



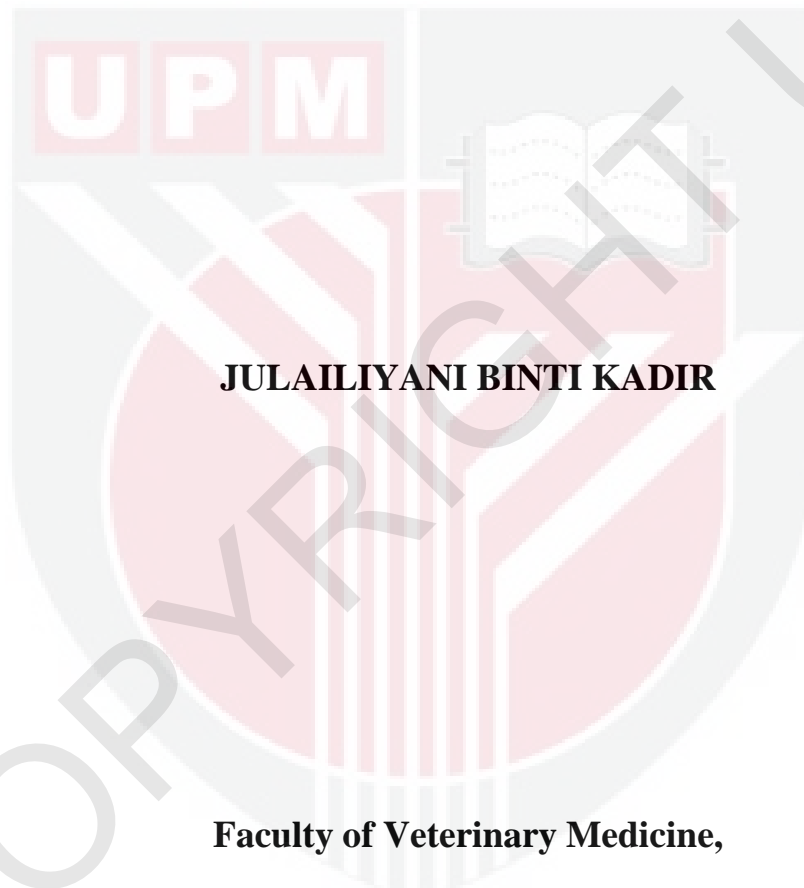
**UNIVERSITI PUTRA MALAYSIA**

***HAEMATOLOGICAL STRESS INDICATOR OF LAYER CHICKENS IN  
OPEN SIDED AND CLOSED HOUSE SYSTEMS***

**JULAILIYANI BINTI KADIR**

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FPV 2015 39**

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CHICKENS IN OPEN SIDED AND CLOSED HOUSE SYSTEMS**



**JULAILIYANI BINTI KADIR**

**Faculty of Veterinary Medicine,  
Universiti Putra Malaysia  
Serdang, Selangor Darul Ehsan**

2015



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**HAEMATOLOGICAL STRESS INDICATOR OF LAYER CHICKENS IN OPEN  
SIDED AND CLOSED HOUSE SYSTEMS**

**JULAILIYANI BINTI KADIR**

**A project paper submitted to the  
Faculty of Veterinary Medicine, Universiti Putra Malaysia  
In partial fulfillment of the requirement for the  
DEGREE OF DOCTOR OF VETERINARY MEDICINE**

**Universiti Putra Malaysia  
Serdang, Selangor Darul Ehsan**

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It is hereby certified that we have read this project paper entitled “Haematological Stress Indicator of Layer Chickens in Open Sided and Closed House Systems”, by Julailiyani Binti Kadir and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999-Final Year Project.

---

**DR. HAZILAWATI HAJI HAMZAH**  
**DVM (UPM), MSc (UPM), PhD**  
**(Murdoch)**  
Senior Lecturer,  
Faculty of Veterinary Medicine  
(Supervisor)

---

**PROFESSOR MOHAMED ARIFF**  
**OMAR**  
**BSc (LOUISIANA), MSc**  
**(OKLAHOMA), PhD (TEXAS)**  
Professor,  
Faculty of Veterinary Medicine  
(Co-supervisor)

---

**DR. SITI NUR AISYAH ISMAN**  
**DVM (UPM)**  
Technical Veterinarian,  
Commercial Poultry Farm  
(Co-supervisor)

---

**DR. HASLIZA ABU HASSIM**  
**DVM (UPM), MSc (UPM), PhD**  
**(Ghent)**  
Senior Lecturer,  
Faculty of Veterinary Medicine  
(Co-supervisor)

**DEDICATION**

I dedicate this paper to both my parents (Kadir Che Kasim and Fatimah Hamad), for their love and everlasting support that made me who I am today. To my siblings; my sister and brothers that inspire me to continue my study in tertiary education. To all my teachers and friends that help me all the way to here. Thank you very much.



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**LIST OF ABBREVIATIONS**

°C	Degree Celsius
AT	Ambient temperature
BCSPCA	British Columbia Society for the Prevention Of Cruelty To Animals
DVS	Department of Veterinary Service
HPA	Hipothalamus-Pituitary-Adrenal
H/L	Heterophil to lymphocyte
kg	kilogram
m/s	meter per second
RBC	Red blood cell
RH	Relative humidity
V	Velocity
WBC	White blood cell

**Abstract****Haematological Stress Indicator of Layer Chicken Raised In Open Sided and Closed House System****Julailiyani Binti Kadir****2015****Supervisor:****Dr. Hazilawati Binti Hamzah,****Co-Supervisors:****Dr. Siti Nur Aisyah Binti Isman, Prof Mohd Ariff Bin Omar, Dr. Hasliza Binti Abu Hassim**

Layer hens in Malaysia are raised in different types of housing, such as open sided house and closed house system. Previous studies have shown layer hens raised in different housing systems experienced different levels of stress. Thus, this study was conducted to evaluate the heterophil to lymphocyte ratio (stress indicator) of layer hens raised in open sided and close house systems. A total of 120 Lohmann Brown birds were chosen randomly and equally (n=20) from two housing types comprised of three open sided houses and three closed houses. Blood samples were obtained once from the birds for the determination of the heterophil to lymphocyte (H/L) ratio. The environmental parameters include temperature, relative humidity and air velocity were taken in the

morning, afternoon and evening on the same day of blood sampling. The heat stress index was calculated by the sum of the temperature and the relative humidity. During sampling, two of six houses, both were open sided houses, were not contracted with disease outbreaks (viral infection). Results showed that H/L ratio of unhealthy birds in the open sided houses was significantly ( $p < 0.05$ ) higher (1.113) compared to the healthy birds (0.646) reared in the similar house type. Meanwhile, the H/L ratio of unhealthy birds in the open sided house was significantly ( $p < 0.05$ ) higher (1.113) as compared to the unhealthy birds in the closed house system (0.836). Although the heat stress index for both types of houses was not exceeded the limit, which is  $107^{\circ}\text{C}$ , the mean value of air velocity in the open sided houses was significantly ( $p < 0.05$ ) lower (0.4 m/s) compared to closed houses (1.8 m/s). Hence, it is suggested that low air velocity in the open sided houses contributed to the ineffective heat regulation by the birds via convection, which lead to stress and caused H/L elevated. Results in this study showed that H/L ratio is a good haematological stress indicator in layer hens, and unhealthy birds particularly the one that reared in an open sided house had higher H/L ratio indicating a greater stress response.

Keywords: heterophil to lymphocyte (H/L) ratio, Lohmann Brown, heat stress index

**Abstrak****Penunjuk Tekanan Secara Haematologi oleh Ayam penelur Yang Dijaga dalam Reban Terbuka dan Tertutup**

Oleh

**Julailiyani Binti Kadir**

2015

**Penyelia:****Dr. Hazilawati Binti Hamzah****Penyelia Bersama:****Dr. Siti Nur Aisyah Binti Isman; Prof. Dr. Mohd Ariff bin Omar; Dr. Hasliza Binti Abu Hassim**

Ayam penelur di Malaysia dijaga dalam pelbagai jenis rumah, seperti rumah reban terbuka dan rumah reban tertutup. Kajian-kajian sebelum ini ada menyatakan bahawa ayam penelur yang dijaga mengalami tahap tekanan berbeza mengikut jenis penjagaan. Oleh itu, kajian ini dijalankan untuk mengkaji nisbah heterofil kepada limfosit (penunjuk tekanan) dalam ayam penelur yang tidak sihat untuk rumah reban terbuka dan tertutup. Sejumlah 120 ayam penelur jenis Lohmann Brown telah dipilih secara rawak dan dalam jumlah yang sama ( $n=20$ ) dari dua jenis rumah terdiri daripada tiga rumah reban terbuka dan tiga rumah reban tertutup. Sampel-sampel darah diambil sekali sahaja daripada ayam untuk mendapatkan nisbah heterofil kepada limfosit (H/L). Parameter

persekitaran termasuk suhu, kelembapan relatif, dan halaju udara telah di ambil pada waktu pagi, tengahari dan petang pada hari yang sama sampel darah di ambil. Indeks tekanan haba dikira dengan mendapatkan hasil penambahan suhu dengan kelembapan relatif. Semasa pengambilan sampel, dua daripada rumah reban yang kedua-duanya rumah reban terbuka, tidak dijangkiti penyakit (jangkitan virus). Keputusan menunjukkan nisbah H/L pada ayam yang tidak sihat di dalam rumah reban terbuka dengan signifikannya ( $p < 0.05$ ) lebih tinggi (1.113) berbanding ayam yang sihat (0.646) yang dijaga di dalam sistem penjagaan yang sama. Sementara itu, nisbah H/L pada ayam yang tidak sihat di dalam rumah reban terbuka dengan signifikan ( $p < 0.05$ ) lebih tinggi (1.113) berbanding kepada ayam yang tidak sihat di dalam sistem rumah reban tertutup (0.836). Walaupun indeks tekanan haba untuk setiap jenis rumah tidak melebihi had, iaitu  $107^{\circ}\text{C}$ , nilai purata halaju udara di dalam rumah reban terbuka dengan signifikan ( $p < 0.05$ ) lebih rendah (0.4m/s) berbanding rumah reban tertutup (1.8m/s). Oleh itu, adalah dicadangkan bahawa halaju angin yang rendah di dalam rumah reban terbuka menyumbang kepada ketidakberkesanan pengawalan haba melalui perolakan, yang menjurus kepada tekanan dan menyebabkan peningkatan H/L. Keputusan dalam kajian ini menunjukkan bahawa nisbah H/L adalah penunjuk tekanan secara hematologi yang bagus di dalam ayam penelur, dan ayam yang tidak sihat terutamanya ayam yang dijaga di dalam rumah reban terbuka mempunyai nisbah H/L yang lebih tinggi menunjukkan respon tekanan yang lebih besar,

**Kata Kunci:** nisbah heterofil kepada limfosit (H/L), Lohmann Brown, indeks tekanan haba

## 1.0 Introduction

Poultry farming has been established for many years. It is expanding in relation to rapidly changing technology. Poultry numbers have increased by more than 50% in the last twenty years due to increasing consumer demand. It includes layer, broiler, duck, turkey and quail, which is categorized into poultry meat and egg production industry.

In layer poultry farming, birds are reared either in natural or controlled environment confinements; open sided or closed housing systems, respectively. Both housing systems practice cage and cage-free egg production systems. The types of cages used in the cage egg production system are conventional and enriched cages, while for the cage-free egg production system the birds are either reared in deep litter or free ranged system. Layer hens reared in the different housing systems are experienced different stages and magnitudes of stress, which influence performance of egg production.

The key differences between open sided and closed housing systems are temperature, relative humidity and air velocity. Increase or decrease level of these parameters are closely related to heat stress in birds. Apart from heat stress, health status also influences level of stress in the birds. Stressed birds have high plasma corticosterone; resulted from release of glucocorticoid after activation of the hypothalamus-pituitary-adrenal (HPA) system. The glucocorticoid will enhance influx of heterophils into blood circulation and suppress lymphocyte proliferation, which lead to increase in the H/L ratio.

Hence, the objectives of this study are 1) to determine and compare the stress levels in layer hens reared in two different housing systems by evaluating the H/L ratio, and 2) to evaluate the usefulness of H/L ratio as a haematological stress indicator.



## **2.0 Literature Review**

### **2.1 Poultry farming**

Poultry broiler and layer farming in Malaysia are aimed to locally produce sufficient chicken meat and egg by ensuring the availability of the product supply, accessibility of product at retail shop, affordability of consumer to purchase product and to ensure the safety of product consumption (Ng et al., 2013). The demands is increased by years, for example 33.63 kg of chicken meat and 253 table eggs per capita was reported in year 2004 as compared to 46.60 kg and 315 in year 2013 (DVS Malaysia, 2013).

The poultry farming is categorized into breeder, broiler, layer, jungle fowl, duck egg and meat, and quail. The farms are specific for breeder, hatchery, broiler, layer, duck, and quail. Breed for broiler breeder breeds are Cobb, Ross, Arbor Acres, and Hubbard. While, layer breeder breeds are Lohmann Brown, Hisex Brown, Isa Brown, Isa Warren, H & N Brown Nick and Hyline. Breeders of jungle fowl come are local jungle fowl, mixed jungle fowl and colour meat jungle fowl; sassa, kabir and hubbard isa (DVS Malaysia, 2012).

The layer farming starts with the purchasing of special chicks of egg producing breeds from hatcheries. These chicks are reared in different stages of management until the point of lay. The young pullets are ready to produce egg for commercialization at age of 19 weeks old. The pullets become hens and it will be kept until the end of laying cycle, which is at the age of 90 weeks old. The hens produce the highest peak of egg production at age of 25 to 36 weeks old (Beutler, 2007).

The day old chicks are purchased and raised at brooding stage. At this period, the most important factors of brooding management are temperature of brooding area, feed and water availability. Normal thermal condition will increase feed intake of the chicks. (Leksrisompong et al., 2009).

In general, layer hens are reared either in cage or non-cage system. For the non-cage system, the hens are usually reared in deep litter or free ranged system. In both systems, the hens are free from cage. In the deep litter system, the hens are placed on a barn floor and kept entirely indoors, while for the free ranged system, the hens are also kept in the barn, but they can get accessed to the outdoor.

There are two types of cage that are usually used in the cage system, namely conventional and enriched cages. The conventional cage system is cage built in a row arrangement, with cage floor and wire are built in by using wire. The cage floor built in slightly loop to influence egg rolled onto automated belt conveyer (Bryden et al., 2006). The enriched cages (also called furnished or modified cages) are similar to conventional battery cages but provide more space and height and contain a range of additional elements intended to enable hens to perform some important natural behaviours. They come in a range of sizes. Smaller ones may house fewer than 10 birds, whilst larger versions may house up to 60 or more birds. The cage system usually applied in both open sided and closed housing system.

Both open sided and closed housing systems, where the hens are kept entirely indoors (BCSPCA, 2009), are used in layer hens. The open sided housing is a type of housing where the air and natural daylight are allowed to enter the house. This type of house is a naturally dependent housing system, where temperature, humidity and air velocity are totally depends on the weather. While, closed housing system is a controlled environment house, or also known as power ventilated house system. The temperature, humidity and air velocity are under controlled of exhaust fan and/or cooling pad (Bryden et al., 2006). The type of flooring in both housing systems is a concrete floor.

## **2.2 Stress in poultry**

Stress factors in poultry are composed of psychological, physiological, physical, environmental, climatic, nutritional, social, and disease (Mohan, 2005). Psychological stress occurs when hens feel fear, while physical stress occurs during handling. For the social stress, it develops from the aggression, feather pecking and/or cannibalism (Denis et al, 2004; Mohan, 2005). The environmental and climate stress on hens usually happened due to extreme hot and cold environment, and high humidity, high stocking density with poor ventilation and high light intensity (Chancellor and Glick, 1960; Regnier and Kelly, 1981; Mohan, 2005).

In poultry industry, the most common stress in hens are climatic and environmental stress, or known as heat stress. The stress occurs as a result of the imbalance between heat released from the hens' body and the amount of heat generated (Defra, 2005).

Amount of heat generated in hens' body resulted from metabolism of body growth, maintenance and egg production (St-Pierre, 2003). Hens have ability to release heat by four main methods which are radiation, conduction, convection, and evaporation (panting) (Halls, 2000; Defra, 2005). Release of heat through radiation occurs when it radiates from the bird's warmer body to a cooler surface, such as air; without using a medium or surface, through rising of their wings to allow heat to radiate from poorly feathered areas, such as under wings. Released of heat through a medium or surface is called conduction; the heat is transferred through warm surface to a cooler surface. The birds will cool down their body by touching cooler materials, such as water pipes or digging into litter to make a direct contact with a cool floor. The laying hens also will try to touch cage wall to conduct the heat through the wall. Another common method of heat released in hens is through convection. Convection occurs when air is moving over the hens' body. Thus, fast moving air will break and push away the warm air around its body (Hall, 2000; Defro, 2005). The fourth way of heat release in birds is through evaporation or panting, which occurs in heat stress condition. The heat stress is worse when both ambient temperature and relative humidity are high. In high humidity condition, hens are unable to generate their heat loss through panting (Hall, 2000; Defra, 2005). Hence, high air velocity provides an aid to the hens to release heat in other way, which is through convection.

Apart from heat stress, any types of stressor will interfere the homeostasis mechanism of maintaining the normal physiological function and regulation of birds. There are three stages of stress regulation mechanism in birds, which are reaction, resistance and

exhaustion. Stage of reaction is a short-term stress regulation that involves neurogenic system. The neurogenic system involves is sympathetic nervous system and adrenal medulla system. These systems ensure bird aware of the stressor and equipped it to the flight or fight reaction in emergency condition. This reaction initiated by the catecholamines commanded by rapid release of glucose in the blood, depletion of liver glycogen, increased peripheral vasomotor activity, altered ventilation rate and increased neuronal sensitivity (Selye, 1950; Siegel, 1980; Mohan, 2005).

Stress regulation mechanism of resistance or adaptation stage is a body response for the long-term stress. It consists mainly endocrine system that is activated by the hypothalamus-pituitary-axis (HPA), characterized by adrenal cortical hypertrophy and increased synthesis and release of adrenal glucocorticoids, known as corticosterone in the bird's blood. As the plasma corticosteroid increased, it will lead to the increased of peripheral circulating leukocytes of heterophils and decreased proliferation of lymphocytes (immunosuppression), which resulted increased of the heterophil to lymphocyte ratio (H/L). The HPA activation is a long-term adaptation towards the stress condition (Selye, 1936; Siegel, 1971, 1980; Puvadolpirod et al, 2000; Mohan, 2005).

The last stage of stress regulation mechanism is known as stage of exhaustion. In this stage, the body did not able to tolerate any more with the stressor, as body reserves energy and hormones did not sufficient to maintain the homeostasis of body response and may lead to death of bird (Brake, 1985; Freeman, 1987; Maxwel, 1993).

Short-term or acute stress is easily determined via assessing the blood glucose level. For the long-term or chronic stress, plasma corticosterone and heterophil to lymphocyte ratio can be used to determine the stress level. High heterophil counts in relation to low lymphocytes reliably correlates with the high glucocorticoid level (Ots et al., 1998; Davis et al., 2008; I, Krams et al., 2012).

The assessment of H/L ratio as a stress indicator in birds has several advantages over hormone assessment. Several researches reported high H/L ratio related with the increased mortality in hens (Davis et al., 2008; Sepp et al., 2010). The ratio resembled the concept of glucocorticoid-induced immunosuppression in chronic stress. This is explained where the ratio of H/L is lower in less stressed bird that have stronger antibodies than the bird with high H/L ratio (Maier et al., 1994; Sapolsky et al., 2000; Dhabhar, 2009; I, Krams, 2012). However, there is a restriction of using H/L ratio in stress assessment in birds, because the heterophilia and lymphopenia are only happened in bird with mild to moderate stress. In extreme life threatening stress condition, the birds will develop heteropenia. This heterophil responds to stimulus in 30 minutes during early inflammatory phase. They are also becomes more sensitive and had selective phagocytizing properties. In seven days, the heterophils becomes unrecognizable, with macrophage recruitment; the characteristic of heterophilic granuloma develops. At this stage and condition the plasma corticosterone are more reliable for stress assessment compared to H/L ratio (Maxwell et al., 1998).

### **3.0 Material and method**

#### **3.1 Animals**

The study was conducted at a commercial poultry farm, located at Malacca, Malaysia. It consists of Lohmann Brown hens and the hens were reared under open sided and closed housing system. In the open sided housing system, the hens were allocated in single-tiered cage system, while for closed housing system, the hens are reared in a multi-tiered cage system up to 7 levels. Five farms of this commercial company were chosen randomly. The hens of farms 1 and 2 were raised in open sided houses, and in farms 4 and 5, the hens were managed in closed house system. Upon randomized selection, only hens in farm 3 were raised in both open sided and closed house systems.

#### **3.2 Environmental data**

Ambient temperature (AT), relative humidity (RH) and air velocity (V) of the farms were recorded at 9.00 am, 1.00 pm and 5.00 pm. These data were recorded at each alleys of laying hens tiers, at points of front, middle and end of laying hen house. For each position, five readings were recorded and mean was determined. The means of each parameter was compared between houses.

Heat stress index in both open sided and closed house systems was calculated by the sum of the AT and RH.

### **3.3 Blood sampling**

A total of 20 laying hens were chosen randomly from each house; the hens were selected at the front, middle and back of the houses. Approximately 0.5 mL of blood was taken from brachial vein venipuncture using 1 mL syringe and half (1/2) inch 25G needle. The blood was collected into EDTA tube. Blood smear was prepared and stained with Wright stain. A total of 200 white blood cells (WBC) were counted and differentiated into heterophils, basophils, eosinophils, lymphocytes and monocytes. Ratio of heterophil to lymphocyte (H/L) was calculated for each bird.

### **3.4 Data analysis**

All environment data, heterophil and lymphocyte count, and H/L ratio were analyzed using non parametric method, which was Mann Whitney Test using SPSS version 22.

## 4.0 RESULT

### 4.1 Environmental Parameters

Result of the environmental parameters include AT, RH and V are shown in Table 1. Result showed temperature of the open sided house was significantly higher ( $p < 0.05$ ) than the closed house, which were  $29.4^{\circ}\text{C}$  and  $28.1^{\circ}\text{C}$ , respectively. There was no significant difference between both houses for the RH, however the V value in the open sided house system was significantly lower ( $0.4 \text{ m/s}$ ) compared to the closed house system ( $1.8\%$ ).

Table 1: Environmental parameters of open sided and closed house systems.

Parameters	Open Sided House	Closed House
Temperature; AT ( $^{\circ}\text{C}$ )	$29.4 \pm 0.07^{\text{a}}$	$28.1 \pm 0.15^{\text{b}}$
Relative Humidity; RH (%)	$59.7 \pm 0.26^{\text{a}}$	$59.4 \pm 0.71^{\text{a}}$
Air Velocity; V (m/s)	$0.4 \pm 0.02^{\text{a}}$	$1.8 \pm 0.01^{\text{b}}$

Values with different superscripts were significantly different ( $p < 0.05$ ) between columns.

### 4.2 Heat stress index

The heat stress index values were 89.1 for open house and 85.5 for closed house system, respectively. It showed no significant difference between the two house types.

### 4.3 Blood Cell Morphology

Figures 1, 2, 3 and 4 show morphology of blood cells of Lohmann Brown layer chicken stained with Wright stain under 100x magnification.

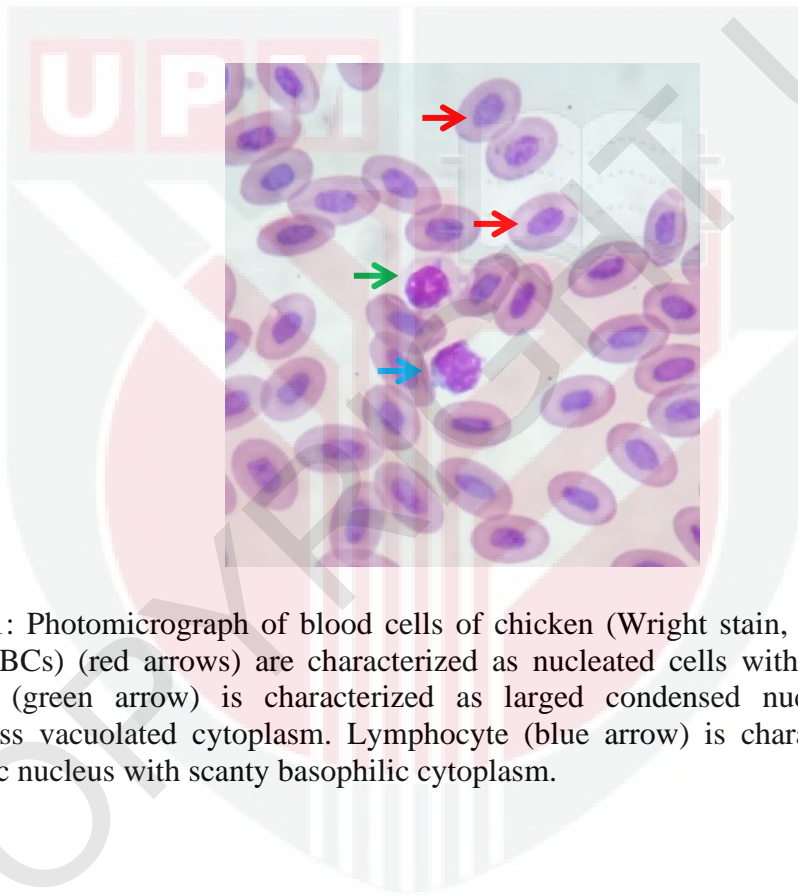


Figure 1: Photomicrograph of blood cells of chicken (Wright stain, 100x). Red blood cells (RBCs) (red arrows) are characterized as nucleated cells with elongated shape. Platelet (green arrow) is characterized as larged condensed nucleated cell with colourless vacuolated cytoplasm. Lymphocyte (blue arrow) is characterized as large eccentric nucleus with scanty basophilic cytoplasm.



Figure 2: Photomicrograph of blood cells of chicken (Wright stain, 100x). Heterophil (black arrow) is characterized as abundant rod needle eosinophilic granules in the cytoplasm which obscured the round eccentric nucleus.

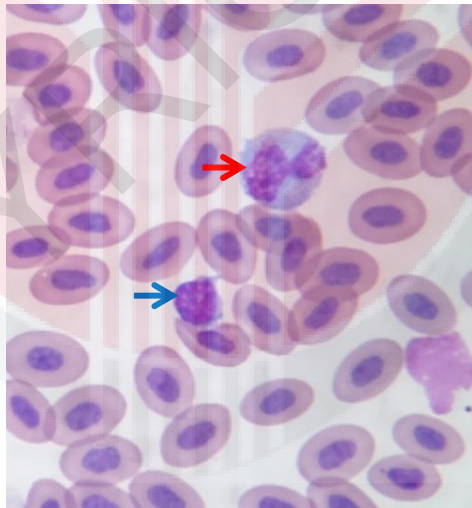


Figure 3: Photomicrograph of blood cells of chicken (Wright stain, 100x). Monocyte (red arrow) is characterized as indented to bilobed nucleus with abundant basophilic cytoplasm. Blue arrow is a lymphocyte with large eccentric nucleus and scanty basophilic cytoplasm.

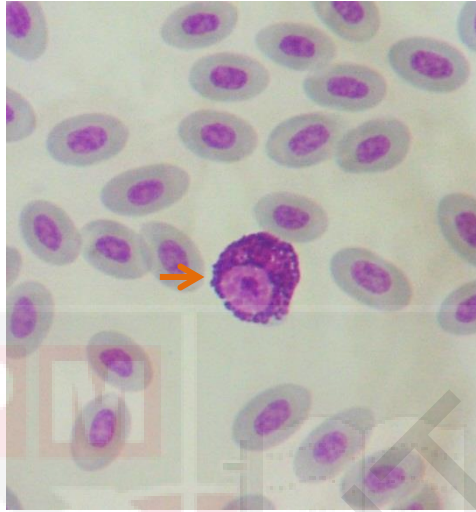


Figure 4: Photomicrograph of blood cells of chicken (Wright stain, 100x). Basophil (orange arrow) is characterized as round shaped of cells with basophilic granules in the cytoplasm with round eccentric nucleus.

#### 4.4 Health Status of the Layer Hens

During blood sampling, two of three layer hens raised in the open sided house were found free from viral disease outbreak, while one of the farms was contracting with the disease. Meanwhile, for the closed house system, one of the farms was contracted with the same outbreak and the other two farms had just recovered from the disease outbreak.

#### 4.5 Comparison of heterophil and lymphocyte percentages and heterophil to lymphocyte ratio

The comparison of heterophil and lymphocyte percentages, and the heterophil to lymphocyte ratio of diseased hens were performed between both housing systems (Table 2), and diseased-free hens and diseased hens in the open sided housing system (Table 3). There was no significant difference ( $p < 0.05$ ) on the heterophil percentage between both housing systems (45.35% for open housed and 39.5% for closed house). However, there was significant ( $p < 0.05$ ) higher percentage of lymphocyte of layer hens reared in open sided house (44.28%) compared to closed house (50.38%). This resulted in a higher ratio of heterophil to lymphocyte in open sided house (1.113) compared to closed house (0.836).

Table 2: Comparison of percentages of heterophil and lymphocyte, and heterophil to lymphocyte ratio of diseased layer hens between open sided and closed house systems.

	Open Sided House	Closed House
Heterophil (%)	45.35±2.1 <sup>a</sup>	39.5±1.55 <sup>a</sup>
Lymphocyte (%)	44.28±1.75 <sup>a</sup>	50.38±1.95 <sup>b</sup>
Heterophil to Lymphocyte Ratio	1.113±1.02 <sup>a</sup>	0.836±0.68 <sup>b</sup>

Values with different superscripts were significantly different ( $p < 0.05$ ) between columns.

The comparison of percentages of heterophil and lymphocyte, and ratio of heterophil to lymphocyte between diseased and diseased-free hens in the open sided houses showed there were significant ( $p < 0.05$ ) higher percentage of heterophil and lower percentage of lymphocyte of diseased hen compared to diseased-free hens. The results led to significant ( $p < 0.05$ ) higher ratio of heterophil to lymphocyte in diseased hen compared to diseased-free hens.

Table 3: Comparison of percentage of heterophil, lymphocyte and heterophil to lymphocyte ratio between diseased and diseased-free hens in open sided house system.

	Diseased Hens	Diseased-free Hens
Heterophil (%)	45.35 $\pm$ 2.1 <sup>a</sup>	33.68 $\pm$ 1.95 <sup>b</sup>
Lymphocyte (%)	44.28 $\pm$ 1.75 <sup>a</sup>	55.4 $\pm$ 1.94 <sup>b</sup>
Heterophil to Lymphocyte Ratio	1.113 $\pm$ 1.02 <sup>a</sup>	0.646 $\pm$ 0.6 <sup>b</sup>

Values with different superscripts were significantly different ( $p < 0.05$ ) between columns.

## 5.0 Discussion

Hens raised in different types of housing system experienced different levels of heat stress and discomfort. The hens experience the discomfort when they cannot regulate their body temperature. The regulation of heat in birds is influenced by ambient temperature and relative humidity, together with air velocity in the house. The sum of the ambient temperature and relative humidity will give a value of heat stress index. The heat stress index is used for an assessment of hen performance. It is divided into three different zones, which are safety zone, caution zone and danger zone. Hens will maintain the performance when they are in the safety zone, where the ambient temperature between 27-35°C and relative humidity between 50-60%. The hens will start lost the performance when temperature is starting to rise above 35°C and relative humidity increases more than 60% (caution zone). The hens are in the danger zone when temperature in the house is increased more than 42°C and relative humidity is more than 65%, which is exceeded heat stress index of 107. Based on results of this study, the heat stress index values calculated in both houses were below than the caution (<95) and danger zones (>107), which were 89.1 for open sided house and 85.5 for closed house system.

Although both housing systems had a safe heat stress index, the H/L ratio values of diseased hens between these two housing systems were significantly different. This might be related to the degree of severity and chronicity of viral infection that diseased hens in the open sided housing system were exposed, which led to a greater stress

response indicated by high H/L ratio, as compared to the diseased hen reared in the closed house system. The usefulness of H/L ratio as a diseased/haematological stress indicator was further supported by results of comparison between diseased free hens and diseased hen reared in open sided houses in this study.

The increase of H/L ratio value is related to the adaptation stage of stress regulation mechanism, which activate the HPA corticol system. The activation of this system leads the hypothalamus to produce corticotrophin-releasing factor that stimulates release of adenocorticotropic hormone (ACTH) by pituitary. As the ACTH released, adrenal corticol tissue starts to proliferate and secrete corticosteroids into blood circulation (Viriden and Kidd 2009). The circulatory corticosteroid had an effect on the immune system via inhibition of the function of lymphocyte proliferation, immunoglobulin production, cytokine production, cytotoxicity and anti-inflammatory agents. The prolonged presence of corticosteroid in blood of hens will lead to depression of lymphoid tissues include thymus, spleen, and bursa. Thus, the depression of the size of these organs, leads to depletion of circulating lymphocyte, and causes increase H/L ratio. The ratio is a reliable stress indicator for mild to moderate stress in hens compared to plasma corticosterone concentration (Maxwell et al., 1992; Campo and Redondo, 1996). The similar trend of high H/L ratio is observed in diseased hens compared to non-diseased hens (Mohan, 2005).

Apart from severity and chronicity of the infection, the significant higher level of air velocity value in the closed house system might also be contributed to a lower stress

response in the birds. Higher air velocity enhances released of body heat through convection in these birds. In contrast to hens in the closed house system, hens in the open sided house could not physiologically release their body heat through convection effectively. Heat released through convection occurs when warm air surrounding the hens' body is broken up by the fast air movement (Tierzucht, 2009 and Balasubramanian et al., 2014).



## 6.0 Conclusion

Although the ambient temperature and air velocity results showed significant differences between open sided and closed house system, the heat stress index values in both houses were in the safety zone of heat stress index. Hence, the significant difference between H/L ratio of diseased hens raised in open sided house compared to closed house system were more related to the severity and chronicity of the viral disease outbreak. The significant higher air velocity in the closed house system might contribute to more comfortable environment to the birds, which contribute to the lower stress response. Results in this study also showed diseased hens reared in the open sided house had higher ratio of heterophil to lymphocyte compared to non-diseased hens reared in the open sided house. Based on the results, it is concluded H/L ratio is a sensitive haematological stress indicator to evaluate diseased-induced stress in layer hens.

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