Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 1, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF DRIED PROBIOTIC ON LIVER GLYCOGENOLYSIS PATHWAY THE END OF THE FINISHER PHASE OF HEAT STRESSED LAYING HENS

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Abstract

Homeothermic livestock such as laying hens have been widely developed and cultivated throughout Indonesia. This group of animals physiologically has a system that is able to maintain a normal body temperature range of 40-42°C. One of the strategies used to reduce the impact of heat stress is the provision of natural feed additives, namely probiotics containing a consortium of bacteria. An experiment was conducted on forty laying hens, which were kept in cages with heat stress. Probiotic feeding was done by dividing four groups of hens each. A probiotic level of 4% is the most effective level. This means that heat stress in laying hens can be overcome by giving probiotics. Probiotics have an important role in preventing changes in the osmotic pressure of body fluids, so that overall, they can overcome metabolic changes associated with heat stress.

***Key words**: heat stress, laying hens, probiotics.*

INTRODUCTION

Indonesia is one of the countries with the highest layer population and egg production in ASEAN. In addition, the high population causes the demand for eggs to increase every year. The biggest challenge for layer productivity in Indonesia is the high ambient temperature (average from 28 to 34°C). Laying hens are able to express their genetic potential in a comfortable environmental zone or commonly called the *thermoneutral zone*, which is 21-25°C.

The impact of temperature has been widely reported by previous researchers. An increase in temperature up to 10°C from the normal threshold causes thermoregulation and hyperthermia in laying yams, characterized by an increase in muscle creatine and HSP expression, up to a 50% decrease in production and a decrease in shell thickness and an increase in the proportion of shell cracks (Mushawwir & Latipudin, 2011; Amin et al., 2014). Furthermore, it was also reported that this situation makes homeostasis very difficult to achieve, even with the provision of vitamins and low energy rations. It is more difficult to achieve optimum production, because in this situation, more energy is supplied to meet the needs of homeostasis processes (Al-Haidary et al., 2001). Many researchers have shown excessive concern in addressing the issue of energy balance under heat stress. The provision of energy through feed intake to help with thermoregulation, on the other hand, has an impact on the incidence of heat increment.

On the other hand, an enhanced risk of DNA mutation and denaturation of body proteins including hormone peptides, cell transporters, and enzymes, among others, is likely to be induced by excessive heat exposure. Other studies have shown that increased free radical production can be induced by heat stress and ultimately result in protein translational failure (Slimen et al., 2016; Mushawwir et al., 2021, 2024). This condition is directly related to metabolism, especially to the shock of energy production in mitochondria, as well as excessive redox activity.

This situation, described in the previous paragraph, causes metabolic regulation to meet energy, to undergo major changes and even accompanied by energy requirements to maintain these alternative mechanisms. The basic mechanism that animals can utilize is carbohydrate catabolism, through the breakdown of glycogen into glucose by the phosphorylation pathway. This catabolic reaction is known as glycogenolysis. Activation of adenylyl cyclase leads to the induction of protein kinase in catalyzing glycogen into glucose and pyruvate (Peace et al., 2013; Mushawwir et al., 2021) either with the help of oxygen (aerobic glycolysis), or without oxygen (anaerobic glycolysis).

An approach presented in this paper to reduce the impact of heat stress is to provide *feed additive*, namely probiotics containing bacterial consortia (Adriani et al., 2023; 2024). Probiotics are non-pathogenic bacteria that have active peptides and active compounds (Adriani et al., 2015; 2020; 2021; Antosiewicz et al., 2006; Tanuwiria et al., 2022). Probiotics in the ration are expected to be able to create better ecological conditions for digestion, especially in the ileum. The microbial consortium should be able to contribute positively to the physiological and biochemical conditions, both of the villi and metabolism as a whole. Improvements in the condition of the villi and metabolism should be able to have a positive impact on the ability of layers in the final phase of production to be able to cope with the heat exposure experienced by reducing the rate of glycogenolysis.

MATERIALS AND METHODS

Animal Samples

In this study, a sample of 70-week-old laying hens, totaling 40 layers, was used. Experiments with the administration of dry probiotics at the Poultry Experimental Center, Faculty of Animal Science, University of Padjadjaran. Chicken samples were placed in individual boxes with battery cages, respectively. These battery cages were placed in a semi-close house system. Each chicken sample received basal feed, with a crude protein content of 20.15%, metabolic energy of 2931 Kcal/kg feed. The microclimate of the housing was recorded during the study with the average temperature and humidity ranging from 37-38.5°C and 82-87%, respectively.

Experimental Design

A completely randomized design (CRD) was used in this experiment. The experimental chickens were distributed into four experimental groups (each consisting of ten sample chickens), namely without and with the administration of dry probiotics. Each experimental unit consisted of 1 laying hen. Treatment was applied from the start of the research. The four treatments applied were P0: Basal diet (BR) and no dry probiotics, and no heat stress; P1: BR contains dry probiotics (DP) 2% of the total ration, with a housing temperature of 38°C; P2: BR contains DP 3% of the total ration, with a cage temperature of 38°C; P3: BR contains DP 4% of the total ration, with a cage temperature of 38°C.

Experiment Protocols

Blood Sample Collected

Glycogenolysis rate analysis was performed by measuring metabolites in blood plasma. Blood samples were collected after 40 days of dry probiotic administration. A sterile thermo-branded syringe was used to draw 3 ml of blood through the flank vein (external pectoralis vein). The blood sample was placed in a sterile thermo-branded EDTA tube, with a gentle swing, the EDTA tube was shaken for approximately 30 seconds. The whole sample was placed in a cool box to prevent damage and blood clotting.

Sample Analysis

The external pectoralis vein is a vein that has been used as a vascular source of blood. Forty 3 mL syringes and 3 mL EDTA-coagulated venojecte tubes, respectively, were used to collect blood samples. The entire process was done carefully and sterile. Blood plasma was obtained by centrifuging each sample at 3500 rpm for 15 minutes. Plasma was removed from the blood cell solids, and then each sample was put into a 3 mL append of tube.

Plasma was used for the analysis of glycogen level and enzyme concentration. Spectrophotometric methods with various types of equipment have been used to examine blood samples. All analyzes were carried out based on analytical techniques based on the instructions in KIT Biolabo and KIT Rendox, following previous research protocols.

Statistical Analysis

Regarding the statistical analysis of the experimental results, the ANOVA (one-way analysis of variance) technique based on a one-way model with high homogeneity, and a significance degree of 95%, was applied in this study to evaluate the effect of probiotics.

All analyses were conducted following the analysis protocol by General Linear Models (GLM) of SAS Version 8.216, for a completely randomized design.

Differences between experimental groups were justified based on Duncan's test (SAS Institute, 2001).

RESULTS AND DISCUSSIONS

The impact of probiotics in the diet on the rate of glycogenolysis of laying hens reared in microclimatic conditions of cages in comfort zone bags, based on the current experiment is shown in Table 1. The average glycogen level of the experimental group of chickens without probiotics and placed in cages with temperatures above the comfort zone was 0.82 mg/dL (Table 1). Glycogen levels in this group appeared to be lower (P<0.05) compared to the experimental group of chickens given probiotics with the same heat exposure.

Table 1. The impact of probiotic administration on metabolite concentrations related to glycogenolysis in laying hens

Metabolites	Dried Probiotics			
	PO	P1	P2	P3
Glycogen (mg/dL)	0.82±0.12^{a}	1.02±0.11^{a}	1.29±0.13^{b}	1.41±0.12^{c}
Glycogen Phosphorylase (IU/dL)	0.22±0,01^{ab}	0.21±0,01^{b}	0.17±0,01^{bc}	0.16±0.01^{c}
Phosphoglucomutase (IU/dL)	0.39±0.02^{a}	0.18±0.03^{b}	0.10±0.02^{c}	0.05±0.02^{d}
Glucose 6-Phosphate (mg/dL)	0.27±0.01^{a}	0.23±0.01^{a}	0.13±0.01^{b}	0.08±0.01^{b}
Glucose 1-Phosphate (IU/dL)	0.37±0,01^{b}	0.19±0.01^{b}	0.14±0.02^{c}	0.07±0.01^{d}
Glucose 6-Phosphate (IU/dL)	0.27±0.01^{a}	0.17±0.01^{a}	0.11±0.02^{b}	0,07±0.01^{b}

Glycogen levels were higher with increasing levels of probiotics in groups P1, P2 and P3, at 1.02 mg/dL; 1.29 and 1.41 mg/dL, respectively. Investigations of laying hens maintained in temperature conditions above their comfort zone resulted in an increased energy requirement.

In an effort to reduce this impact, glycogen catabolism is a good alternative. The effectiveness of probiotic administration was seen in the group of chickens given probiotics as much as 4%. The decrease in plasma glycogen levels in this study, illustrates an indication that the heat stress exposed to the research chickens, urges the need for alternative energy availability for homeostasis (Al-Haidary et al., 2001; Latipudin & Mushawwir, 2010; 2011), as well as maintaining physiological stability (Slimen et al., 2016), increased glucose synthesis (Amin et al., 2014; Ao et al., 2010; Mushawwir et al., 2011; Rahmania et al., 2022).

Based on the results shown in Table 1, it can be explained that heat stress increases the metabolic rate mainly to provide energy related to the homeostasis process. This condition is characterized by enzyme activity related to the glycogenolysis pathway that increases in the treatment group without probiotics, then the effect of heat decreases on the activity of glycogenolysis with the administration of probiotics.

Homeostasis increases under heat stress (Aritonang et al., 2024), aiming to maintain biochemical and physiological processes for survival (Muhammad et al., 2023; Kharazi et al., 2022) and reproduction (Nelson et al., 2008; Loyau et al., 2014). Heat radiation to the internal milieu of livestock instinctively causes livestock to reduce their *feed intake* or ration consumption. This decrease aims to prevent heat from food digestion (*heat increment*) in the gut (Kumalasari et al., 2023; Ao et al., 2010) as well as metabolic heat (Mushawwir et al., 2018; 2020) and increased free radicals (Royer et al., 2016; Tanuwiria et al., 2011; Mushawwir et al., 2010; Mushawwir et al., 2011; Mushawwir et al., 2020). Animal activate the glucogenolysis mechanism to compensate for the decrease in feed intake. This is triggered by an increase in neural stimulants through neurotransmitters (Royer et al., 2016; Adriani et al., 2015), thus increasing levels of the hormone epinephrine (Slimen et al., 2016).

Probiotics were effective in reducing the rate of glycogenolysis under heat stress. The results (Table 1) showed that probiotics decreased ($P<0.05$) the intermediate metabolite compounds in the breakdown of glycogen to glucose, as well as the enzymes that catalyze the breakdown reactions.

The effectiveness of probiotic utilization appeared to be optimum at 4% (P3). The optimization of the role of probiotics in reducing the rate of glycogenolysis is shown by the glycogen levels of laying hens under heat stress conditions without probiotics, significantly different ($P < 0.05$) from the glycogen levels of laying hens experiencing heat stress but given 2-4% probiotics (P1-P3). This optimization was also shown by the levels of catalyzing enzymes and intermediate metabolite compounds, which showed differences with the experimental hen groups in heat stress, without and with probiotic feeding.

Overall, probiotics are effective in reducing the rate of glycogenolysis, suggesting that probiotics can improve metabolic balance (Aritonang et al., 2024; Al-Haidary et al., 2001; Mushawwir et al., 2018) related to heat regulation and homeostasis (Slimen et al., 2016; 2016). Continuous heat stress in high conditions causes *panting* behavior to evaporate body heat. *Panting* is the behavior of releasing heat through breathing by panting (breathing fast and short) or hyperventilating. This results in an increase in blood pH (Mushawwir et el., 2010; Adriani et al., 2021; 2023) compared to quail that are not exposed to excessive heat.

One important factor that plays a role in blood acidity is environmental temperature. The effect of high ambient temperature causes *panting* behavior. Through this behavior, the release of H_2O and CO_2 compounds becomes excessive (Mushawwir & Latipudin, 2011; Royer et al., 2016), causing the formation of bicarbonate (H_2CO_3) to decrease (Adriani et al., 2024; Mushawwir et al., 2024). Bicarbonate is an H^+ proton donor and forms carbonic acid (HCO_3^-). The ability of active peptides in probiotics with a feeding level of 2-4% to mitigate the effects of heat on the metabolic system of laying hens, confirms that probiotics have the ability to bind to proteins especially at the H atom of proteins, causing reduced protein denaturation (Pearce et al., 2013; Ao et al., 2010; Kharazi et al., 2022; Rahmania et al., 2022). This means reducing cell death and maintaining protein function (Royer et al., 2016; Adriani et al., 2024; Mushawwir et al., 2024). Both positive effects are simultaneously able to maintain proteins in the erythropoesis system and blood cell proteins (erythrocytes and leukocytes). The results of research by Pearce et al. (2013), showed the role of probiotic active compounds in maintaining blood precursor proteins from damage due to reactive compounds (ROS) and cellular level energy stress.

The role of active peptides in probiotics in controlling and overcoming heat stress is related to their ability to increase reaction kinetics with H_2O. The high binding energy of active peptides with H_2O makes it difficult to be evaporated and excreted through the kidneys, resulting in a decrease in body fluid loss. In addition, biomolecules present in blood plasma that are amphiphilic in nature lead to the formation of very favorable interactions with active compound carrying charged S and O atoms. This increases the pattern of electrostatic interactions. Mushawwir et al. (2018) state that these electrostatic interactions are able to maintain the structure of proteins, carbohydrates, and lipids to which they bind. Indirectly, it reduces the risk of heat-induced damage to biomolecules, and increases the capability of body fluids to retain heat.

In addition, the ability of the active peptides in these probiotics, either by enhancing the reaction kinetics with H_2O or by their electrostatic interaction patterns, both have an impact on heat stress management through enhanced body fluid adaptation (Royer et al., 2016). It directly impacts on increasing the retention of body fluid cations, especially Na^+ so as to maintain the osmotic pressure of body fluids. Good body fluid adaptation also maintains water retention so that extracellular fluid volume can be maintained.

CONCLUSIONS

The probiotic level of 4% was the most effective, so this means that heat stress in laying hens can be overcome by probiotics. Probiotics have an important role in preventing changes in the osmotic pressure of body fluids, so that overall it is able to cope with metabolic changes related to heat stress.

ACKNOWLEDGEMENTS

This research was supported by Universitas of Padjadjaran, through funding from the Padjadjaran Research Grant (HRU). The author also highly appreciates the role and assistance of all undergraduate and master students, as well as laboratory assistants and cage technicians who have been involved in this research.

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