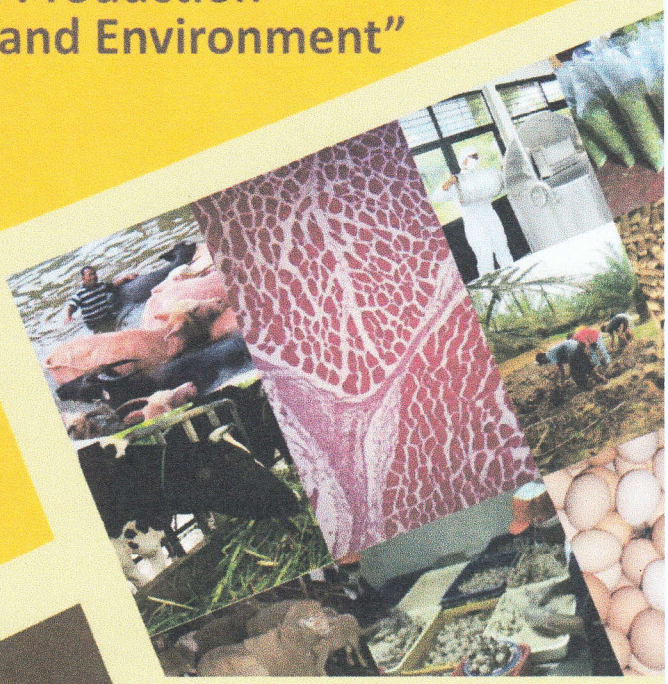
ISBN: 978-602-96530-4-5

PROCEEDING

isai 3rd
International Seminar on Animal Industry 2015

THE THIRD INTERNATIONAL SEMINAR ON ANIMAL INDUSTRY

"Sustainable Animal Production
for Better Human Welfare and Environment"



September, 17-18 2015
IPB International Convention Center
Bogor-Indonesia

Organized by:

Sponsored by:

SieradProduce

FACULTY OF ANIMAL SCIENCE
BOGOR AGRICULTURAL UNIVERSITY
2015

LIST OF EDITORS

Proceeding of the 3rd International Seminar on Animal Industry, Bogor, 17-18 September 2015

Scientific Editors

Chief : Prof. Dr. Ir. I Komang G. Wiryawan
Secretary : Prof. Dr. Ir. Cece Sumantri, M.Agr.Sc
Members : Prof. Dr. Ir. Bas. Kemp. (Netherland)
Prof. Myunggi Baik (Korea)
Prof. Wayne Pitchford (Australia)
Prof. Dr. Ir. Wasmen Manalu, M.Sc
Prof. Dr. Ir. Iman Rahayu H.S., MS
Prof. Dr. Ir. Nahrowi Ramli, M.Sc
Prof. Dr. Ir. Muladno, MSA
Prof. (R)Dr. Ir. Bess Tiesnamurti
Prof. Dr. Ir. Dewi Apri Astuti, MS
Dr. Jean Pierre Bidanel (France)
Dr. Anjas Asmara Samsudin (Malaysia)
Dr. Kai J. Kuehlmann (Germany)
Dr. Ir. Idat Galih Permana, M.Sc.Agr
Dr. Tuti Suryati, SPt, MSi
Dr. Indah Wijayanti, S.Tp, M.Si
Ir. Anita Tjakradidjaja S., M.Rur.Sc

Technical Editors

Windi Al Zahra, S.Pt, M.Sc.Agr
Irma Nuranthy Purnama, SPt, MSi
Fitri M. Manihuruk, S.Pt
Himmatul Khasanah, S.Pt
Reikha Rahmasari, S.Pt, M.Si
Rika Zahera, S.Pt, M.Si

List of Reviewers

Prof. Dr. Ir. Sumiati, M.Sc
Prof. Dr. Ir. Dewi Apri Astuti, MS
Prof. Dr. Ir. I Komang G. Wiryawan
Prof. Dr. Ir. Bas. Kemp. (Netherland)
Prof. Myunggi Baik (Korea)
Prof. Dr. Ir. Erika B. Laconi, MS
Prof. Dr. Ir. Cece Sumantri, M.Agr.Sc
Prof. Dr. Ir. Iman Rahayu H.S., MS
Prof. Dr. Ir. Yuli Retnani, M.Sc
Prof. Dr. Ir. Wasmen Manalu, M.Sc
Prof. Dr. Ir. Panca Dewi M.H.K, MS
Prof. Dr. Ir. Luki Abdullah, M.Sc.Agr
Prof. Dr. Ir. Yuli Retnani, MSc
Dr. Jean Pierre Bidanel (France)
Dr. Anjas Asmara Samsudin (Malaysia)
Dr. Kai J. Kuehlmann (Germany)
Dr. Ir. Asnath M. Fuah
Dr. Indah Wijayanti, S.Tp., M.Si
Dr. rer.nat. Nur Rochmah Kumalasari, S.Pt, M.Si
Dr. Ir. Lilis Khotijah, MS
Dr. Ir. Asep Sudarman, M.Sc
Dr. Ahmad Yani, S.TP, M.Si
Dr. Ir. Muhammad Ridla, M.Agr
Dr. Ir. Widya Hermana, M.Si
Dr. Ir. Afton Atabany, M.Si
Dr. Ir. Didid Diapari, MS
Ir. Burhanudin, MM
Dr.Agr Asep Gunawan, S.Pt, M.Sc
Dr. Tuti Suryati, S.Pt, M.Si
Ir. Anita Tjakradidjaja S., M.Rur.Sc
Dr. Irma Isnafia Arief, S.Pt, M.Si
Dr. Ir. Heri Ahmad Sukria, M.Sc.Agr
Dr. Ir. Rudi Afnan, M.Sc.Agr
Dr. Anuraga Jayanegara, S. Pt, M.Sc
Dr. Ir. Henny Nuraini, MS
Dr. Ir. Rudy Priyanto
Dr. Sri Suharti, SPt, M.Si
Dr. Ir. Idat Galih Permana, MSc Agr
Dr. Ir. Rita Mutia, M.Agr
Dr. Ir. Dwierra Evvyernie A, MS, M.Sc
Dr. Ir. Hotnida H. C. Siregar, M.Si
Dr. Ir. Suryahadi, DEA
Ir. Lucia Cyrilla, E.N.S, M.Si
Dr.Ir. Sri Darwati, MSi
Dr. Epi Taufik, S.Pt, MVPH, M.Si
Dr. Ir. Moh. Yamin, M.Agr.Sc
Dr. Despal, S.Pt, M.Sc.Agr
Dr. Ir. Niken Ulupi, M.Si
Dr. Jakaria, S.Pt, M.Si
Dr. Iwan Prihantoro, S.Pt, M.Si
Drh. Agus Setiono, MS, Ph.D
Dr. Ir. Sri Mulatsih, M.Sc.Agr
Maria Ulfah, SPt, MSc.Agr
Yuni Cahya E., S.Pt, M.Si

LIST OF CONTENTS

THEME G.
ANIMAL PHYSIOLOGY, BEHAVIOUR, AND WELFARE

Proceeding of the 3rd International Seminar on Animal Industry,
Bogor, 17-18 September 2015
Indonesia

Level of Malondialdehyde (MDA), Uric Acid and Lymphocyte: Neutrphyl Ratio of Laying Hen in The Different Temperature Humidity Index (THI)

*D. Latipudin[1], L. Adriani[2] & R. Permana**
[1] *Doctorate Student of Animal Science, Bogor Agricultural University, Indonesia*
[2] *Animal Physiology and Biochemistry, Faculty of Animal Science, Padjadjaran University, Indonesia*
email: ronnie_permana@yahoo.com

Abstract

This study is conducted to investigate the effect of THI on the concentration of MDA, uric acid blood plasma and lymphocytes: heterophil ratio of laying hen. One hundred laying hens arerandomly divided into two treatments, each treatment consist 50 birds. Treatments in this work are two levels of temperature humidity index (THI = 89 and 72), which calculated based on dry and wet bulb thermometer in laying housing, located in Kuningan and Bandung. The experiment period lasted 8 weeks, blood samples were collected weekly. Malondialdehyde, uric acid and lymphocytes: heterophilratio is recorded by spectrophotometer following Biolabo Kit. Data are analysed using paired t-test method. Based on results of this study, the level of MDA and uric acid significantly increase ($P < 0.01$) in the high THI (89). On the other hand, lymphocytes/ heterophils (H/L) ratio decreases ($P < 0.05$). These data suggest that high THI (89) contributes of laying stress is caused by the level of temperature and humidity in environment and inside the housing.
***Keywords**: H/L, laying hen, malondialdehyde, THI, uric acid*

Introduction

Laying hens is the land fowl which has the highest sensitivity towards environmental heat stress, either caused by metabolic heat production or environmental stress in cages (Mushawwir and Latipudin, 2012). The environmental factors which often change are temperature and humidity. The temperature and humidity of environment are able to determine a comfort zone for livestock whose index is usually called by Temperature-Humidity Index (HTI).

High temperature is one of environmental factors which affects to the metabolic profile in cattle's body and causes the heat stress. The heat stress is exacerbated by increased humidity. This condition is indicated by the increase of THI index.

Chicken are homoioterm animal that always try to maintain their body temperature which one of the ways is by increasing the secretion of glucocorticoids carried out by adrenal cortex and epinephrine by the adrenal medulla (Mushawwir dan Latipudin, 2012). The secretion occurred is due to the increase of adrenocorticotropic hormon (ACTH) that is caused by environmental stress (Wilson *et al.*, 2007; Wheelock *et al.*, 2010; Won *et al.*, 2012).

The increase of glucocorticoids secretion results the gluconeogenesis and turns up the protein metabolism. However, it also decreases the anabolism, so that the synthesis of lymphocytes is decreasing while heterophilsis increasing. The protein metabolism can form urea. Urea is an end product of protein metabolism in which if the profile in body is too high, it is excreted through the urine. Several amino acids, besides forming the urea, they also can become a precursor of other compounds, for example purine, pyrimidine, and hormones (Mushawwir *et al.*, 2010). Purine and pyrimidine bases are converted into uric acid in the body. Uric acid is an end product or waste product resulted by metabolism/breakdown of purines. The too high-uric acid profile in the body causes pro oxidant and endangers the livestock and causes free radicals.

Free radicals are increasing along with the increase of heat stress as a result of oxidative phosphorylation which produces Reactive Oxygen Species (ROS). The ROS causes the lipid peroxidation rate increases. Product of this oxidation is malondialdehyde (MDA). The levels of ROS are equal to the levels of MDA.

Based on abovementioned descriptions, it is necessary to examine the blood uric acid, the levels of MDA, and the ratio of lymphocyte as a stress indicator due to heat stress for layer-phase laying hens on the different temperature-humidity index (THI).

Materials and Methods

Livestock Samples

This research examines 100 laying hens leghorn which are each 50 hens placed in a cage in Kuningan and Cililin (Bandung), in which with the average of Temperature-Humidity Index (THI) is 89 (in Kuningan), and 72 (in Cililin, Bandung). The livestock were kept for two months.

Blood Collection

The collection of blood sampling is carried out on each chicken samples for two times, which are on the 30th day and the 60th day. Each blood samples is collected as many as 3 mL using venojet with EDTA.

Blood Analysis

Analyzing blood aims to know the levels of Melondiadehide (MDA) and uric acid whose process is carried out by following the procedure of KIT-BIOLABO. The recording of MDA and uric acid is conducted by using a spectrophotometer with wavelength of each is 532 nm and 540 nm. The measurement of lymphocytes and heterophils levels is performed by injecting "whole blood" into hematology analyzer Mindray BC-2800.

Data Analysis

The data obtained (MDA levels, Uric Acid levels, and Lymphocytes ratio: Heterophil) are analyzed by using method of unpaired T-Student with IBM SPSS Statistic 21.

Results and Discussion

Based on results of the research, the effects of THI towards MDA levels, Uric Acid levels, and Heterophils Lymphocytes ratio is shown on the following Table:

Tabel 1. The MDA levels, Uric Acid levels, and Heterophyl Lymphocytes Ratio of Laying Hens in the locations with the different Temperature Humidity Index (THI)

Blood Indicator	THI		p-value
	89	72	
MDA ($nm.M^{-1}.cm^{-1}$)	2.271^{a}	1.055^{b}	< 0.01
Uric Acid ($mg.dL^{-1}$)	7.426^{a}	4.893^{b}	< 0.01
Lymphocytes:Heterophils (ratio)	0.312^{a}	0.152^{b}	< 0.05

Note: The average levels of MDA and Uric Acid is highly significant different ($p<0.01$), while the ration of Lymphocytes: Heterophilsis significantly different ($p<0.05$).

The result of analysis shows that the uric acid level in the maintenance locations with THI=89 is highly significant different ($p <0.01$) which is 2.271 mg/dL. It is higher than laying hens at THI=72, which is 1.055 mg/dL. The increase of blood uric acid in layer-phase laying hens is caused by chicken responds to the heat stress by activating the neurogenic system to stimulate Corticotropin Releasing Factor (CRH), so that pituitary anterior issues Adenocorticotropin (ACTH). Furthermore, medulla adrenal secretes the epinephrine which functions to stimulate the second messenger, namely adenylate cyclase (Mushawwir dan Latipudin, 2013). The adenylate cyclase catalyzes the formation of cAMP which moreover, the cAMP will activate the protein kinase A which plays a role in the regulations of metabolic-enzymes and gene transcription, such as triggering glycogenolysis.

The increase of cAMP can improve the uric acid formed. The increase can improve the synthesis of AMP, which is one of purine nucleotide. Moreover, AMP is deaminated to inosine which is later being hydrolyzed to produce hypoxanthine and D-ribosa. Furthermore, hypoxanthine is converted to xanthine, and xanthine gets converted to uric acids by xanthine oxidase.

The enzyme Hypoxanthine-guanine phosphor ribosyl transferase (HGPRT) is one of enzyme involved in the reaction of purine bases utilization into nucleotides. This enzyme plays a role in converting purines into purine nucleotides, so it can be reused as a constituent of DNA and RNA. If this enzyme is deficient, purine in the body can be increased for it is not metabolized by enzyme HGPRT which later caused the purine will be metabolized by enzyme xanthine oxireductase (XOR) into uric acid.

The increase of ambient temperature and heat stress on the chickens triggers the increase of enzyme XOR's activity (Donsbough *et al.*, 2010; Mushawwir *et al.*, 2011; Settle *et al.*, 2012). This condition will trigger the increase of uric acid which circulates in blood. In the same condition, it will be known that heat stress decreases the activity of enzyme XOR, so this condition can be ascertained will give impacts towards the increase of uric acid level in blood.

The increase of THI also causes an increase of Reactive Oxygen Species (ROS) which affects to the formation of MDA. MDA levels of laying hens at THI=89 which is 7.426 is highly significant different ($p<0.01$), is higher than laying hens at THI=72 which is 4.893.

ROS is a derivative of oxygen which is more reactive than oxygen in the basic conditions. ROS can get into the blood stream in which if the condition is high prooxidant activity due to free radicals, it often causes oxidative stress. According to Bottje (1995), ROS induces peroxidation of fatty acids with protein, cellular nucleic acids, fats, especially PUFA, hence resulting lipid peroxidation. The main target of lipid peroxidation by ROS is PUFA in membrane lipids. The PUFA degraded by ROS will cause the form of aldehyde like MDA. MDA is a highly reactive three carbon dialdehyde which can be obtained from hydrolysis of pentose, deoxyribose, hexoses, amino acids, and DNA (Evans, 1991; Bottje *et al.*, 1995).

The increase of MDA as an impact of increasing THI shows that the increase of environmental stress is a combination of temperature and humidity. The high environmental stress leads the trajectories of catabolism (Mushawwir *et al.*, 2010) and anabolism (Hardy *et al.*, 2005; Favlik *et al.*, 2007) are activated at the same time, in order to homoestasis defends the normal physiological conditions and the provision of energy.

The increase of free radicals also can be triggered by the rising of trajectory of glycolysis and glycogenolysis, and also oxidative phosphorylation in mitochondria matrix for ATP biosynthesis. The concentration of free radicals (ROS) becomes a key trigger for formation of MDA.

The high concentration of ROS results a reaction with fat, protein, cellular nucleic acids. It then causes a local damage and particular organ dysfunction. Fats are biomolecules that are susceptible towards free radical attack. The component of animal cell membranes contains many sources of PolyUnsaturated Fatty Acid (PUFA). Uppu (2010) in line with Mushawwir and Latipudin (2012) state that PUFA is biomulecules which easily damaged by oxidizing substances whose process commonly called by lipid peroxidation. Evans (1991) reports that breaking the bonds of carbon during lipid peroxidation leads to the formation of aldehyde, such as malondialdehyde (MDA).

Based on the hormonal perspective, heat stress caused by highly THI (89) causes the secretion of Adrenocotrico Tropik hormon (ACTH) becomes increased, thus it stimulates the secretion of hormone streroid, namely cortisol. This cortisol obstruct the anabolism which affects to the decrease of lymposit (L) and the increase of heterophils level (H). So that, the ratio of H/N is higher ($p<0.05$) at THI = 89 which is 0.312, than at THI = 72 which is 0.152.

The high temperature with highly environmental humidity can worsen the condition of heat stress (Marai dan Haeeb, 2010). In addition, Marai and Haeeb (2010) state that level of cortisol will increase during acute heat stress condition. This is caused by glucocorticoids can lead to the transport of amino acids and fats from cells. So, it can be used directly as a source of energy and for synthesis of other compounds including glucose which is needed by various tissues of the body (gluconeogenesis).

The high cortisol can cause damage of lymphoid glands (thymus) and extension of heterophils' age. This change also results an increased number of heterophils in blood circulation (Kim *et al.*, 2006; Ogura *et al.*, 2007). The results of previous studies as reported by Zapata et al., (2004) and Milne et al., (2012) show that the increase of glucocorticoid (cortisol) can reduce the secretion of cytokines IL-2 which leads to the decrease of lymphocytes proliferation and makes cells of lymphocytes become delicate towards apoptosis. There by it reduces the amount in circulation.

Conclusion

The high Temperature-Humidity (THI) index up to 80 leads to the increase of MDA and Uric Acid levels, and also the decrease of the ratio of lymphocytes to heterophils. This condition shows that index of THI up to 89 can cause a heat stress for layer-phase laying hens.

References

Bottje W, B Enkvetchakul, R Moore. 1995. Effect of α-tocopherols on antioxidants, lipid peroxidation, and the incidence of pulmonary hypertensio syndrome (ascites) in broilers. Poult. Sci. 74: 1356-1369.

Donsbough, ALS Powell , A Waguespack, TD Bidner, LL Southern. 2010. Uric acid, urea, and ammonia concentrations in serum and uric acid concentration in excreta as indicators of amino acid utilization in diets for broilers. Poult. Sci. 89: 287-294.

Favlik A, M Poklodova, D Zapetal, P Jelinek. 2007. Effect of Housing System on Biochemical of Blood Plasma in Laying Hens. Acta Vet. Brno 2007, 76: 339- 347.

Hardy MP, HB Gao, Q Dong, R Ge, Q Wang, WR Chai, X Feng, C Sottas. 2005 . Stress hormone and male reproductive function. Cell Tissue Res. 322: 147-153.

Kim JW, I Tchernyshyov, GL Semenza, CV Dang. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 3: 177-185.

Milne KJ, S Wolff, EG Noble. 2012. Myocardial accumulation and localization of the inducible 70-kDa heat shock protein, HSP70, following exercise. J. Appl. Physiol. 113: 853-860.

Mushawwir A, D Latifudin. 2012. Respon Fisiologi Thermoregulasi Ayam Ras Petelur Fase Grower dan Layer. Proceeding of National Seminar on Zootechniques for Indogenous Resources Development. Faculty of Animal Agriculture Diponegoro University and Indonesian Society of Animal Agriculture, Semarang.

Mushawwir A, D Latipuddin. 2013. Biologi Sintesis Telur, perspektif Fisologi, Biokimia dan Molekuler Produksi Telur. Penerbit Graha Ilmu, Yogyakarta.

Mushawwir A, L Adriani, KA Kamil. 2011. Prediction Models For Olfactory Metabolic and Sows $\%Rna_{reticulocyt}$ (Rna_{rt}) by Measurement of Atmospheric Ammonia Exposure and Microclimate Level. Journal of Indonesia Tropical Animal Agriculture. 36: 14-20.

Mushawwir A, YK Yoong, L Adriani, E Hernawan, KA Kamil. 2010. The Fluctuation Effect of Atmospheric Ammonia (NH_3) Exposure and Microclimate on Hereford BullsHematochemical. Journal of Indonesian Tropical Animal Agriculture. 35: 232-238

Ogura Y, Y Naito, T Tsurukawa, N Ichinoseki-Sekine, N Saga, T Sugiura, S Katamoto. 2007. Microwave hyperthermia treatment increases heat shock proteins in human skeletal muscle. Br. J. Sports Med. 41: 453-455.

Settle T, MD Carro, E Falkenstein, W Radke, H Klandorf. 2012. The effects of allopurinol, uric acid, and inosine administration on xanthine oxidoreductase activity and uric acid concentrations in broilers. Poult. Sci. 91: 2895-2903.

Uppu RM, AN Murthy, WA Pryor, NL Parinandi. 2010. Free Radicals and Antioxidant Protocols. 2nd Edition. Humana Press, New York USA.

Wheelock JB, RP Rhoads, MJ VanBaale, SR Sanders, LH Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93: 644-655.

Wilson DC, AD Corbett, C Steel, R Pannirselvam, DL Bovell. 2007. A preliminary study of the short circuit current (Isc) responses of sweat gland cells from normal and anhidrotic horses to purinergic and adrenergic agonists. Vet. Dermatol. 18: 152-160.

Won SGL, G Xie, R Boddicker, JN Rhoades, MC Lucy, TJ Safranski, JT Selsby, S Lonergan, LH Baumgard, JW Ross, RP Rhoads. 2012. Acute duration heat stress alters expression of cellular bioenergetic-associated genes in skeletal muscle of growing pigs. J. Anim. Sci. 90 (E-Suppl. 3): 573.