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The relationship of hematological values with Newcastle disease antibody in Thai indigenous chicken: strain Leung Hang Khao

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Abstract The relationship between hematological values and Newcastle disease immunity of Thai indigenous chicken was studied in strain Leung Hang Khao which attractive to consumers in Thailand. The blood and serum samples were collected from 153 chickens of this strain (mixed sex: male = 70, female = 83) at 4 and 7 months of age raising in free-range condition. Hematological value of the strain Leung Hang Khao at 4 month of age showed the mean corpuscular volume (MCV) in male (126.80±0.51 fl.) was statistically significant (p<0.05) higher than in female (123.30±0.46 fl.). While, the total red blood cell (TRBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell count (TWBC), heterophil, lymphocyte, monocyte and H:L ratio were no statistically significant (p>0.05) between sexes at 7 month of age. The average titers were no statistically significant (p>0.05) between sex at 4 month (male: 806.40±97.70 and female: 756.70±87.65) and 7 month (male: 5,372±799.08 and female: 6,561±709.28) of age, respectively. In addition, the hematological values of this strain at 4 month of age was relative to Newcastle disease immunity. The titer was statistically significant (p<0.05) correlation with the hematological value. The titers with the low, medium and high levels of immunity (63.15±23.47, 421.77±28.04 and 1525±131.92, respectively) were negative relationship with lymphocyte (71.00±2.46, 69.79±0.81 and 67.72±1.29 percentages of white blood cell (WBC), respectively) but statistically significant positive (p<0.05) relationship with heterophil (25.17±2.34, 27.76±0.86 and 29.41±1.05 percentages of WBC, respectively). The relationship between the amounts of lymphocyte and heterophil, and Newcastle disease immunity were indicated with the coefficients of phenotypic correlation of -0.17 and 0.18, respectively. From these results showed that, the relationship between hematological values and Newcastle disease immunity could be used as a primary health assessment Newcastle disease index for Thai indigenous chicken strain Leung Hang Khao.

Keywords: Thai indigenous chicken, immunology, hematological values, Newcastle disease

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Introduction

Thai indigenous chicken is very popular to feed in Thailand due to low cost of egg production and housing enables low-income farmers to provide a source of meat protein with low investment. These chicken are rared not for desmetic consumption but also for expert in Asean (Singapore, Brunei, Malaysia, Phillippines, Vietnam, Laoss, Cambodia and Myanmar) (Jaturasitha *et al.,* 2016). In general, the chickens are reared by free ranging under backyard system or on the farm as live birds and slaughtered a few at a time as needed. The Thai indigenous chicken (*Gallus domesticus*) originates from the Red jungle fowl (*Gallus gallus*) of Southern Asia (Parkhurst and Mountney, 1988; Wight and Siller, 1997). One of the most popular Thai indigenous chicken is Leung Hang Khao because it is a good fighting cock strain. This strain has been selected as a pure breed line by Department of Livestock Development. The special properties of this chicken are easy to parenting, big shape, good growth and high disease resistance (Duangjinda, 2015). Therefore, it is suitable for feeding in a tropical weather condition like Thailand. In addition, its meat has unique taste and texture with low cholesterol, free of antibiotic and hormones chicken production. This leads to become very popular among Thai consumer and a higher price than commercial broilers. However, one of the important problems in the indigenous chicken is outbreak of virus infectious disease due to the lack of control and poor disease prevention (Duangjinda *et al.,* 2009).

The seriously disease in Thai indigenous chicken is Newcastle disease, which is found worldwide and including Thailand. Newcastle disease virus (NDV) contains of one serotype and is also known as avian paramyxovirus serotype 1 (PMV-1). It is the most common RNA virus causes of respiratory problems in chicken. This disease is found to be compatible with the chicken of any age, but in the chicks will have the illness and high mortality rate may be up to 100 % (Horning *et al.,* 2003; Alders and Peter, 2001). Breeding of the chicken with a good immune level and high disease resistance is the alternative way to solve this problem in farming system. The vaccination of NDV vaccines in the chicken is one of the best way to protect Thai indigenous chicken from Newcastle disease. The NDV vaccines stimulate an immune response that minimizes or completely prevents the occurrence mortality of the disease (Miller *et al.,* 2009). This method is not only helpful in diagnosis of specific poultry diseases but also provide basic knowledge for study the immunology in chicken. The study on hematology of Thai indigenous chicken could be help for protection and reduction of this disease. The hematological data is one of the significant basic data that indicates animal health called “Health Indices” and

some blood biochemical and electrolyte contents are also indicate the deviation from normal organ function.

Since, there is a little information about the basic biological data of Thai indigenous chicken, the aims of this study was to examine the average standards of hematological values and the relationship between hematology and Newcastle disease immune system in Leung Hang Khao strain.

Materials and methods

Chicken, vaccine and nutrition

One hundred fifty-three Thai indigenous chickens (*Gallus domesticus*) with 1 day to 7 month of age were provided from Kabinburi Animal Husbandry and Research Center Thailand. These Thai indigenous chickens were housed using semi intensive rearing system in the farm of Suranaree University of Technology, Nakhon Ratchasima, Thailand. For the first 3 weeks they were fed by starter diet containing 21% crude protein after that the diet was contained 17% crude protein. The chickens were vaccinated with the live vaccine against Newcastle disease (ND: Lasota strain) and Infectious bronchitis (IB: H 120 strain) at 1, 2, 8 and 12 weeks. After that they were vaccinated every 3 months according to the recommendation from the local veterinarians.

Sample collection

The serum samples were collected for examination the presence of antibodies against Newcastle disease virus. Firstly, the 3 mL of blood samples were collected from the jugular vein using sterile disposable needles and syringes into the microtubes from the chickens at 4 to 7 months. After a few minutes the blood samples were formed a clot. Next, the serum was separated from the clot by centrifugation at a speed of 3,000 rpm for 15 minutes at 25◦C. Finally, the supernatant (serum) was transferred into the new microtube and stored at -20 ◦C to determine the Newcastle disease antibodies by ELISA kit for assessing antibody titer against NDV. (Synbiotics Corporation, San Diego, Canada).

Hemotological technique

The differential WBC counts were analyzed by spreading blood on monolayer blood films, fixed and stained with Giemsa-Wright's stain. For total red blood cell (TRBC) and total white blood cell counts (TWBC) were

determined using haemacytometer (Campbell, 1995; Pierson, 2000). The packed cell volume (PCV) was measured using microhematocrit capillary tubes and centrifugated at a speed of 2,500 rpm for 5 minutes. In addition, the hemoglobin concentration (Hb), erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were examined using HYCEL® automatic blood counting machine (Pierson, 2000).

Statistical analysis

All hematological data were expressed as mean ± standard error. The comparison of hematological data both sex and age was statistically analyzed by student's *t*-test using SPSS Statistics software (Version 20) (SPSS, Inc., Chicago, III, USA). Pearson's correlation coefficients (r) was analysed to determine the relationship between titer antibody and hematological parameters of the chicken at the age of 4 month. The Differences among the mean values were tested by Duncan's multiple range test.

Results

Titer and Hematological parameters

The average hematological values in Thai indigenous chickens strain Leung Hang Khao age of 4 month by sex were shown in Table 1. The results indicated the MCV in male (126.80±0.51 fl.) was statistically significant ($p<0.05$) higher than female (123.30±0.46 fl.). While, TRBC, HGB, HCT, MCH, MCHC, TWBC, heterophil, lymphocyte, monocyte and H: L ratio were no statistically significant ($p>0.05$) between sexes. The average titers of this strain were no statistically significant (male: 806.40±97.70 and female: 756.70±87.65).

At 7 month of age, the TRBC, HGB, HCT, MCV, MCH, MCHC, TWBC, heterophil, lymphocyte, monocyte and average titers (male: 5,372±799.08 and female: 6,561±709.28) were no statistically significant ($p>0.05$) between sexes (Table 2).

The relationship of Hematology with Newcastle disease immunity in Thai indigenous chickens strain Leung Hang Khao

The results showed that the titer was statistically significant ($p<0.05$) relative with hematological values. At 4 month of age the titer of this strain

Table 1. The hematological values and titers of Thai indigenous chicken strain Leung Hang Khao at 4 month of age and other strains

Parameters	Sex		Total (n=153)	Range	Simaraks *et al.* (2004)
	Male (n=70)	Female (n=83)			
Total RBC ($\times 10^6$ µl)	4.20±0.84	4.09±0.76	4.15±0.80	1.5-5	2-3
Hemoglobin (g/dl)	10.98±0.21	11.34±0.19	11.16±0.20	7.6-14	8-10
Hematocrit (%)	30.46±0.61	31.83±0.55	31.15±0.58	21-40	28-37
MCV (fl)	126.80±0.51**	123.30±0.46	125.05±0.49	116-147	126-163
MCH (pg)	45.78±0.42	44.21±0.37	45.00±0.40	39-60	35-45
MCHC (g/dl)	35.70±0.42	35.83±0.38	35.77±0.40	25-36	24-31
Total WBC ($\times 10^4$ µl)	13.07±0.60	14.19±0.53	13.63±0.57	6-25	1.6-2.5
Lymphocyte (%)	69.32±0.70	68.82±0.64	69.07±0.67	38-83	54-73
Heterophil (%)	28.17±0.67	28.31±0.61	28.24±0.64	14-44	16-31
Monocyte (%)	2.47±0.17	2.54±0.16	2.51±0.17	1-6	1-7
H: L ratio	0.42±0.01	0.24±0.01	0.42±0.01	0.17-0.82	0.23-0.57
Titer	806.40±97.70	756.70±87.65	781.55±88.19	62.1-1,694	-

Table 2. Comparison of hematological values between Thai indigenous chicken strain Leung Hang Khao at 7 month of age and other species

Parameters	Thai indigenous chickens strain Leung Hang Khao		Thai fighting strain[/2]		Vietnam fighting strain[/2]		Thai indigenous chickens in northeastern Thailand[/3]			Jain (1993)
	Male (n=56)	Female (n=67)	Male (n=15)	Female (n=15)	Male (n=15)	Female (n=15)	Male (n=20)	Female (n=20)	Range	Range
Total RBC ($\times 10^6$ µl)	2.84±0.10	2.72±0.09	2.99±0.16	3.16±0.12	2.79±0.14*	2.04±0.10*	2.32±0.31	2.19±0.26	2-3	2.5-3.5
Hemoglobin (g/dl)	13.24±0.53	12.73±0.47	12.90±0.40*	11.40±0.20*	12.20±0.30*	8.90±0.40*	9.27±1.42*	8.52±0.85	8-10	7.0-13.0
Hematocrit (%)	36.38±1.29	34.07±1.14	40.10±1.10*	35.70±0.70*	38.90±1.00*	29.70±0.90*	33.55±4.72*	30.80±3.96	28-37	22.0-35.0
MCV (fl)	126.40±18.08	144.10±16.04	137.60±6.40*	115.00±4.50*	144.30±8.40	152.50±12.10	147.87±18.43	141.39±19.17	126-163	90.0-140.0
MCH (pg)	45.70±1.16	44.61±1.03	44.30±2.30*	36.70±1.51*	45.30±2.80	46.10±4.20	40.26±5.16	39.11±4.42	35-45	33.0-47.0
MCHC (g/dl)	36.25±0.81	35.83±0.38	32.20±0.70*	31.90±0.60*	31.50±0.90	30.00±0.70	27.93±4.56	27.79±1.77	24-31	26.0-35.0
Total WBC ($\times 10^4$ µl)	18.08±1.46	16.23±1.29	11.81±1.04*	21.04±1.69*	18.42±1.86*	17.32±1.37*	2.05±0.39	2.05±0.53	1.6-2.5	1.2-3.0
Lymphocyte (%)	-	-	49.10±3.40	49.30±2.50	57.30±0.50	57.30±3.20	60.30±5.33	67.05±1.49*	54-73	45.0-70.0
Heterophil (%)	-	-	43.30±3.60	38.80±3.30	33.00±3.00	37.30±3.30	25.40±5.12	22.00±8.78	16-31	15.0-40.0
Monocyte (%)	-	-	1.80±0.20*	2.50±0.20*	6.30±1.30*	3.30±0.60*	4.35±3.15	4.05±3.39	1-7	5.0 - 10.0
H:L ratio	-	-	-	-	-	-	0.43±0.12	0.36±0.21	0.23-0.57	-
Titer	5,372±799.08	6,561±709.28	-	-	-	-	-	-	-	-

[1]/* Mean ± SE with superscript, within row between sex in each parameter differ significantly ($p< 0.05$); [2]/ Reference values of Salakij (2004); [3]/ Reference values of Simaraks *et al.* (2004)

could be divided into 3 levels including low (63.15±23.47), medium (421.77±28.04) and high (1,525±131.92), respectively. The lymphocyte was negative statistically significant (p<0.05) relationship with Newcastle disease immunity (71.00±2.46 69.79±0.81 and 67.72±1.00 percentages of WBC, respectively), but positive statistically significant (p<0.05) relationship was found in heterophil (25.17±2.34, 27.76±0.86 and 29.41±1.05 percentages of WBC, respectively) (Table 3). The relationship between the amounts of lymphocyte and heterophil, and Newcastle disease immunity were indicated with the correlation coefficient of -0.17 and 0.18, respectively (Table 4).

Table 3. The relationship of immunity group to titer, lymphocyte and heterophil of Thai indigenous chicken strain Leung Hang Khao at 4 month of age

Group of Titer (N=153)	Titer		Lymphocyte		Heterophil	
	Male	Female	Male	Female	Male	Female
Low (N=8) 0-100	64.19±20.48	62.10±26.45	73.00±2.16	69.00±2.79	25.00±2.04	25.33±2.63
	Average = 63.15±23.47		Average = 71.00±2.46		Average = 25.17±2.34	
Medium (N=91) 101-800	438.18±28.50	405.35±27.57	69.93±0.82	69.64±0.79	27.46±0.87	28.06±0.84
	Average = 421.77±28.04		Average = 69.79±0.81		Average = 27.76±0.86	
High (N=54) >800	1694±146.76	1356±117.08	67.76±1.43	67.67±1.14	29.91±1.16	28.91±0.93
	Average = 1525±131.92		Average = 67.72±1.29		Average = 29.41±1.05	

Discussion

Thai indigenous chicken is in the group of *Gallus domesticus* which containing of various chicken species and each species have a different characteristics. The 8,600 species of poultry group have a different anatomy and physiological characteristics and hematological value. Therefore, these characteristics are widely studied at the present in order to animal health check and farming development for using as food. However, it is still a lack of information about the effects of season, age, gender and reproduction which are initially studied and these data will support for further development of this knowledge (Bounous and Stedman, 2000). The biological information of indigenous chicken is studied to farming development for using as food. Recently, Thai indigenous chickens are increased a role as live stock which are developed for Thai farmer. The results of this study are very useful for feeding Thai indigenous chicken both at the present and in the future.

Table 4. Coefficients of phenotypic correlation between titer and haematological parameters of Thai indigenous chickens at 4 month of age

	Titer	Total RBC ($\times 10^6$ µl)	Total WBC ($\times 10^4$ µl)	Hemoglobin (g/dl)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Lym (%)	Het (%)	Mono (%)	H:L
Titer	1.00											
Total RBC	0.04	1.00										
Total WBC	0.12	0.02	1.00									
Hemoglobin	0.09	0.08	0.20*	1.00								
Hematocrit	0.05	0.03	0.18*	0.91**	1.00							
MCV	0.09	-0.01	-0.08	-0.44**	-0.41**	1.00						
MCH	0.13	0.01	-0.01	-0.16*	-0.50**	0.47**	1.00					
MCHC	0.09	0.11	0.03	-0.03	-0.25**	-0.03	0.47**	1.00				
Lym	-0.17*	-0.01	0.01	-0.21**	-0.19*	0.11	0.03	-0.01	1.00			
Het	0.18*	0.04	0.06	0.18*	0.14	-0.11	0.02	0.01	-0.86**	1.00		
Mono	0.00	-0.13	-0.09	0.05	0.04	0.08	0.07	-0.19*	0.10	-0.37**	1.00	
H:L	0.19*	0.04	0.03	0.20*	0.20*	-0.12	0.01	0.08	-0.95**	0.97**	-0.28**	1.00

[1]/** Mean ± SE with superscript, in each parameter differ very significantly ($p \leq 0.01$);[2]/* Mean ± SE with superscript, in each parameter differ significantly ($p \leq 0.05$)
RBC = Total red blood cell; WBC = Total white blood cell; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; Lym = Lymphocyte; Het = Heterophil; Mono = Monocyte; H:L = Heterophil to lymphocyte ratio

Lumsden (2000) reported in the study on biological standard value for using as a reference value of each animal species which could be indicated abnormal in the animals by comparison of the minimum and maximum values of normal animals (normal values). Therefore, normal values of normal animals was important and must be accurate and reliable affecting by various parameters including methods of analysis, sample collection and maintenance,

and statistical analysis. In addition, the normal values were affected by animal species, age, gender, feed stock and farm system. The findings were basic information of Thai indigenous chicken under free-range and semi free-range raising systems.

The relationship between hematological values and Newcastle disease immunity in Thai indigenous chickens strain Leung Hang Khao was determined to find the average hematological value and titer for use as a primary health assessment Newcastle disease index of Thai indigenous chicken. The hematological values of Thai indigenous chicken strain Leung Hang Khao at 4 month of age showed the highly statistically significant ($p<0.01$) of MCV between male and female chickens. The amount of MCV was higher in male more than in female. The high MCV values were found in Thai indigenous chicken which could be used as a tool for chicken health check. If the MCV value was lower than normal value (small red blood cells) may be caused by anemia from lack of iron of disorder of blood cells. In contrast, if the MCV values was higher than normal value (big red blood cells) may be due to the abnormal of liver. In this study, at 4 month of age male indicated higher MCV than female but MCV values were in the standard range which is similar to the report of Jain (1993) (126-163 and 90-140, respectively).

The comparison of hematological values between Thai indigenous chicken strain Leung Hang Khao and other chicken strains was performed. The different of haematological values in each chicken strains was found and the average values were shown in Table 2. The titer and haematological values were no statistically significant ($p>0.05$).

The amount of lymphocyte and heterophil were relative to Newcastle disease immunity with the coefficients of phenotypic correlation of -0.17 and 0.18, respectively. In Table 4, chicken with high titer was negative relative to lymphocytes but positive relative to heterophil. These hematological values could be used as additional information for evaluation the state of Newcastle disease immunity. The function of heterophil in chicken are a part of immune that helps fight infection, defend the body against other foreign materials and uses to diagnose body infection. Using hematological value to determine the state of Newcastle disease immunity in the vaccinated Thai indigenous chicken must be very difficult. The amount of lymphocytes and heterophil were not identified that directly produced by immune system due to the very low coefficient correlation of immunity and amounts of lymphocytes and heterophil. In addition, the separation of blood cell type in chicken was no tools to get the accuracy and use a long time to count. Therefore, the evaluation of immunity level in chicken using ELISA remains a cost effective method to save time, precision and specification at the present.

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