

Analysis of Temperature-Humidity Index, HSP70, and Cortisol Levels in Tropical Holstein Friesian Crossbreed Dairy Cows

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Abstract

Holstein Friesian crossbred dairy cattle are a breed of cattle farmers in Indonesia widely raised. Indonesia's tropical climate is increasing milk productivity, and reproductive productivity in Holstein Friesian crossbred dairy cattle. The health status of dairy cattle in the livestock area can be determined by measuring and turning on levels of THI, HSP70 protein, and cortisol hormone. High environmental temperature and humidity can trigger heat stress in dairy cattle. In heat stress conditions, dairy cattle will show a decrease in milk productivity, reproductive disorders occur, and decreased food intake. Environmental conditions are known by measuring the temperature Humidity Index (THI) using a thermometer, hygrometer, and altimeter. The levels of HSP70 and cortisol hormone in the blood are high if dairy cattle get stressed. HSP70 and cortisol hormones are indicators of stress in livestock that can be measured using the Enzyme-linked immunosorbent assay (ELISA). This book chapter will explain THI as a parameter of heat stress, the structure and function of HSP, the role of HSP70 in heat stress, the measurement of HSP70 and cortisol levels, and the impact of cortisol regulation on dairy cows experiencing heat stress.

Keywords: Cortisol, HSP70, PFH cattle, Temperature-Humidity Index

Cite this Article as: Madyawati SP, Srianto P, Sindhuranu IWA, Shober ABQ, Prastiya RA, Kholik K, Pradana M, Haddar MA and Munawarroh M, 2025. Analysis of temperature-humidity index, HSP70 and cortisol levels in tropical holstein friesian crossbreed dairy cows. In: Kausar R, Nisa ZU, Jamil M and Bashir I (eds), Integrated Health and Sustainability: Plants, Wildlife, and Genetic Resilience. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 97-102. <https://doi.org/10.47278/book.HH/2025.68>



A Publication of
Unique Scientific
Publishers

Chapter No:
25-014

Received: 12-Jan-2025
Revised: 05-March-2025
Accepted: 12-May-2025

Introduction

Dairy cows raised by Indonesian farmers are generally Holstein Friesian crossbreed dairy cows, which have undergone extensive crossbreeding and are commonly known as crossbreed dairy cows. The difference in climate and temperature between Indonesia and the cattle's country of origin leads to lower milk production (Atabany et al., 2011). Low milk production is also caused by the lack of knowledge of farmers about the effect of climate and temperature on reproductive activity, which has an impact on low reproductive efficiency (Sudrajad et al., 2018). Low reproductive efficiency is an indication of the disorders. The reproductive disorders that often occur in cow farms are the case of repeat breeding, which is a condition in female cows that are not pregnant after being inseminated more than twice or more with a normal breeding cycle (Pothmann et al., 2015). Generally, repeat breeding in Holstein Friesian crossbreed dairy cows is characterized by 18 to 24 months of calving interval, the conception rate is less than 40%, and service per conception is more than 2 (Prihatno et al., 2013).

Factors causing repeat breeding include endocrine dysfunction, the environment in this case temperature and humidity, poor nutrition, and mismanagement that will cause fertilization failure and result in early embryonic death (Yaginuma et al., 2019, Prihatno et al., 2013). Geographical differences such as differences in the altitude of dairy cow rearing locations above sea level will cause differences in weather and microclimate, especially temperature and humidity (Mulia et al., 2011). The correlation of air temperature and humidity is called the Temperature-Humidity Index (THI). THI can illustrate the heat stress level of dairy cows (Bernabucci et al., 2014). Effective air temperature and humidity indices are environmental factors that affect the welfare and reproductive performance of dairy cows (Kargar et al., 2015). THI more than 72 can cause the cow to stress.

One of the proteins that can be used as an indicator of stress that can be observed in dairy cows is the heat shock protein (HSP) of 70. HSP is produced by the heat shock response (HSR). HSR expression is due to a genetic-based response that appears to induce genes encoding

molecular chaperones, proteases, and other proteins that function in recovery and defense mechanisms against cellular injury associated with protein misfolding (Qureshi et al., 2023). HSP70 at the transport of intracellular by maintaining proteins in an inactive form and preventing protein degradation (Zang et al., 2022). HSP 70 has been in Holstein Frisian dairy cows with weather differences ranging from 536.88 to 1532.04 ng/mL (Pathirana et al., 2022).

Heat stress also responded to by the body of dairy cows will bully the hormone cortisol produced by the adrenal glands. Stress suppresses reproductive function through inhibition of the hypothalamic-pituitary-gonadal pathway by inhibiting the release of gonadotropin-releasing hormone (GnRH). Cortisol inhibits the secretion of GnRH thus decreasing the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the ovaries (Narayan and Parisella, 2017). Based on the literature study, the measurement of the THI, HSP70, and cortisol levels in Holstein Frisian crossbreed dairy cows is essential as an indicator of stress levels which can have an impact on reducing cow production and reproduction. This chapter will explain THI as a heat stress parameter, the structure and function of HSP, the role of HSP70 in heat stress, HSP70 and cortisol levels measurement, and cortisol regulation in dairy cows in heat stress.

Temperature Humidity Index as a Parameter of Heat Stress

Heat stress is a condition in which animals are unable to release heat from the body that is needed to maintain the body's thermal balance (Rasool et al., 2023). Heat stress in cows can analyzed by temperature and humidity index (THI). THI is an indicator that can be used to determine and evaluate the environmental temperature conditions of dairy cattle farms. THI is the result of a combination of temperature values and humidity values (Bernabucci et al., 2014). Many studies have shown a correlation between THI, heat stress, and milk production in dairy cattle. High milk production in cows can result in a decrease in output in THI conditions reaching 68 (Collier et al., 2012). Osei-Amponsah et al. (2020) also reported that an increase in THI resulted in a decrease in daily milk production of 14%. Meanwhile, Wildridge et al. (2018) stated the clinical symptoms seen in dairy cattle in moderate and high THI conditions are panting with an open mouth and hypersalivation. Measurement of THI was carried out using a room thermometer left in the cage for 3-5 minutes. Measurement of humidity using a hygrometer placed inside the cage for 3-5 minutes. Measurement of area altitude using an altimeter. Calculation of THI according to Categories of Livestock Weather Safety Index Associated with THI based on Collier et al. (2012) (Figure 1).

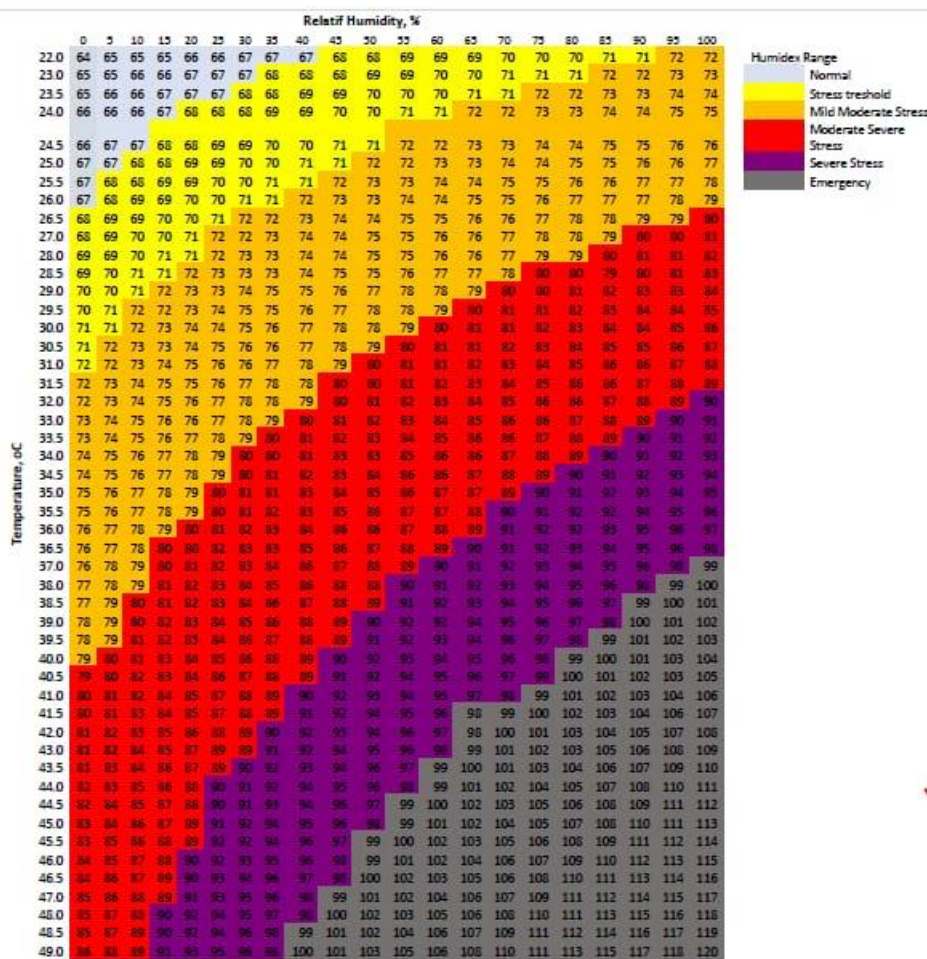


Fig. 1: Temperature-Humidity Index (THI) chart

Structure and Genetic Variant of HSP (70) in Dairy Cow

High environmental temperatures require animals to be able to adapt to continue living. The body's mechanism due to high temperatures is the ability of cells to release a protein that is named HSP. HSP can be identified in cells of animals, plants, and microorganisms (Hasan et al.,

2019). HSP has been divided into 5 families based on the weight of the molecule. Proteins that have 70 kDa of molecular mass are called HSP70 which is highly conserved. The structural components of HSP70 are a nucleotide-binding domain (45 kDa), a substrate-binding domain (15 kDa), and a C-terminal cap region (Yu et al., 2015). HSP70 has been identified in cattle are HSP70-1, HSP70-2, HSP70-3, and HSP70-4 (Gade et al., 2010). Genetic heterogeneity among each animal can result in varying thermal stress tolerance capabilities. The systems of physiological control with thermoregulation gene are necessary to understand in order genotype-environment interactions (Hariyono, 2022).

The Function of HSP70

HSP70 can involve in protecting cells against heat stress and is required to control the immune system to establish protein folding, HSP70 also regulates the apoptosis of cells. The main functions of the HSP70 protein include the following:

1. HSP70 as Heat Tolerance

HSP70 is involved in heat tolerance in cells and protects the denaturation of polypeptides through the stress of heat. Hot temperatures cause heat stress, which can interfere with the ability of cells to carry out the processes of material transport, replication of DNA, the transcription of DNA, and synthesis of polypeptides. The presence of stress can cause protein misfolding at the level of cellular. HSP is active during stress conditions to prevent the accumulation of denatured or abnormal proteins in cells. Bhat et al. (2016) found that this approach increases the survival of cells through oxidative and stress thermal. Hot environmental temperature conditions will stimulate HSP70 expression in cells by binding damaged proteins and then preventing them from accumulating in cells. Proteins that aggregate due to heat will cause significant problems in cells; therefore, HSP70 is connected to provide heat tolerance of cells (Mishra and Palai, 2014).

2. HSP70 as a Molecular Chaperone by Protecting Cellular Compartments from Thermal Stress

HSP70 protects from destroying newly formed polypeptides by proper folding of the protein. The protection on HSP70 activity is helping the folding of non-native proteins are: helping the folding of the protein to the native state, avoiding aggregation, and solubilizing and refolding of protein aggregations. These activities are utilized to monitor the integrity of misfolded proteins and to fold newly formed polypeptides after translation. HSP70 prevents non-native protein aggregation via binding to a repellent region of water. This function is accommodated by the domain-J protein chaperone. In addition, HSP70 contributes to inefficient or irreversible protein cleavage (Yakota and Fuji, 2010).

3. HSP70 as Cellular Apoptosis

Apoptosis is a process to maintain homeostasis of tissue and remove abnormal cells. HSP70 regulates apoptosis by interacting with various proteins involved in the apoptotic pathway, modifying various signaling cascades affecting various steps in the pathway, and helping cells survive after exposure to harmful stimuli. (Rakib et al., 2024). The mechanism of HSP70 prevents apoptosis by inhibiting caspase activation and inhibiting mitochondria to secrete cytochrome C (Beere and Green, 2001).

The Role of HSP70 In Cattle During Heat Stress

Hot conditions of the environment will cause livestock to experience stress, which has a negative impact on productivity and reproduction. Livestock needs a body defence mechanism to reduce stress to maintain physiological balance. Heat stress due to environmental conditions can cause decreased production of milk and problems of cow health (Bernabucci *et al.*, 2014). Around 35°C of environmental temperature has been triggered by stress in dairy cows (Berman, 2005). The presence of stress in animals due to extreme environmental temperature conditions will cause an HSP70 expression to increase, which acts as the first line of defence to reduce heat stress (Page et al., 2006). The body's response to increased environmental temperature will induce the expression of HSP genes, including HSP70, which has regulatory pathways for cell responses. In addition, the HSP70 gene was induced by responses to the physiological and stress of thermal (Hariyono *et al.*, 2022). HSP70 also acts as a biomarker of acute and chronic heat stress in cows (Gaughan et al., 2013). HSP70 levels in animal blood have been identified as a biomarker of cellular heat tolerance that is correlated with stress. In addition, to increasing protein integrity, HSP70 can also prevent cell death (Min et al., 2015). HSP70 expression can indicate an increase in body temperature above 38.6°C.

Measurement of HSP70 Level

Dairy cattle blood serum can be tested for HSP70 levels with Sandwich Enzyme-linked immunosorbent assay (ELISA). The ELISA product used a Bovine HSP70 ELISA kit. The ELISA examination procedure was as follows: 100µL sample mixture was correctly added into the wells and incubated at 37°C for 90 minutes.

After that, the well was washed, labeled with antibodies, and then incubated at 37°C for 60 minutes. After that, the well was washed and then labeled by an antibody with wash buffer, HRP-Streptavidin Conjugate of each well, and then incubated. Then, the plate was washed with wash buffer and leave the wash buffer in the wells for 1-2 minutes. Then, 90µL TMB substrate was added to each well, and incubated at 37°C in the dark for 10-20 minutes. At the end, 50 µL of stop solution was added to each well. The color will immediately turn yellow. The addition of the stop solution should be the same as the TMB substrate solution. The Optical densities of the samples test and standards kit were read at 450 nm using a microplate reader.

The Impact of Cortisol Hormone Regulation on Dairy Cows Experiencing Heat Stress

Cows with high stress during the estrus period will affect the secretion of the hormone ACTH, the ACTH will control in the release of cortisol in the cortex adrenal (Ferro et al., 2010). High levels of the hormone cortisol can hurt endocrine, immune, and neurological functions critical to animal health and productivity (Thau et al., 2023). High cortisol levels in cows suffering from repeat breeding will inhibit the secretion of GnRH and LH from the hypothalamus so that follicular growth is inhibited due to a lack of Adenosine Triphosphate (ATP) which functions

to activate cyclic Adenosine Monophosphate (cAMP) as an intracellular messenger. Besides that, cows that are undergoing repeat breeding will have an impact on their production. Zhao et al. (2019) stated that heat stress in dairy cows will give negative feedback to the productivity of milk.

Gonadotropins play certain functions in the ovaries such as regulating growth and ovulation follicles and also the development of the corpus luteum (Wolfenson and Roth, 2019). The presence of stimuli such as heat stress will change steroid levels so that there will be adverse effects on ovarian function. As a result, with higher progesterone levels, LH secretion is inhibited, ultimately disrupting the growth of dominant follicles and oocyte fertilization (Khan et al., 2020). In addition, the direct effect of heat stress is that suppressed follicle dominance results in decreased plasma inhibin levels and consequently higher FSH concentrations (Roth et al., 2000). The mechanism of high temperature-induced heat stress, release of cortisol, and effects on ovarian function in cows can be seen in Figure 1. Figure 1 is a modification figure from Lemal et al. (2021) and Naranjo-Gómez et al. (2021).

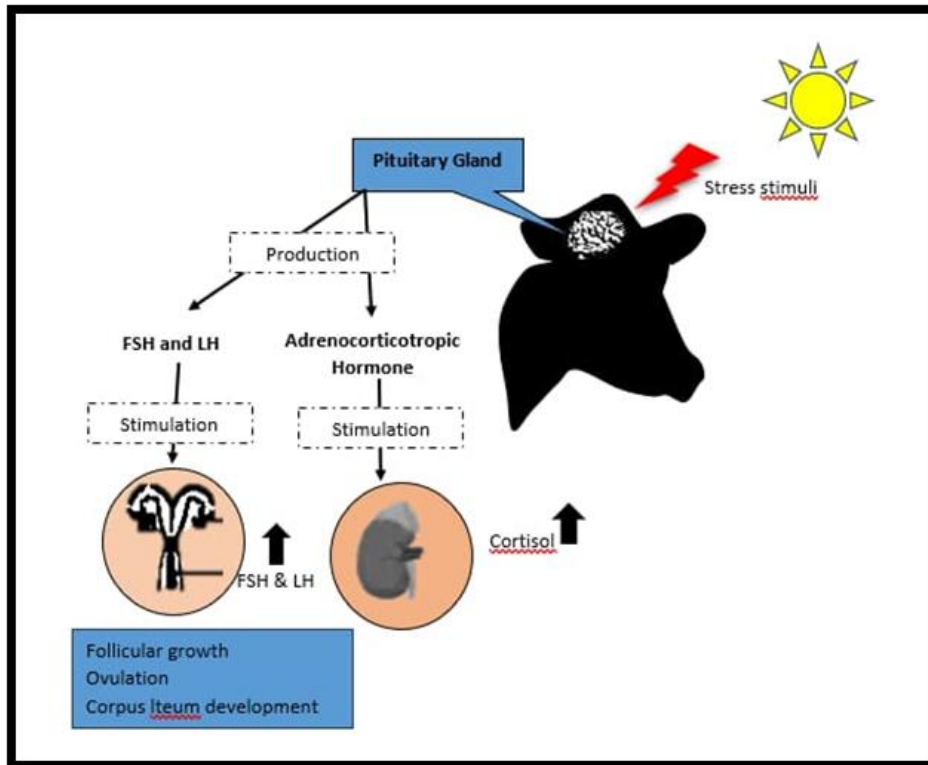


Fig. 1: Mechanism of high temperature-induced heat stress, release cortisol, and effects on ovarian function in the cow

Measurement of Cortisol Level

Cortisol levels can be measured by the ELISA method from the serum of Holstein Friesian crossbred dairy cows. The kit used is Bovine cortisol (COR). The procedure works as follows: Microplate wells were marked according to the sample number, then 50 uL of each standard control was added according to the numbering, and 40 uL of sample serum was added to the wells according to the numbering, the next step was added 10 uL of cortisol anti-antibody and 50 uL of enzyme conjugate into each well and then closed. To mix well, shaker mixing was done for 10 seconds. Next, incubate for 60 minutes at 37°C, after the incubation period is complete, immediately throw out the contents of the well. Then rinse the microplate with washing solution 5 times, then 50 uL of substrate solution is added to each well and incubated for 10 minutes at room temperature. The final stage is to add 50 uL of stop solution to each well to stop the enzymatic reaction. Wait for 30 minutes and read the results using an ELISA Reader at Optical Density (OD) of 450 nm.

Holstein Friesian Crossbred Dairy Cows

The use of some cattle breeds to adapt to tropical weather is now problematic because of the inverse link between environmental temperature heat tolerance and productivity level (Renaudeau et al., 2012). Milk and meat production is important to increase in tropical areas for the next few years so it is important to choose a breed of cattle that is suitable for tropical areas. Certain breeds of cattle developed in tropical areas must be able to adapt to the environment and feed. This is intended to improve the quality and digestibility of livestock for the consumption of various plants that are resistant to heat and drought (Hernandez-Castellano et al., 2019). Tropical areas have a climate that is less suitable for European cattle because of the different characteristics of livestock and areas of origin. One way to increase livestock production in hot areas is by crossbreeding. Crossbreeding can be done by mating high-producing European breeds with local breeds that are resistant to heat (such as Holstein Friesian Crossbred Dairy Cattle).

Conclusion

HSP70 has an important role in determining the condition of Holstein Friesian Crossbred dairy cows when experiencing heat stress on farms. This protein can function as heat tolerance in cells. HSP 70 also can protect polypeptides from denaturation during heat stress and as

a molecular chaperone by protecting cell compartments from thermal stress. An increase in HSP70 is associated with increasing cortisol levels as the body responds to heat stress due to increased environmental temperature. The negative impact of the hormone cortisol high level is that it causes cows to experience repeated mating and decreased milk production. Therefore, maintaining the environmental conditions of Holstein Friesian Crossbred dairy cows needs to be done to anticipate heat stress which can result in decreasing productivity. Improvement of the cage management and handling of repeat breeding cases in Holstein Friesian Crossbred dairy cows is essential to reduce stress which can impact the production and reproduction of these cows.

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