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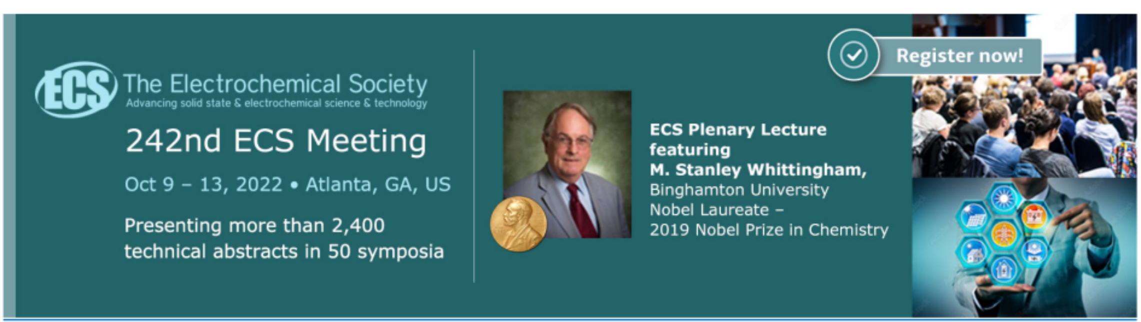

The hematological profile and immune response treated by heat stress on ''Gaok''native chickens

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Abstract: The study was conducted to evaluate the effect of heat stress on hematological profile (hemoglobin, erythrocytes, hematocrit, MCV, MCH, MCHC, leucocyte, granulocyte, lymphocytes, MID) in blood of Native chickens. Total of 32 ''Gaok'' native chickens (18-week) were redesigned into CRD (completely randomized design) 2x2 factor arrangement. The first factor was the temperature condition in indoor and outdoor pen. The second factor was with and without supplementation Vitamin C. Blood samples were collected on 3 days during treatment. Results indicated that treated with heat stress was showed not significant effect on Hemoglobin, erythrocytes, MCV, MCH, MCHC, leucocyte, lymphocytes, granulocytes. Heat stress pressure had significantly ($p<0.05$) increase in the water consumption and feed intake. The result provides a new information that Gaok chickens was resistant to heat stress without any effect on immune response and blood cell metabolic.

1. Introduction

The earth's temperature has increased up to 0,8^{O}C, in two decades. In prediction, the earth's surface temperature will go up further to 1,88 - 4,08^{O}C [1]. The effect of climate will cause heat stress and decreased poultry productivity. The estimated calculation for poultry production, 50% of the meat supplied by tropical country thus the influence of global warming could have a negative impact on global food supply [2]. The reducing poultry productivity which caused by high ambient temperature drives concern in the animal welfare and trigger effort to maintain the thermal comfort in tropical country [3]. Heat stress is the exposure caused by up normal environment temperature. Comfort zone temperature for layer is 18-22^{O}C and 18-30^{O}C for broiler respectively [4].

The effect of heat stress seen in poultry is decreased feed consumption and poultry production [5], hampers the development of poultry [6], and decrease egg production and egg shell quality [7]. Heat stress increased activating hypothalamic pituitary adrenal hormone (HPA), which stimulates the release of corticotrophin releasing hormone and adrenocorticotropic hormone from hypothalamic and pituitary cells [8]. Improved poultry immunity be marked with increased cell lymphocyte and Heterophil /Lymphocyte (H/L) ratio, which is effect from secretion of costiteron hormone [9]. Feed supplements have been used to alleviate negative effect of heat stress on poultry production for example yeast [10] and vitamin C, which can limit reducing of the systemic immune response.

Indonesia is one of the tropical countries that have high temperature and relative humidity, mainly during the dry season. In an attempt to expand the Indonesia native chicken still need to be explored chicken breed that tolerance to high environmental temperatures. One of the indigenous Indonesia's Chicken is Gaok, which has potential as a producer of meat. Gaok is Indonesia native chicken from Madura Island East Java Indonesia. There is limited information available about immune system of heat-stressed Indonesia native chickens specially Gaok. The data about Gaok animal responses to heat stress can be used as a basis for development of heat-resistant native chicken strains. Therefore the present study was conducted to elucidate how heat stress affects the immune system of Indonesia native chicken Gaok on the hematology profile. The hypothesis of this study is that heat stress will reduce immune system and performance of Gaok chicken.

2. Materials and Methods

2.2. Animal and diets

A total of 32- 15 week –old –mixed sex Gaok native chickens (1.22 ± 0.26 kg) were obtained from Madura Island. Gaok chickens was maintained in Pen (H 150 cm and W 60 cm x D 80 cm) and placed in indoor and outdoor pen. All birds were randomly assigned to 4 treatments, and 4 replicate pens per treatment. This study was designed in a CRD with a 2 x 2 factorial arrangement. The first factor was the temperature condition in indoor and outdoor. The second factor was with and without supplementation Vit C. The amount feed were measured each in morning before feeding. All nutrients contained in the basal diet met or exceeded the requirements suggested by the NRC 1994 [11]. The ingredients and chemical composition of the basal diet are shown in **Tables 1.**

2.3. Collection of Performance Data

The initial body weight and feed consumption of the Gaok chickens were recorded in each pen on week 21 to week 23 and these values were used to calculate the average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) of the broilers (FI:ADG) for the periods between week 21- week 23.

2.4. Blood samples

The blood sampled were taken in d 3 after heat stress treatment. The blood was collected according to protocol [12]. Briefly, the samples were collected as soon as possible from the wing vein (three/treatment) using a 1 mL syringe with a 18 gauge needle. From this, 1 mL was carefully moved into heparinized tubes for measuring for hematology profile test. An automated hematology analyzer using Analyzer Xs, XS series XS-1000i/XS-800i, sysmex Corporation according ISO standard ISO9001:2015 for analysis the concentrations (cells/µl) of WBC and RBC. Blood smears were prepared on slides and painted by Gimsa method. 100 leukocytes per sample were counted by heterophile to lymphocyte separation under an optical microscope.

2.5. Statistical Analysis

Data on FI, AVG, FCR, initial weight, final weight, water intake and blood profile were analyzed using completely randomized procedure with ANOVA. The Minitab software package was used for statically analysis (Minitab Inc, USA). The post hoc Duncan New Multiple Range Test was calculated, at a statistical significance of P<0.05.

Tabel 1. Analyzed composition of experimental diets

Item	Prelayer phase
Ingredients (%)	
Meal Corn	55.00
Complete Concentrate	33.00
Rice hull meal	10.00
Coconut oil	1.00
Premixes)*	1.00
Essential Amino acid (DL Methionin and L Lysin	0.06
Composition analysis	
Protein (%)	21.0
Fat (%)	5.0
Fiber (%)	3.0
Calcium (Ca) (%)	2.0
Phospor (P) (%)	0.7
Energy (Kcal/Kg)	2.850

*) Premixes as feed supplement contains : Vitamin A, 8.723 IU; Vitamin D 3.311 IU Vitamin K 17 mg, Vitamin E 21 mg; Vitamin K3, 6 mg; riboflavin, 5.0 mg, cobalamin, 0.1 mg; choline chloride 500 mg, Fe77 mg,

3. Results and Discussion

The average environmental temperature in indoor and outdoor pen during the experiment is shown in **Figure 1**. From the data, it can be seen that the average temperature difference between indoor and outdoor cages, especially at 08.00 -13.00 hours with an average temperature increase of 6-7^{o}C at outdoor temperatures compared to indoor temperature (25-36^{o}C vs 23-29^{o}C). The temperature difference lasts for 5-6 hours every day. The temperature increase indicator is simulated as heat stress in chickens. According to Aengwanich [14], a temperature of more than 32^{o}C indicates heat stress, which is indicated by an increase in body temperature. So that in this study an increase in temperature or temperature outside the door in heat stress conditions.

The performance data for chickens reared in indoor and outdoor pens are shown in **Table 2**. The data shows that there is no interaction between the treatment of temperature differences and the addition of vitamin C supplements (P> 0.05). This study clearly shows that an increase in temperature tended to have an effect on increasing feed intake (P <0.07) which is accompanied by a tendency to increase in water consumption (P <0.06). Meanwhile, the addition of vitamin C had no significant effect on FI, FCR and water consumption (P> 0.05). Increased consumption and drinking water are thought to be related to efforts to overcomes with hot temperatures. Increasing temperature can cause chickens to lose energy, so that the chicken consumes higher feed than the temperature in the indoor pen. By increasing consumption, it will increase the energy that can be used to overcome the energy lost during the exposure to heat stress and efforts to reduce body temperature. This increase in consumption is thought to prove that raising native chickens outside the cage will increase feed costs which will be related to the economic calculation.

Figure 1. Temperature profile (°C) in indoor and outdoor pen during 24 hours

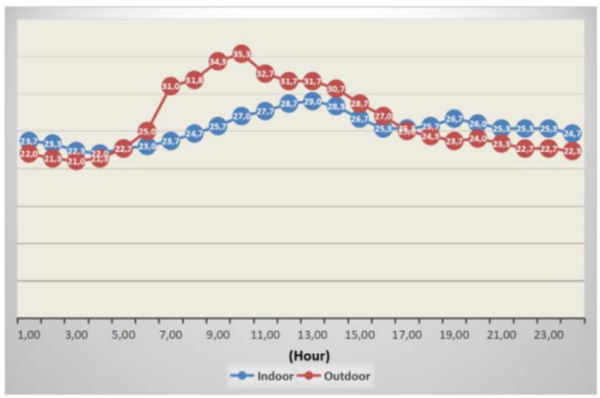

Table 2. Gaok performans in indoor an outdoor with and without vitamin C supplementation

Item	Indoor		Outdoor		SEM	*P value*		
	-Vit C	**+ Vit C**	**-Vit C**	**+ Vit C**		**T**	**S**	**TxS**
Initial weight (g)	1659	1532	1608	1796	283	0,466	0,831	0,288
Final Weight (g)	1765	1655	1734	1919	290	0,438	0,804	0,329
FI (g)	643	572	710	726	110	0,069	0,628	0,450
ADG (g)	106	122	126	122	43	0,659	0,785	0,659
FCR : FI/ADG	9,35	5,06	8,40	7,77	3,96	0,665	0,238	0,375
Water intake (ml)	2507	2101	2587	2978	464	0,062	0,974	0,112

SEM : Standart error mean
FI: feed intake; ADG : average daily gain; FCR;: feed conversion ratio

Table 3. Blood profil were maintenance in indoor and outdoor pen with and without vitamin C supplementation

	Indoor		Outdoor			*P value*		
Item	**-Vit C**	**+ Vit C**	**-Vit C**	**+ Vit C**	**SEM**	**T**	**S**	**TxS**
Haemoglobin (HGB) (g/dl)	13,9±2,79	14,9±1,69	13,12±1,15	13,52±1,13	0,456	0,231	0,457	0,748
Erytrocytes (RBC)(x 10^6)	2,53±0,51	2,69±0,33	2,4±0,23	2,395±0,13	0,083	0,218	0,641	0,620
Haematocrit (%)	31,3±6,63	32,75±4,33	28,95±2,24	29,5±2,77	1,087	0,222	0,654	0,839
MCV (fl)	123,5±2,85	121,3±2,89	120,9±5,79	123,1±5,79	1,142	0,868	0,987	0,368
MCH	55,3±1,45	55,6±1,07	54,9±2,24	56,4±2,50	0,478	0,858	0,365	0,542
MCHC (g/dl)	44,8±0,48	45,8±1,07	45,4±0,62	45,82±0,60	0,731	0,428	0,071	0,466
Leucocyt(WBC (x 10^3/Ml)	4,99 1,02	6,14 6,50	5,40 1,04	5,23 0,75	2,21	0,577	0,290	0,164
Lymphocyte (%)	91,95±1,48	90,9±1,03	91,35±2,35	91,2±1,14	0,589	0,853	0,465	0,582
ranulocyt (%)	1,87±0,97	2,05±0,71	2,2±1,51	1,92±0,80	0,263	0,854	0,924	0,675
MID (%)	6,17±0,63	7,05±0,40	6,45±1,06	6,9±0,31	0,167	0,855	0,071	0,538
Trombosit (Plt)(x 10^3)	6,5±1,0	6,7±2,0	6,7±0,9	5,5±0,5	3,18	0,448	0,448	0,262

SEM : Standart error mean
MCH: mean corpuscular hemoglobin, RDW-CV: red blood cell distribution width coefficient variation; MCHC: mean corpuscular hemoglobin concentration, RBC: red blood cell, WBC: white blood cell, MCV: Mean corpuscular volume

The results on blood analysis of blood profiles are shown in **Table 3**. The data shows that there is no interaction between the treatment of temperature differences and the addition of vitamin C supplements (P> 0.05). In this study, the effect of heat pressure has not affected the blood profile. From this condition, the metabolic processes in the body have not had an impact on the blood profile of the Gaok chicken. According to Cooper et al [13], the exposure to heat stress conditions can cause disturbances as indicated by the decrease in MCV content increasing while MCHC and MCH decreases. According to Aengwanich [14], changes in MCHC occurred at 38 ^{o}C, in this study the temperature was still in the range of 36^{o}C, so there was no significant effect. From the content of Lympocites does not change with temperature treatment, it is suspected that heat stress has not affected hormonal. Heat stress can activate the hyphothalamic pituitary adrenal hormone (HPA), which functions in the release of corticotrophin, releasing hormone and adrenocorticotropic hormone from the hypothalamus and pituitary cells [8]. Thus the condition of increasing temperature has not affected the immune system in Gaok chickens.

4.Conclusion

The result of study provides a new information that Gaok native chickens was resistant to heat stress without any effect on immune response and blood cell metabolic.

5. Acknowledgments

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