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Evaluation of Cardiovascular Biomarkers and Lipid Regulation in Lactation Friesian Holstein at Different Altitude in West Java, Indonesia

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ABSTRACT

Altitude contributes and plays an important role in the development dairy cows in Indonesia. Altitude is directly related to temperature and humidity, both of which affect cardiovascular function and energy metabolism in dairy cattle. The 120 samples, consisted of 4th-5th lactation Friesian Holstein dairy cows were used in this study. The 40 samples of cattle each spread in three maintenance sites with different altitudes, namely 300 to 500 m above sea level (a.s.l.); 600-900 m a.s.l. and >1,000 m a.s.l. All the study sites located in West Java, Indonesia, to study the effect of altitude on the cardiovascular biomarker and lipid regulation in the dairy cow. Based on the result in this experiment showed that the CRP high sensitivity, H-FABP, homocysteine, and γ-Glutamyl Transpeptidase in Frisian Holstein dairy cows expressed higher levels (P <0.05) at low altitude site (300-500 m a.s.l.) than dairy cows at altitude sites higher (600-900 and >1,000 m a.s.l.), These results indicated, these compounds can be biomarkers for cardiovascular function. This study also showed, the lipid regulation also showed higher levels (P <0.05) at low altitude sites than altitude sites higher.

1. Introduction

West Java Province is a center for the development and production of milk in Indonesia. The topography of West Java varies from the lowlands to the highlands. Nevertheless, the maintenance of dairy cows is abundant in all these topographies. Previous studies have shown that the productivity of dairy cows is greatly influenced by topography or altitude.

The humidity and temperature are physical factors that play an important role in animal metabolism. Dairy cows as homoiothermic animals are very sensitive to these two climate factors (humidity and temperature). Many studies show metabolic changes during climate fluctuation. Cheng *et al.* (2014) and Allen *et al.* (2015) reported a reduction in immunity and blood profile (Roland *et al.* 2016). Climate changes related to the enclosure in housing. Mushawwir *et al.* (2010 and 2011) showed that microclimates are strongly related to NH_3 levels of the cage, olfactory receptor response, and also hematologic and biochemical of blood plasma in poultry (Slimen *et al.* 2016; Mushawwir *et al.* 2018).

Altitude is closely related to the temperature and humidity of the environment. High temperatures generally occur in areas with low altitude, while high altitudes generally exposed low temperatures. Temperature and humidity that are not suitable for dairy cows cause an increase in the rate of thermoregulation.

Thermoregulation is needed to maintain a normal temperature range so that the minimum metabolic process can be maintained. This process requires a large amount of energy and interactions throughout the body's tissues. Previously report demonstrated that heat stress increases lipid retention in beef cattle at low altitude (Nasr and El-Tarabany 2017; Jiangjing *et al.* 2019), in the chicken (Geraert *et al.* 1996; Abou¬Elkhair *et al.* 2014; Adriani *et al.* 2015; Lee *et al.* 2017) and pig (Qu and Ajuwon 2018). The other report showed that a high temperature or heat stress at low altitude stimulates muscle cells increase in lipolysis and triglyceride degradation (Tanuwiria *et al.* 2011). Based on the results of previous studies,

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reports on the involvement and expression of lipid transport proteins during altitude stress in animals merely limited information.

Physiological responses related to altitude in dairy cows also have an impact on the physiology of the cardiovascular. The heart pump rate increases in high temperatures to accelerate the flow of blood to the peripheral blood vessels to be evaporated (Rejeb *et al.* 2016), either through sweating or by breathing evaporation (Li *et al.* 2016). Previous research has shown that a high pacemaker rate increases the risk of cell death (Lambertz *et al.* 2014), tissue damage (Kang *et al.* 2016), necrosis (Gernand *et al.* 2019), apoptosis (Chmielewski *et al.* 2009), also increased the risk of heart failure (Geraert *et al.* 1996). but no previous study showed cardiovascular biomarker associated heart physiology in the dairy cow.

Therefore, this experiment was conducted to evaluate cardiovascular biomarkers and lipid regulations in Friesian Holstein lactation at different altitudes.

2. Materials and Methods

2.1. Animal Sample and Study Site

The 120 samples, consisted of 4th-5th lactation Friesian Holstein dairy cows were used in this study. The 40 samples of cattle each spread in three maintenance sites with different altitudes, namely 300 to 500 m above sea level (a.s.l.); 600-900 m a.s.l. and >1,000 m a.s.l. All the study sites located in West Java, Indonesia. Samples of Dairy cows were kept intensively, with open housing system. The ration provided consisted of grass and concentrate. Observation and blood sampling of dairy cow samples were carried out for 6 months, every 3 months during the rainy season and the dry season.

2.2. Blood and Sample Analysis

Blood samples (5 ml) were collected with EDTA tube and sterilized syringe at wk 4, 8, 12, 16, 20, and 24. The blood samples were taken in the morning before the animals were feeding, from the front tail vein. The collected blood sample was immediately put into a thermos filled with ice gel. The samples were taken to the laboratory. The blood samples were centrifuged (3,000 × g, 10 min) within 30 min after collection and the plasma was stored in a freezer at −20°C until analysis.

The blood plasma was used to analyze cardiovascular parameters and lipid regulation profiles. All analysis procedures (reagent and absorbance) were carried out based on the user manual as stated in the biochemical kit from Randox Laboratories LTD, UK, and Biolabo Biochemistry, France.

2.3. Statistical Analysis

All data collected were presented as average (mean) ± standard error (SE). To examine differences in cardiovascular character and lipid regulation, the multivariate analysis of variance for repeated measurements (MANOVA) was using with altitude as the main factor in the model. The Wilcoxon rank test that was signed for paired samples was used to analyze the differences in the concentration of each parameter analyzed in each experimental animal in different altitude groups. All statistical analysis procedures were performed with the SPSS statistical software for Windows (Version IBM 21; SPSS Inc., Chicago, IL), with significance level was set to $P < 0.05$ for all tests.

3. Results

The maintenance of dairy cattle at different altitudes has an impact on its physiological response. Altitude causes a difference in heart rate. A high heart rate causes a higher potential for cardiovascular cellular damage compared to a dairy cow with a normal heart rate. In Table 1, showed the response parameters associated with the physiological function of the heart of a dairy cow.

In Table 1, showed that the all cardiovascular parameters, included of CRP high sensitivity, H-FABP, homocysteine, and γ-Glutamyl Transpeptidase in

Table 1. The response of dairy cattle to biomarkers of heart failure based on the altitude of the maintenance site

Cardiovascular biomarkers	Altitude		
	300 to 500 m a.s.l.	600 to 900 m a.s.l	>1,000 m a.s.l
CRP high sensitivity (mg/l)	18.83 ± 1.34^{a}	10.73 ± 1.03^{b}	9.68 ± 1.04^{c}
H-TFABP (ng/ml)	7.24 ± 1.01^{a}	5.62 ± 0.62^{b}	4.17 ± 0.13^{b}
Homocysteine (µmol/l)	16.52 ± 3.06^{a}	15.84 ± 5.04^{a}	9.23 ± 0.16^{b}
γ-Glutamil transpeptidase (IU)	48.41 ± 2.57^{a}	26.46 ± 3.07^{b}	23.93 ± 2.02^{b}
sPLA2-IIA (ng/dl)	89.05 ± 3.04^{a}	46.81 ± 2.07^{b}	45.19 ± 3.38^{b}

CPR high sensitivity = C-reactive protein, H-TFABP = Heart-type fatty acid-binding protein, sPLA2-IIA = Secretory phospholipase-A2-IIA

[a,b,c] Means in the same row with a different letter of superscripts are significantly different ($p < 0.05$), values are given in Mean ± SD

Frisian Holstein dairy cows expressed higher levels (P <0.05) at low altitude site (300-500 m a.s.l.) than dairy cows at altitude sites higher (600-900 and >1,000 m a.s.l.). In Table 1, also showed that the expression of cardiovascular biomarkers in dairy cows at an altitude of 600-900 m a.s.l. with >1,000 m a.s.l. were not significantly different (P >0.05), except CRP high sensitivity and homocysteine level higher significantly different (P <0.05) at >1,000 m a.s.l. than 600-900 m a.s.l.

The response of dairy cows on lipid regulation at different altitude site was showed in Table 2. lipid transport protein levels include types of apolipoprotein, HDL, and LDL in the group of the dairy cow at an altitude of 300-500 m a.s.l., showed higher levels, significantly different (P <0.05) compared to the dairy cattle groups at the other two altitudes. Lipid degradation appears to be increased in dairy cows that were kept at low altitude locations (300-500). This phenomenon was indicated by a decrease in triglyceride levels and an increase in NEFA levels in their blood plasma.

Table 2. The response of dairy cattle to lipid regulation based on the altitude of maintenance site

Lipids regulation	Altitude		
	350 to 650 m a.s.l.	700 to 1,000 m a.s.l	>1,050 m a.s.l
Adiponectin (µg/ml)	11.03±0.44^{a}	9.25±0.25^{b}	9.31±0.09^{b}
Apolipoprotein A-I (g/l)	2.68±0.19^{a}	2.01±0.14^{a}	1.98±0.11^{b}
Apolipoprotein A-II (g/l)	2.81±0.89^{a}	2.13±0.78^{a}	1.03±0.08^{b}
Apolipoprotein B (g/l)	1.93±0.08^{a}	1.16±0.07^{a}	1.13±0.08^{a}
Apolipoprotein C-II (g/l)	2.67±0.11^{a}	2.52±0.09^{b}	2.47±0.81^{b}
Apolipoprotein C-III (g/l)	2.18±0.59^{a}	1.74±0.60^{a}	1.72±0.09^{a}
Apolipoprotein E (g/l)	2.88±0.08^{a}	2.46±0.07^{b}	2.39±0.11^{b}
Cholesterol HDL (mg/dl)	89.35±3.04^{a}	76.84±3.05^{b}	74.16±2.96^{c}
Cholesterol LDL (mg/dl)	101.67±4.06^{a}	98.73±3.95^{b}	91.38±4.02^{c}
Cholesterol total (mg/dl)	191.78±5.02^{a}	187.36±7.14^{a}	174.92±4.02^{b}
Triglycerides (mg/dl)	268.61±5.07^{a}	282.47±5.62^{b}	283.15±5.25^{b}
NEFA (mg/dl)	78.81±4.06^{a}	68.25±3.26^{b}	68.07±4.13^{b}

HDL = high density lipoprotein, LDL = low density lipoprotein, NEFA = non esterified fatty acid

^{a,b,c}Means in the same row with a different letter of superscripts are significantly different (P <0.05), values are given in Mean ± SD

The results in Table 1, as a whole also showed that the rate of lipid regulation was higher significantly different (P <0.05) in dairy cows at low altitude locations than in dairy cows at high altitude locations. The specific proteins which function as lipid transport were generally not different (P >0.05) in the group of dairy cows at altitude locations of 600 to 900 and >1,000 m a.s.l.

4. Discussion

4.1. Cardiovascular

The altitude is closely related to macroclimates, including temperature and humidity. The housing temperature environment above the comfort zone or upper thermoneutral zone causes an increase in the pacemaker rate. A high pacemaker rate every day in dairy cows has an impact on increasing cardiovascular cellular damage. Compound markers of heart damage have been reported, among others with CRP (Zhu *et al.* 2017). C-Reactive Protein (CRP) is a protein that is released by the liver and is produced in large amounts during infections and heat stress (Zhao *et al.* 2017). Conversely, in inflammation that occurs in the process of developing atherosclerosis, an increase in CRP concentration is much smaller (Wheeler *et al.* 2014; Xu *et al.* 2015). Nevertheless, the increase is quite significant when compared to normal conditions.

High-sensitivity C-reactive protein (hs-CRP) measures the low amount of CRP in the blood. In this study, this test was used to determine the risk of heart problems, especially those that were combined with other risk factors such as cholesterol (Mohammed *at al.* 2015), age (Kayadoe *et al.* 2019), blood pressure (Song *et al.* 2015; Tanuwiria *et al.* 2022). This test was used in this investigation to find out the effect of an increased risk of sudden on cardiac problems of the dairy cow, such as heat stress. Although, the relationship between high CRP levels and heart disease risk is not well understood (Kou *et al.* 2016).

Homocysteine is a natural amino acid, which, when in high levels in the blood, can increase the risk of clogged arteries (arteriosclerosis). This condition is known as hyperhomocysteinemia (Gonzalez-Rivas *et al.* 2016). Based on the result of this investigation showed that dairy cow with high homocysteine levels triggers arterosclerosis in veins, such as deep vein thrombosis (Anderson *et al.* 2013; Cheng *et al.* 2014; Fournel *et al.* 2017) and pulmonary embolism or in the arteries (Ikewaki 2014). It is known that a high amount of homocysteine can damage the lining of blood vessels. This damage can cause arterosclerosis. The report of previous studies also showed a close relationship between heat stress and homocysteine levels (Kubow *et al.* 2015; Kang *et al.* 2016; Maskal *et al.* 2018).

Many previous studies have shown that biological markers of phospholipase A2 (PLA2) have been found to play an important role in the inflammatory pathway (Kang *et al.* 2016; Lee *et al.* 2017; Jae-Sung *et al.* 2020). PLA2 is classified in the acute phase protein group. PLA2 reported triggers the host's inflammatory response to infection (Pruzanski and Vadas 1991). As an inflammatory mediator, the release of arachidonic acid from the phospholipid membrane of cells catalyzed by intracellular PLA (Leach and Cowen 2014), thereby initiating prostaglandin and leukotriene synthesis (Johnson *et al.* 2015). As a result, this stimulated an increase in many physiological responses in animals, such as vasodilation in the heat-stressed dairy cow (Geraert *et al.* 2016), inhibition of platelet aggregation (Bertocchi *et al.* 2014).

The release of sPLA2-IIA in low altitude or high temperature can be induced by several specific proteins among other inflammatory cytokines, such as the group of interleukin (interleukin IL-6, IL-1β, and also tumor necrosis factor/TNF-α). These proteins were key factors in the process of neutrophil adhesion and migration (Jiangjing *et al.* 2019). While the exact role of SPLA2-IIA for livestock is still being discussed. Although, several studies have shown small clinical trials that sPLA2-IIA plasma levels show a positive correlation with stress (Lambartz *et al.* 2014; Roland *et al.* 2016; Mushawwir *et al.* 2021a).

The H-FABP biomarker of heart in this study was analyzed. Zhao *et al.* (2017) showed that heart-FABP is low molecular weight (15 kDa) cytoplasmic protein found in high concentrations in cardiac muscle tissue. Heart-FABP is released from the liver during cell necrosis faster than other markers (Zhu *et al.* 2017; Hernawan *et al.* 2017; Mushawwir *et al.* 2021b). Its small size and its location in the cytoplasm, it can be expelled rapidly into the bloodstream after damage to the heart muscle. Plasma H-FABP levels not only rise early but normalized after 24 hours it is possible to detect recurrent myocardial infarction (Slimen *et al.* 2016). The advantage of H-FABP was dominant in the early stages of myocardial infarction. The initial combination of H-FABP after the onset of symptoms of discharge, rapid screening of the kidneys from blood circulation, and high cardiac characteristics shows strong power for diagnostic tools that are useful in early detection of heart muscle damage.

4.2. Lipid Regulation

Biochemical efforts of dairy cattle to achieve homeostasis under heat stress conditions required a lot of energy. During the thermoregulation process for dairy cows at low altitudes, the site was a complicated chemical pathway in the tissue cells. In this intricate problem, dairy cows reduce feed intake to avoid metabolic heat production (Qu and Ajuwon 2018), but at the same time, the dairy cow also required high feed intake to produce milk. The involvement of lipid metabolic pathways was an alternative supply of energy precursors.

Apolipoprotein or apoprotein is known as a protein group in lipoprotein. The function of apolipoprotein is to transport fat into the blood (Sato *et al.* 2016; Adriani and Mushawwir 2020) because fat is not soluble in water, then the way it is transported in water-based blood, this fat will be bound by a protein which then forms a complex called a lipoprotein that can mix with water. Sierra-Johnson *et al.* (2009) reported that apolipoprotein consists of apolipoprotein A-I, A-II, B, C-I, C-II, and E. Apolipoprotein B (apo B), showing protein structure for particles atherogenic, VLDL, IDL, LDL, small dense LDL (sdLDL). Whereas apo A-I is the main structural protein for HDL and reflects the atheroprotective side of lipid metabolism (Yew Tan *et al.* 2015; Kamil *et al.* 2020). Both of these apolipoproteins can also indicate cardiovascular risk more accurately than LDL-C and another lipid. The apoB/apo A-I ratio is strongly associated with the risk of myocardial infarction (MI).

The level of apolipoprotein E (apoE) based on the result in this study showed also increased. This lipid transport was a protein constituent of plasma lipoprotein which has several functions including its role in cholesterol metabolism (Zahner *et al.* 2004) and as an important ligand in lipoprotein clearance (Kohler *et al.* 2013). Apolipoprotein E was first identified as a constituent of very-low-density lipoprotein (VLDL) that functions as triglyceride transport from the liver to peripheral tissue (Quispe *et al.* 2015).

The correlation of the lipid regulation with heart physiologic, reported by Zahner *et al.* (2004), demonstrated an associated lipid regulation with biomarkers of heart failure. In cattle, CRP levels increase with increasing age, indicating that high levels of apoE occur before inflammation occurs (Jiang *et al.* 2019; Tanuwiria *et al.* 2020). Previously investigated also showed that the biological activity of apoE can be influenced by modifications to its structure and or quantity (Emoto *et al.* 2013;

Chmielewski 2018). Structural changes can occur in the apoE polymorphism, which encodes apoE2, apoE3, and apoE4. Apolipoprotein E2 showed lower affinity to LDL receptors, resulting in apoE clearance which was slower and increases plasma apoE levels (Emoto *et al.* 2013; Quispe *et al.* 2015; Sato *et al.* 2016; Adriani *et al.* 2021). This situation will then be responded by regulating LDL receptors in the liver to reduce cholesterol levels. Apolipoprotein E4 was instead taken more efficiently, resulting in lower apoE levels and increasing cholesterol levels, and both related to altitude (Nasr and El-Tarabany 2017; Qu *et al.* 2018)

Therefore, variations in genetics that affect lipid metabolism will change the risk of cardiovascular disease. Although, heat stress enhanced lipid metabolism effectively. The degradation of triglycerides showed by the decrease in plasma triglycerides into NEFA, based on the results of this study, illustrated that lipid regulation was an efficient alternative to supply energy precursors.

Adiponectin was a good indicator for estimating the complications of metabolic syndrome. Many studies show the use of adiponectin in the body as a marker for metabolic syndrome (Cruzen *et al.* 2015; Catapano *et al.* 2016; Mushawwir *et al.* 2020). Decreased plasma adiponectin (hypoadiponectinemia) is associated with an increase in Body Mass Index (increased incidence of obesity) (Berry *et al.* 2002), decreased insulin sensitivity (increased incidence of diabetes) (Gornaik *et al.* 2014), unwanted fat profile, and increased risk of heart disease in claves (Roland *et al.* 2016).

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