	41.08±3.55 ^c	44.76±4.15 ^a	44.19±3.75 ^a	0.015
PO ₂ (mm Hg)	27.81±2.69 ^a	18.07±1.85 ^c	21.39±1.77 ^b	14.24±2.01 ^d	13.75±0.78 ^c	0.005
pH	7.07±0.05 ^b	7.42±0.05 ^a	7.43±0.04 ^a	7.48±0.05 ^a	7.48±0.04 ^a	0.002
TCO ₂ (mmol l ⁻¹)	20.78±5.03 ^c	25.78±4.13 ^b	25.88±3.38 ^b	26.78±3.56 ^a	26.23±4.01 ^a	0.004

Control (thermoneutral temperature-26°C); Metabolic energy groups exposed to heat stress (36°C from 11:00 to 16:00 hrs). ¹HCO₃: Bicarbonate; PCO₂: Partial pressure of carbon dioxide; PO₂: Partial pressure of oxygen; TCO₂: Total carbon dioxide. Values are mean of eight replicates ± S.E. ^{a,b,c,d} Different letters indicate significant level between different groups ($p < 0.05$)

Table 7: Effects of different metabolic energy on levels of blood immunoglobulin (IgG) and corticosterone in ducks exposed to heat stress (ng ml⁻¹)

Items	Metabolic energy (kcal kg ⁻¹)					p-value
	Control	2900	3000	3100	3200	
IgG	175.8±10.16 ^{a1}	264.6±9.33 ^b	220.4±8.98 ^c	277.3±7.09 ^a	276.7±8.75 ^a	0.013
Corticosterone	30.13±5.02 ^d	67.17±4.89 ^b	55.40±4.68 ^c	87.71±4.54 ^a	86.35±4.88 ^a	0.005

Control (thermoneutral temperature-26°C); Metabolic energy groups exposed to heat stress (36°C from 11:00 to 16:00 hrs). Values are mean of eight replicates ± S.E. ^{a,b,c,d} Different letters indicate significant level between different groups ($p < 0.05$)

metabolic disturbances in broiler chicken (Sayed and Downing, 2011). However, there are few studies on metabolic energy levels in heat stress exposed meat ducks. Levels of blood electrolyte are known to be increased when poultry including ducks are exposed to heat stress compared to normal control (Park and Park, 2017).

Blood electrolytes play an important role in metabolism due to their participation in osmotic balance, acid-base balance, and transport of substances across cell membranes (Gamba et al., 2015). Heat stressed poultry have shown increased excretion of Na⁺ and K⁺ in their feces and urine compared to normal control. Heat stress broiler chickens are known to have higher blood gas except for PO₂ compared to normal control (Bang et al., 2015). Compared to the control group, (Table 7) heat stress groups showed a significant increase in blood IgG and corticosterone levels ($p < 0.05$). Among heat stress groups, blood IgG and

corticosterone levels were significantly lower in heat stress 2900 and 3000 groups than those in heat stress 3100 and 3200 groups ($p < 0.05$). However, they were not significantly different between heat stress 2900 and 3000 or between heat stress 3100 and 3200. It has been reported that heat stress can increase the levels of stress hormone corticosterone and decrease immune substance concentrations in laying hens compared to normal control (Fraisie and Cockrem, 2006).

In heat stress groups, excessive energy mobilization can break homeostasis of blood parameters by reducing immunity with increased secretion of IgG and corticosterone compared to normal control (Park and Park, 2017). However, few studies have reported the effect of increasing dietary metabolic energy levels on corticosterone or IgG levels in broiler chickens or meat-type chickens exposed to heat stress compared to normal control

(Shin *et al.*, 2011). In the present study, higher lipids level in heat stress groups compared to those in normal control group might enhance corticosterone release (Habibian *et al.*, 2014). Therefore, determining the appropriate dietary metabolic energy levels is crucial to relieve the heat stress of meat ducks.

In conclusion, the findings of this study suggest that it is very important to strengthen the immune response while maintaining homeostasis of blood biochemical parameters by providing diet containing 3000 kcal kg⁻¹ of metabolic energy to alleviate thermal stress in ducks during hot summer.

Acknowledgments

This research was supported by Kangwon National University 2019, Republic of Korea.

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