Original Article

Region-specific hematological and biochemical reference intervals for southern Thai fighting bulls

Dhiravit Chantip[1] Nantaporn Chooruang[2] Kitikarn Sakuna[3]
Niramol Thongtem[3] Wiruntita Bohman[3*]

Abstract

Accurate health assessments are vital for the performance and welfare of fighting bulls. Existing hematological and biochemical reference intervals (RIs) for cattle may not adequately reflect the unique characteristics of fighting bulls due to breed, age, and geographical differences. This study aimed to develop and apply region-specific RIs for hematological and biochemical parameters in fighting bulls from southern Thailand. A retrospective analysis of laboratory records was conducted on 1,460 healthy fighting bulls enrolled in an annual health check program at the Livestock Animal Hospital, Faculty of Veterinary Sciences, Rajamangala University of Technology Srivijaya, from January 2016 to December 2023. Blood samples were analyzed for hematological and serum chemistry profiles using automated analyzers. Statistical evaluations identified significant geographical, seasonal, and age-related variations. Region-specific RIs were established, revealing slight elevations in red blood cell (RBC) indices, including RBC count, hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentrate (MCHC), as well as serum chemistry parameters such as blood urea nitrogen, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein (TP), compared to previously reported values for other cattle breeds. Older bulls exhibited lower RBC indices and leukocyte counts ($P<0.05$), while western coast bulls showed distinct variations in MCV, leukocyte counts, neutrophils, eosinophils, and ALP compared to eastern counterparts ($P<0.05$). Seasonal effects included higher MCHC, monocyte counts, and TP during the rainy season, whereas red cell distribution width and neutrophil counts increased significantly during summer ($P<0.05$). These RIs provide valuable benchmarks for health assessment, disease diagnosis, and physiological monitoring, enhancing the management and welfare of fighting bulls in southern Thailand.

Keywords: blood chemistry, fighting bulls, health assessment, hematology, reference intervals

[1]*Department of Veterinary Bio-Medical Sciences, Faculty of Veterinary Sciences, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand*
[2]*Laboratory and Diagnostic Center of the Teaching Animal Hospital and Community Services, Faculty of Veterinary Sciences, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand*
[3]*Department of Veterinary Clinical Sciences, Faculty of Veterinary Sciences, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand*
***Correspondence**: wiruntita.c@rmutsv.ac.th* (W. Bohman)
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Introduction

Hematological and biochemical profiles are cornerstone diagnostic tools in veterinary medicine, offering invaluable insights into animal health status (Cozzi *et al*., 2011; Herman *et al*., 2018; Chen *et al*., 2022). These profiles serve as the foundation for disease diagnosis, treatment monitoring, and overall health assessment. Establishing reference intervals (RIs) for these parameters is critical for accurate interpretation and effective healthcare management. While existing research has established RIs for various cattle breeds (Guyot *et al*., 2024; González–Garduño *et al*., 2023; Sofyan *et al*., 2020), these intervals can be significantly influenced by factors like age, sex, breed, geographical location, and season (Kim *et al*., 2014; Mirzadeh *et al*., 2010b).

Southern Thai fighting bulls, a distinct indigenous beef cattle breed, are specifically selected and rigorously trained for bullfighting competitions (Chodchaey *et al*., 2022). The training of these bulls begins at the age of 4-5 years, with competition participation occurring between the ages of 5-10 years or even older (Taweephol *et al*., 2023). This comprehensive training program focuses on physical conditioning to enhance strength, agility, and resilience-crucial qualities for success in these contests. The bullfighting industry in southern Thailand supports livestock trade, breeding programs, and veterinary services, with elite bulls valued at over 1 million Thai Baht. These events also drive revenue through ticket sales, wagering, and tourism, contributing to regional economic growth (Rakjan, 2024). Despite their cultural significance and specialized training, there is a critical gap in our understanding of the normal hematological and biochemical profiles of healthy Southern Thai fighting bulls. The absence of region-specific RIs for this unique population hampers the accurate interpretation of diagnostic tests, posing significant challenges for veterinarians in monitoring and managing their health effectively. Establishing these RIs is essential for improving the healthcare and overall well-being of these bulls throughout their training and competitive careers.

The need to establish RIs specific to Southern Thai fighting bulls is paramount for effective veterinary care. Understanding these parameters will provide veterinarians with essential benchmarks for interpreting blood test results, enabling accurate diagnosis and management of health issues. This, in turn, will optimize the health and welfare of these animals throughout their training and competitive careers. Establishing these benchmarks will contribute valuable information to the field of veterinary medicine and enhance the overall health management of these animals within the context of southern Thai bullfighting traditions. Therefore, the objectives of this study were to establish baseline RIs for hematological and blood chemical parameters in healthy Southern Thai fighting bulls and to analyze potential factors influencing these parameters in this specific population.

Materials and Methods

Ethical approval: Approval for this retrospective study was obtained from the Institutional Animal Care and Use Committee of the University of Technology Srivijaya, Thailand, under the authorization number IAC 05-09-2024.

Study area: The study involved fighting bulls participating in the annual health check program at the Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya (Nakhon Si Thammarat campus), located in central southern Thailand (latitude 5°-12° N, longitude 98°-103° E). The bulls originated from various provinces within this area (Fig. 1) characterized by a tropical monsoon climate with distinct summer (January-April) and rainy (May-December) seasons. Temperatures typically range from 23-33°C throughout the year. The region receives annual rainfall between 2,000 and 3,000 mm with high humidity levels (70–90%) (Worlddata.info, 2024).

Data retrieval: Blood samples were obtained retrospectively from clinically healthy fighting bulls during routine health examinations at the Livestock Animal Hospital, Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat campus, Thailand. The study period encompassed January 2016 to December 2023. To ensure the health status of the sampled bulls, licensed veterinarians conducted comprehensive physical examinations and demographic data, including age and other pertinent details, were systematically collected from the hospital's electronic database. Bulls presenting with records of hemoparasitic infections, intestinal parasitism, abnormal clinical signs, or any history of illness were excluded from the analysis.

Sample collection: A cohort of 1,460 healthy bulls was enrolled in this study. Blood samples (10 mL) were collected aseptically from the jugular vein of each bull by a qualified veterinarian. The samples were then divided into aliquots: 3 mL for hematological analysis in EDTA-containing tubes and 6 mL for serum chemistry evaluation in serum collection tubes with a clot activator. All samples were immediately transported to the Laboratory and Diagnostic Center of the Teaching Animal Hospital, Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, for subsequent analysis.

Hematological and blood chemistry analyses: Hematological parameters were assessed using an automated hematology analyzer (ProCyte Dx Hematology Analyzer, IDEXX, Maine, USA). The analysis included erythrocyte indices (red blood cell count [RBC], hematocrit [HCT], hemoglobin concentration [HGB], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red blood cell distribution width [RDW], and reticulocyte count), platelet indices (platelet count [PLT], platelet distribution width [PDW], mean platelet volume [MPV], platelet large cell ratio [P-LCR], and platelet crit [PCT]), and leukocyte indices (white blood cell count [WBC], neutrophils, lymphocytes, monocytes, eosinophils, and basophils). Serum chemistry profiles were evaluated concurrently using an automated clinical chemistry analyzer (Clinical Chemistry Automatic PKL PPC 125, Paramedical srl, Campania, Italy). The analysis encompassed blood urea nitrogen (BUN), creatinine, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein (TP).

Statistical analysis: Descriptive statistics, including sample size (n), mean, median, standard deviation (SD), minimum, maximum, and 95% confidence intervals (CI) for the mean, were calculated for all parameters using SPSS version 20. Due to the significant skewness of the data, reference intervals for the fighting bull population were established using percentiles. The 2.5th and 97.5th percentiles were used as the lower and upper limits of reference intervals, respectively, encompassing the middle 95% of the data. Age was categorized into three groups: 1.0-5.0, 5.1-8.0, and over 8.0 years, corresponding to distinct physiological and training stages in fighting bulls. The 1.0-5.0 years group represents the early training and developmental phase, 5.1-8.0 years marks peak physical performance, and bulls over 8.0 years are in the late-stage or post-competition phase. This classification facilitates a more precise evaluation of age-related physiological variations. One-way ANOVA was used to analyze the differences in hematological and blood chemistry parameters among the age groups. Independent t-tests were employed to compare hematological and blood chemistry values between bulls from the East and West coastlines, as well as between the rainy and summer seasons. Statistical significance was considered at a P-value less than 0.05.

Results

Hematological and biochemical parameters: This study established RIs for various hematological (Table 1) and biochemical parameters (Table 2) in 1,460 healthy southern Thai fighting bulls. The observed values were compared to existing literature (Cornell University College of Veterinary Medicine, 2024a; Cornell University College of Veterinary Medicine, 2024b; Guyot *et al*., 2024). Slight elevations were observed in RIs for several erythrocyte and leukocyte indices, including RBC, HGB, HCT, MCV, MCH, RDW-CV, MPV, WBC, neutrophil count, monocyte count, eosinophil count, and the percentages of neutrophil, monocyte, and eosinophil. Additionally, RIs for BUN, CREA, ALT, AST, ALP, and TP were also slightly elevated compared to the reported values in the literature. Despite these minor deviations, the wide range of normal values observed for all parameters indicates significant individual variability.

Effects of age: A significant age-related effect was observed on multiple hematological and biochemical parameters (Table 3). Bulls older than 8.0 years exhibited lower levels of several erythrocyte indices, including RBC, HGB, MCH, RDW-CV, and reticulocyte count, compared to younger bulls ($P<0.05$). Conversely, bulls aged 5.0-8.0 years displayed elevated MCV compared to other age groups ($P<0.05$). Leukocyte indices, including WBC and differential counts for neutrophil, lymphocyte, and monocyte, exhibited a decreasing trend with increasing age ($P<0.05$). Conversely, bulls aged 1.0-5.0 years demonstrated a significantly higher percentage of lymphocytes than other age groups ($P<0.05$). The percentage of eosinophils was higher in bulls older than 5.0 years compared to younger bulls. While most clinical biochemistry levels remained unaffected by age, ALT, ALP activity, and TP were significantly lower in older bulls ($P<0.05$).

Effects of geographical location: Geographical location within southern Thailand had a minimal impact on most hematological and blood chemical parameters (Table 4). Bulls from the West Coast exhibited significantly higher values for MCV, WBC, neutrophil count, and eosinophil count compared to bulls from the East Coast ($P<0.05$). Conversely, lymphocyte percentage and ALP activity were significantly lower in West Coast bulls ($P<0.05$).

Effects of season: Seasonal variations significantly impacted most hematological and blood chemical parameters (Table 5). During the rainy season, several parameters, including MCHC, PDW, monocyte count and percentage, BUN, CREA, ALT, and TP, were significantly higher than during the summer ($P < 0.05$). Conversely, RDW and neutrophil count were higher in the summer ($P<0.05$).

Table 1 Reference interval of hematological parameters for healthy fighting bulls in Southern Thailand and comparison with other references.

Parameter	Unit	*n*	Mean	Median	SD	Min	Max	95% CI (Upper – Lower)	Reference Interval (2.5th– 97.5th percentile)	Reference[1]	Reference[2]
RBC	x10⁶/µL	1,460	7.10	7.06	0.91	3.92	9.82	7.05 – 7.15	5.42 – 8.89	5.0 – 7.2	4.7 – 7.5
HGB	g/dL	1,460	13.04	13.00	1.54	6.90	18.30	12.94 – 13.11	10.00 –16.00	8.7 – 12.4	9.2 – 13.1
HCT	%	1,460	38.78	38.70	4.90	21.40	49.90	38.64 – 39.20	29.21– 48.30	25.0 – 33.0	26.0 – 37.0
MCV	fL	1,460	54.74	54.70	4.89	5.90	74.70	54.69 – 55.24	46.20 – 63.60	38.0 – 51.0	40.3 – 57.1
MCH	pg	1,460	18.44	18.40	1.38	10.90	24.50	18.34 – 18.49	15.90 – 21.10	14.0 – 19.0	14.9 – 20.8
MCHC	g/dL	1,460	33.70	33.50	1.90	21.20	45.10	33.44 – 33.59	31.05 – 36.80	34.0 – 38.0	34.2 – 36.3
RDW– SD	fL	1,428	36.91	36.70	3.13	18.40	54.00	36.67 – 37.04	31.57 – 43.93	NA	NA
RDW– CV	%	1,460	24.11	24.10	2.36	6.00	40.60	24.11 – 24.33	18.66 – 28.55	15.0 – 19.4	19.0 – 24.0
Reticulocyte#	x10³/µL	1,407	3.32	2.70	2.87	0.07	63.00	3.14 – 3.51	0.70 – 8.28	NA	NA
Reticulocyte	%	1,408	0.05	0.04	0.08	0.01	2.60	0.04 – 0.05	0.01 – 0.12	NA	NA
PLT	x10³/µL	1,460	282.40	277.50	81.60	72.00	673.00	278.00 – 287.29	132.00 – 459.48	252.0 – 724.0	83.0 – 650.0
PDW	fL	1,441	8.21	7.90	1.64	5.10	16.70	7.98 – 8.11	6.30 – 13.30	NA	NA
MPV	fL	1,442	6.89	6.70	3.52	4.10	123.00	6.77 – 7.00	5.40 – 8.40	5.7 – 8.0	3.5 – 5.8
P– LCR	%	1,409	7.56	6.80	3.70	1.10	26.00	7.35 – 7.80	2.40 – 17.70	NA	NA
PCT	%	1,442	0.19	0.19	0.05	0.06	0.50	0.19 – 0.19	0.10 – 0.31	NA	NA
WBC	x10³/µL	1,460	9.25	8.97	2.34	3.57	19.40	9.05 – 9.34	5.44 – 14.99	5.9 – 14.0	4.4 – 13.3
Neutrophil#	x10³/µL	1,460	4.51	4.19	1.76	0.11	15.18	4.33 – 4.54	1.85 – 9.20	1.8 – 7.2	1.1 – 6.1
Lymphocyte#	x10³/µL	1,460	3.19	3.04	1.05	0.03	13.03	3.15 – 3.27	1.70 – 5.60	1.7 – 7.5	1.3 – 5.2
Monocyte#	x10³/µL	1,460	0.45	0.44	0.24	0.00	2.87	0.44 – 0.47	0.04 – 0.95	0.0 – 0.9	0.0 – 2.0
Eosinophil#	x10³/µL	1,428	1.11	0.99	0.65	0.00	7.75	1.06 – 1.14	0.17 – 2.72	0.0 – 1.3	0.0 – 0.1
Basophil#	x10³/µL	1,428	0.01	0.00	0.02	0.00	0.36	0.01 – 0.01	0.00 – 0.02	0.0 – 0.3	0.0 – 2.0
Neutrophil	%	1,460	48.16	48.10	10.53	1.20	85.30	46.85 – 48.07	28.35 – 68.85	25.0 – 62.0	NA
Lymphocyte	%	1,460	34.98	34.30	8.53	5.70	86.20	34.96–35.94	21.10 – 54.20	29.0 – 66.0	NA
Monocyte	%	1,460	4.98	4.90	2.53	0.00	27.00	4.91 – 5.22	0.50 – 10.30	2.0 – 9.0	NA
Eosinophil	%	1,428	12.06	11.50	6.05	0.00	40.70	11.64 – 12.35	1.87 – 25.80	1.0 – 12.0	NA
Basophil	%	1,428	0.07	0.00	0.19	0.00	4.60	0.06 – 0.08	0.00 – 0.30	0.3 – 1.4	NA

Abbreviation: RBC: Total number of erythrocytes; HCT: Hematocrit value; HGB: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW– SD: Red blood cell distribution width– Standard Deviation; RDW– CV: Red blood cell distribution width– coefficient of variation; PLT: Total number of platelets; PDW: Platelet distribution width; MPV: Mean platelet volume; P– LCR: Platelet large cell ratio; PCT: Plateletcrit value; # : total count number; NA: No available and 95%CI: 95% confidence interval of mean.

[1] Cornell University College of Veterinary Medicine, 2024b.

[2] Guyot *et al.*, 2024 (Belgian blue cows).

Table 2 Reference interval of blood chemical values for healthy fighting bulls in Southern Thailand and comparison with other references.

Parameter	Unit	*n*	Mean	Median	SD	Min	Max	95% CI (Upper – Lower)	Reference Interval (2.5th– 97.5th percentile)	Reference[1]	Reference[2]
BUN	mg/dL	1,233	14.25	13.00	7.77	2.00	69.00	13.73 – 14.66	4.00 – 36.00	7.0 – 19.0	NA
CREA	Umol/L	1,235	1.76	1.80	0.59	0.30	3.00	1.71 – 1.78	0.70 – 3.00	0.4 – 0.9	1.72 – 2.96
ALT	U/L	1,148	29.63	28.00	12.74	2.00	97.00	29.16 – 30.68	11.00 – 65.00	NA	NA
AST	U/L	1,237	78.05	73.00	37.56	5.00	285.00	76.64 – 81.38	19.00 – 175.00	54.0 – 135.0	67.0 – 304
ALP	U/L	1,217	85.28	80.00	28.44	5.20	210.00	83.51 – 87.04	43.00 – 154.10	27.0 – 127.0	NA
TP	g/dL	1,220	8.84	8.60	1.45	0.09	13.80	8.80 – 8.99	6.35 – 11.85	6.7 – 8.8	6.33 – 8.45

Abbreviation: BUN: Blood Urea Nitrogen; CREA: Creatinine; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; NA: No available; and 95%CI: 95% confidence interval of mean.

[1] Cornell University College of Veterinary Medicine, 2024a.

[2] Guyot *et al.*, 2024 (Belgian blue cows).

Table 3 Normal hematological and blood chemical values of fighting bulls in different age groups.

Parameter	Unit	1.0– 5.0 yr (n=340)		5.1– 8.0 yr (n=841)		> 8.0 yr (n=251)		*P*– value
		Mean±SEM	**95% CI**	**Mean±SEM**	**95% CI**	**Mean±SEM**	**95% CI**	
RBC	x10^{6}/µL	7.19 ± 0.05^{a}	7.09 – 7.29	7.12 ± 0.03^{a}	7.06 – 7.18	6.94 ± 0.06^{b}	6.83 – 7.06	0.004
HGB	g/dL	13.05 ± 0.09^{a}	12.88 – 13.22	13.12 ± 0.05^{a}	13.02 – 13.22	12.80 ± 0.10^{b}	12.61 – 13.00	0.013
HCT	%	38.82 ± 0.28	38.28 – 39.36	39.03 ± 0.16	38.71 – 39.35	38.25 ± 0.30	37.65 – 38.85	0.082
MCV	fL	54.19 ± 0.26^{a}	53.68 – 54.70	55.03 ± 0.15^{b}	54.74 – 55.32	54.86 ± 0.39^{a}	54.09 – 55.64	0.025
MCH	pg	18.21 ± 0.07^{a}	18.07 – 18.36	18.52 ± 0.05^{b}	18.42 – 18.61	18.49 ± 0.08^{b}	18.33 – 18.65	0.002
MCHC	g/dL	33.68 ± 0.11	33.46 – 33.89	33.71 ± 0.06	33.59 – 33.84	33.52 ± 0.10	33.32 – 33.72	0.344
RDW– SD	fL	37.09 ± 0.18	36.73 – 37.45	36.90 ± 0.10	36.70 – 37.10	36.76 ± 0.21	36.35 – 37.17	0.440
RDW– CV	%	24.53 ± 0.12^{a}	24.28 – 24.77	24.04 ± 0.08^{a}	23.89 – 24.20	23.85 ± 0.14^{b}	23.58 – 24.12	0.000
Reticulocyte#	x10^{3}/µL	3.56 ± 0.22^{a}	3.13 – 3.99	3.31 ± 0.09^{a}	3.13 – 3.49	2.93 ± 0.11^{b}	2.72 – 3.14	0.034
Reticulocyte	%	0.05 ± 0.00	0.04 – 0.05	0.05 ± 0.00	0.04 – 0.06	0.04 ± 0.00	0.04 – 0.05	0.431
PLT	x10^{3}/µL	288.48 ± 4.53	279.56 – 297.39	280.80 ± 2.74	275.42 – 286.18	276.98 ± 4.83	267.47 – 286.49	0.182
PDW	fL	8.26 ± 0.09	8.08 – 8.45	8.18 ± 0.05	8.07 – 8.28	8.00 ± 0.08	7.83 – 8.16	0.123
MPV	fL	6.78 ± 0.04	6.69 – 6.87	6.99 ± 0.16	6.68 – 7.30	6.74 ± 0.04	6.66 – 6.82	0.493
P– LCR	%	7.63 ± 0.22	7.20 – 8.06	7.56 ± 0.13	7.31 – 7.81	7.17 ± 0.21	6.75 – 7.58	0.261
PCT	%	0.20 ± 0.00	0.19 – 0.20	0.19 ± 0.00	0.19 – 0.19	0.19 ± 0.00	0.18 – 0.19	0.070
WBC	x10^{3}/µL	9.79 ± 0.14^{a}	9.53 – 10.06	9.34 ± 0.08^{b}	9.18 – 9.49	8.22 ± 0.12^{c}	7.99 – 8.45	0.000
Neutrophil#	x10^{3}/µL	4.69 ± 0.10^{a}	4.50 – 4.89	4.60 ± 0.06^{b}	4.48 – 4.72	3.96 ± 0.08^{c}	3.80 – 4.11	0.000
Lymphocyte#	x10^{3}/uL	3.52 ± 0.07^{a}	3.39 – 3.65	3.17 ± 0.03^{b}	3.10 – 3.23	2.78 ± 0.06^{c}	2.66 – 2.89	0.000
Monocyte#	x10^{3}/µL	0.47 ± 0.01^{a}	0.44 – 0.50	0.45 ± 0.01^{a}	0.44 – 0.47	0.41 ± 0.01^{b}	0.38 – 0.43	0.003
Eosinophil#	x10^{3}/µL	1.14 ± 0.04	1.06 – 1.21	1.12 ± 0.02	1.07 – 1.16	1.07 ± 0.04	0.99 – 1.15	0.482
Basophil#	x10^{3}/µL	0.01 ± 0.00	0.01 – 0.01	0.01 ± 0.00	0.01 – 0.01	0.01 ± 0.00	0.00 – 0.01	0.080
Neutrophil	%	47.39 ± 0.58	46.25 – 48.53	48.58 ± 0.36	47.87 – 49.30	47.95 ± 0.59	46.79 – 49.11	0.188
Lymphocyte	%	36.39 ± 0.44^{a}	35.52 – 37.27	34.60 ± 0.29^{b}	34.03 – 35.16	33.97 ± 0.54^{b}	32.91 – 35.03	0.001
Monocyte	%	4.90 ± 0.14	4.62 – 5.17	5.01 ± 0.09	4.84 – 5.19	5.00 ± 0.15	4.71 – 5.29	0.758
Eosinophil	%	11.48 ± 0.32^{a}	10.86 – 12.11	12.03 ± 0.20^{a}	11.63 – 12.43	12.94 ± 0.42^{b}	12.11 – 13.77	0.014
Basophil	%	0.08 ± 0.01	0.05 – 0.11	0.06 ± 0.00	0.05 – 0.06	0.08 ± 0.02	0.05 – 0.12	0.061
BUN	mg/dL	14.25 ± 0.46	13.34 – 15.16	14.03 ± 0.27	13.50 – 14.56	14.96 ± 0.65	13.69 – 16.23	0.314
CREA	Umol/L	1.79 ± 0.04	1.71 – 1.86	1.75 ± 0.02	1.71 – 1.79	1.75 ± 0.04	1.67 – 1.83	0.634
ALT	U/L	31.03 ± 0.83^{a}	29.38 – 32.67	29.90 ± 0.50^{a}	28.92 – 30.87	27.02 ± 0.76^{b}	25.52 – 28.51	0.003
AST	U/L	74.55 ± 2.05	70.51 – 78.59	79.69 ± 1.44	76.87 – 82.51	77.44 ± 2.57	72.38 – 82.50	0.139
ALP	U/L	92.48 ± 1.87^{a}	88.79 – 96.17	83.94 ± 1.02^{b}	81.94 – 85.95	77.99 ± 1.69^{c}	74.64 – 81.33	0.000
TP	g/dL	8.65 ± 0.08^{a}	8.49 – 8.82	8.90 ± 0.05^{b}	8.79 – 9.00	8.91 ± 0.10^{b}	8.71 – 9.11	0.041

Abbreviation: RBC: Total number of erythrocytes; HCT: Hematocrit value; HGB: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW– SD: Red blood cell distribution width– Standard Deviation; RDW– CV: Red blood cell distribution width– coefficient of variation; PLT: Total number of platelets; PDW: Platelet distribution width; MPV: Mean platelet volume; P– LCR: Platelet large cell ratio; PCT: Plateletcrit value; # : total count number; BUN: Blood Urea Nitrogen; CREA: Creatinine; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; and 95%CI: 95% confidence interval of mean.

a, b and c: different letters on the same line show significant differences ($P < 0.05$).

Table 4 Normal hematological and blood chemical values of fighting bulls in different coastline of southern Thailand.

Parameter	Unit	East coast (n=883)		West coast (n=316)		*P*– value
		Mean±SEM	95%CI	Mean±SEM	95%CI	
RBC	x10^6/µL	7.11 ± 0.03	7.05 – 7.17	7.05 ± 0.05	6.95 – 7.15	0.298
HGB	g/dL	13.04 ± 0.05	12.93 – 13.14	12.99 ± 0.08	12.83 – 13.15	0.658
HCT	%	38.75 ± 0.17	38.42 – 39.07	38.85 ± 0.26	38.35 – 39.36	0.726
MCV	fL	54.63 ± 0.16[a]	54.31 – 54.95	55.41 ± 0.26[b]	54.90 – 55.92	0.011
MCH	pg	18.40 ± 0.05	18.31 – 18.49	18.53 ± 0.08	18.37 – 18.70	0.160
MCHC	g/dL	33.72 ± 0.07	33.58 – 33.86	33.54 ± 0.10	33.34 – 33.73	0.128
RDW– SD	fL	36.88 ± 0.11	36.66 – 37.09	37.06 ± 0.18	36.71 – 37.41	0.382
RDW– CV	%	24.11 ± 0.08	23.95 – 24.26	23.99 ± 0.14	23.71 – 24.27	0.484
Reticulocyte[#]	x10^3/µL	3.18 ± 0.10	2.99 – 3.38	3.40 ± 0.19	3.02 – 3.78	0.318
Reticulocyte	%	0.04 ± 0.00	0.04 – 0.05	0.06 ± 0.01	0.04 – 0.07	0.190
PLT	x10^3/µL	286.11 ± 2.68	280.85 – 291.37	279.47 ± 4.64	270.35 – 288.59	0.215
PDW	fL	8.25 ± 0.06	8.14 – 8.36	8.09 ± 0.09	7.91 – 8.26	0.119
MPV	fL	6.83 ± 0.07	6.69 – 6.98	7.10 ± 0.37	6.36 – 7.83	0.492
P– LCR	%	7.54 ± 0.12	7.30 – 7.79	7.31 ± 0.22	6.88 – 7.74	0.350
PCT	%	0.19 ± 0.00	0.19 – 0.20	0.19 ± 0.00	0.18 – 0.19	0.082
WBC	x10^3/µL	9.16 ± 0.08[a]	9.01 – 9.32	9.50 ± 0.14[b]	9.23 – 9.77	0.034
Neutrophil[#]	x10^3/µL	4.43 ± 0.06[a]	4.32 – 4.55	4.71 ± 0.10[b]	4.51 – 4.91	0.020
Lymphocyte[#]	x10^3/uL	3.20 ± 0.04	3.13 – 3.27	3.15 ± 0.05	3.04 – 3.25	0.443
Monocyte[#]	x10^3/µL	0.46 ± 0.01	0.45 – 0.48	0.46 ± 0.01	0.43 – 0.48	0.586
Eosinophil[#]	x10^3/µL	1.08 ± 0.02[a]	1.04 – 1.12	1.21 ± 0.04[b]	1.12 – 1.29	0.007
Basophil[#]	x10^3/µL	0.01 ± 0.00	0.00 – 0.01	0.01 ± 0.00	0.01 – 0.01	0.987
Neutrophil	%	47.76 ± 0.35	47.08 – 48.44	48.98 ± 0.57	47.86 – 50.10	0.068
Lymphocyte	%	35.49 ± 0.29[b]	34.92 – 36.05	33.64 ± 0.42[a]	32.82 – 34.46	0.000
Monocyte	%	5.12 ± 0.08	4.97 – 5.28	5.06 ± 0.15	4.76 – 5.35	0.702
Eosinophil	%	11.91 ± 0.21	11.50 – 12.31	12.54 ± 0.34	11.88 – 13.21	0.108
Basophil	%	0.06 ± 0.01	0.05 – 0.07	0.06 ± 0.00	0.05 – 0.07	0.831
BUN	mg/dL	14.09 ± 0.28	13.54 – 14.65	14.09 ± 0.43	13.24 – 14.94	0.995
CREA	Umol/L	1.74 ± 0.02	1.70 – 1.78	1.72 ± 0.04	1.64 – 1.79	0.539
ALT	U/L	29.94 ± 0.45	29.05 – 30.82	29.90 ± 0.81	28.31 – 31.48	0.967
AST	U/L	78.50 ± 1.24	76.06 – 80.93	81.60 ± 2.46	76.77 – 86.43	0.260
ALP	U/L	85.09 ± 0.98[b]	83.17 – 87.00	81.48 ± 1.58[a]	78.36 – 84.59	0.053
TP	g/dL	8.87 ± 0.05	8.78 – 8.97	9.07 ± 0.10	8.87 – 9.26	0.080

Abb**reviation:** RBC: Total number of erythrocytes; HCT: Hematocrit value; HGB: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW– SD: Red blood cell distribution width– Standard Deviation; RDW– CV: Red blood cell distribution width– coefficient of variation; PLT: Total number of platelets; PDW: Platelet distribution width; MPV: Mean platelet volume; P– LCR: Platelet large cell ratio; PCT: Plateletcrit value; # : total count number; BUN: Blood Urea Nitrogen; CREA: Creatinine; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; and 95%CI: 95% confidence interval of mean.
a and b: different letters on the same line show significant differences ($P < 0.05$).

Table 5 Normal hematological and blood chemical values of fighting bulls in different seasons of southern Thailand.

Parameter	Unit	Rainy season (n = 884)		Summer (n = 315)		*P*– value
		Mean±SEM	95%CI	Mean±SEM	95%CI	
RBC	x10⁶/µL	7.09 ± 0.03	7.03 – 7.15	7.10 ± 0.05	7.00 – 7.21	0.846
HGB	g/dL	13.04 ± 0.05	12.94 – 13.14	12.98 ± 0.09	12.81 – 13.15	0.527
HCT	%	38.78 ± 0.16	38.46 – 39.10	38.77 ± 0.27	38.23 – 39.30	0.968
MCV	fL	54.88 ± 0.15	54.58 – 55.18	54.70 ± 0.30	54.10 – 55.29	0.587
MCH	pg	18.47 ± 0.05	18.37 – 18.56	18.36 ± 0.07	18.22 – 18.51	0.233
MCHC	g/dL	33.72 ± 0.07[b]	33.58 – 33.87	33.53 ± 0.07[a]	33.39 – 33.66	0.046
RDW#	fL	36.96 ± 0.11	36.75 – 37.18	36.82 ± 0.18	36.47 – 37.16	0.482
RDW	%	24.00 ± 0.09[a]	23.83 – 24.17	24.29 ± 0.10[b]	24.09 – 24.50	0.029
Reticulocyte#	x10³/µL	3.25 ± 0.10	3.04 – 3.45	3.23 ± 0.17	2.89 – 3.56	0.930
Reticulocyte	%	0.05 ± 0.00	0.04 – 0.05	0.05 ± 0.00	0.04 – 0.05	0.800
PLT	x10³/µL	283.54 ± 2.74	278.17 – 288.91	286.66 ± 4.38	278.04 – 295.29	0.546
PDW	fL	8.31 ± 0.06[b]	8.18 – 8.43	7.93 ± 0.06[a]	7.81 – 8.04	0.000
MPV	fL	6.83 ± 0.07	6.69 – 6.97	7.12 ± 0.38	6.37 – 7.86	0.456
PLCR	%	7.59 ± 0.13	7.34 – 7.84	7.18 ± 0.19	6.80 – 7.56	0.073
PCT	%	0.19 ± 0.00	0.19 – 0.19	0.19 ± 0.00	0.19 – 0.20	0.296
WBC	x10³/µL	9.17 ± 0.08	9.02 – 9.32	9.46 ± 0.15	9.17 – 9.76	0.086
Neutrophil#	x10³/µL	4.43 ± 0.06[a]	4.32 – 4.54	4.71 ± 0.11[b]	4.49 – 4.94	0.027
Lymphocyte#	x10³/uL	3.17 ± 0.04	3.10 – 3.24	3.22 ± 0.06	3.11 – 3.33	0.447
Monocyte#	x10³/µL	0.48 ± 0.01[b]	0.47 – 0.50	0.40 ± 0.01[a]	0.38 – 0.43	0.000
Eosinophil#	x10³/µL	1.11 ± 0.02	1.07 – 1.16	1.11 ± 0.04	1.04 – 1.18	0.924
Basophil#	x10³/µL	0.01 ± 0.00	0.00 – 0.01	0.01 ± 0.00	0.00 – 0.01	0.791
Neutrophil	%	47.80 ± 0.35	47.13 – 48.48	48.85 ± 0.58	47.71 – 49.98	0.121
Lymphocyte	%	35.05 ± 0.28	34.50 – 35.61	34.86 ± 0.46	33.96 – 35.75	0.715
Monocyte	%	5.36 ± 0.08[b]	5.21 – 5.52	4.37 ± 0.14[a]	4.10 – 4.65	0.000
Eosinophil	%	12.15 ± 0.20	11.75 – 12.55	11.87 ± 0.35	11.18 – 12.55	0.483
Basophil	%	0.06 ± 0.01	0.05 – 0.08	0.06 ± 0.00	0.05 – 0.07	0.455
BUN	mg/dL	14.62 ± 0.30[b]	14.04 – 15.21	12.69 ± 0.33[a]	12.04 – 13.35	0.000
CREA	Umol/L	1.77 ± 0.02[b]	1.73 – 1.81	1.65 ± 0.03[a]	1.58 – 1.71	0.003
ALT	U/L	30.60 ± 0.47[b]	29.67 – 31.53	28.11 ± 0.69[a]	26.74 – 29.47	0.003
AST	U/L	79.90 ± 1.36	77.22 – 82.58	77.82 ± 1.92	74.05 – 81.60	0.379
ALP	U/L	84.24 ± 0.98	82.31 – 86.17	83.84 ± 1.56	80.76 – 86.92	0.829
TP	g/dL	8.99 ± 0.05[b]	8.89 – 9.09	8.74 ± 0.09[a]	8.57 – 8.92	0.013

Abbreviation: RBC: Total number of erythrocytes; HCT: Hematocrit value; HGB: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW– SD: Red blood cell distribution width– Standard Deviation; RDW– CV: Red blood cell distribution width– coefficient of variation; PLT: Total number of platelets; PDW: Platelet distribution width; MPV: Mean platelet volume; P– LCR: Platelet large cell ratio; PCT: Plateletcrit value; # : total count number; BUN: Blood Urea Nitrogen; CREA: Creatinine; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TP: Total protein; and 95%CI: 95% confidence interval of mean.
a and b: different letters on the same line show significant differences ($P < 0.05$).

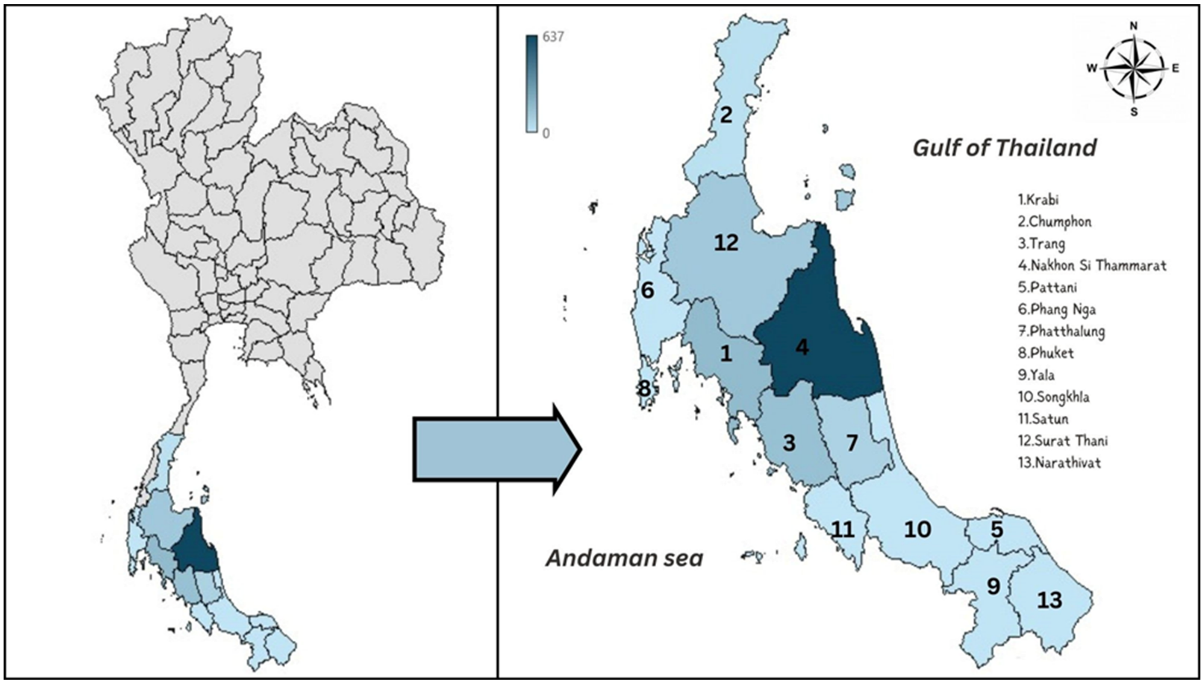

Figure 1 The map illustrates the geographical distribution of fighting bulls in southern Thailand, with color gradients indicating their density in each province.

Discussion

Breed-specific hematological and biochemical reference intervals: This study established RIs for hematological parameters in fighting bulls from southern Thailand, providing valuable data for this specific population. While most parameters aligned with expected reference ranges, several erythrocyte and leukocyte indices exhibited slight deviations such as RBC, HGB, HCT, MCV, MCH, WBC and neutrophil count from previously reported values in dairy cattle (Cornell University College of Veterinary Medicine, 2024a; Cornell University College of Veterinary Medicine, 2024b) and Belgian blue cattle (Guyot *et al*., 2024). These discrepancies likely stem from the distinct physiological characteristics of fighting bulls, which differ significantly from those of dairy and beef breeds. Fighting bulls are selectively bred and conditioned for endurance, strength, and aggression, which may drive hematological adaptations. Elevated erythrocyte indices suggest an enhanced oxygen transport capacity, enabling fighting bulls to meet the high metabolic demands associated with sustained physical exertion (Pellegrini *et al*., 2000). The observed increases in MCV and MCH values may represent a compensatory adaptation to optimize oxygen delivery in response to intensive physical activity (Laeto *et al*., 2023). Additionally, the elevated WBC and neutrophil counts likely reflect an adaptive immune response to recurrent injuries and training-induced physiological stress (Neves *et al*., 2015). Unlike dairy cattle, which are managed for lactation efficiency, or beef cattle, selected for rapid growth and carcass yield, fighting bulls experience continuous physiological stressors that are likely to contribute to these hematological differences. Additionally, genetic factors play a crucial role in shaping these variations. Fighting bulls exhibit hematological profiles distinct from breeds such as Yunling cattle, a three-way hybrid (half Brahman, one-fourth Murray Grey, and one-fourth Yunnan Yellow), which display higher RBC and WBC counts, likely as an adaptation to high altitudes and disease resistance in subtropical regions (Yang *et al*., 2022). In contrast, fighting bulls may prioritize muscle efficiency and anaerobic metabolism over heightened erythropoiesis, leading to the observed differences in RBC parameters. Moreover, creatinine levels in fighting bulls were comparable to those in Belgian Blue cattle, a breed characterized by myostatin-related muscle hypertrophy (Guyot *et al*., 2024). This suggests a strong correlation between muscle mass and creatinine production, as greater muscle turnover leads to higher creatinine synthesis (Megahed *et al*., 2019). Such metabolic differences emphasize the necessity of breed-specific RIs to ensure accurate diagnostic interpretation, as standard RIs derived from conventional beef or dairy cattle may not fully capture the metabolic profiles of highly muscular or performance-driven breeds. These findings underscore the importance of establishing breed-specific RIs for precise clinical assessments in veterinary medicine. Future research should focus on expanding these reference intervals to include a broader range of hematological and biochemical parameters, thereby optimizing health monitoring, performance evaluation, and disease detection in fighting bulls

Age-related variations and importance of age-specific Ris: Age significantly influenced hematological parameters in bulls, particularly erythrocyte indices. Older animals showed reduced levels of RBC, HGB, HCT, and WBC (Kim *et al*., 2014; González *et al*., 2023). These changes underscore the importance of establishing age-specific reference intervals to enhance the accuracy of health assessments in cattle. The decline in erythrocyte parameters likely reflects physiological and metabolic changes associated with aging (Kim *et al*., 2014). Leukocyte indices also varied with age (Yaqub *et al*., 2013). WBC, along with neutrophil, lymphocyte, and monocyte counts, were lower in older bulls, indicating a potential decline in immune function and production (Roland *et al*., 2014). In contrast, eosinophil percentages were higher in older bulls, which may suggest a response to chronic inflammation or parasitic infections. This could be related to the high prevalence of gastrointestinal parasites observed in older Thai fighting bulls (Chantip *et al*., 2024). Additionally, the aging immune system's reduced efficiency likely contributes to the observed decreases in lymphocyte and neutrophil counts (Herman *et al*., 2018; Bedenicki *et al*., 2014). While stress-induced increases in white blood cell counts are common in younger animals, older cattle often exhibit decreased bone marrow activity, leading to lower overall WBC (Roland *et al*., 2014). Interestingly, our findings revealed lower ALP levels in older fighting bulls, a result that contrasts with the established trend of higher ALP levels in younger animals observed across various species, including beef cattle (Saraiva *et al*., 2014). This discrepancy may be attributed to the unique physical demands placed on fighting bulls, such as intense training and frequent high-impact activities, which are likely to enhance osteoclastic activity and influence bone remodeling dynamics (Doblaré *et al*., 2004). Unlike beef cattle, the management of fighting bulls emphasizes performance overgrowth, which may affect bone metabolism markers, including ALP. Furthermore, hormonal changes associated with aging, such as decreases in growth hormone and testosterone levels, could also contribute to the observed trend (Roth *et al*., 2016). These findings suggest an age-related shift in bone turnover dynamics in fighting bulls. Further investigation through longitudinal studies examining bone turnover markers and structural changes in bone tissue is warranted to better understand this phenomenon. The observed alterations underscore the critical need for age-specific RIs to avoid potential misinterpretations of health status in fighting bulls. Implementing age-related RIs would enable veterinarians to more accurately assess health concerns in older animals, which might otherwise be overlooked when relying on reference ranges established for younger cattle.

Geographical and seasonal influences on blood profiles: Our study identified significant geographical and seasonal variations in the hematological profiles of fighting bulls in southern Thailand. Bulls from the West Coast exhibited markedly higher MCV, WBC, neutrophil, and eosinophil counts compared to their East Coast counterparts. In contrast, east coast bulls demonstrated a significantly higher lymphocyte percentage and ALP level. Variations in climate, pasture quality, and management practices across different regions likely contribute to the observed geographical differences in blood profiles. Studies suggest that climate (Mazzullo *et al*., 2014) and pasture quality (Gonçalves *et al*., 2018) can influence immune responses, hematopoiesis (blood cell production), and protein metabolism, potentially explaining the higher MCV, WBC, neutrophil and eosinophil count observed in west coast bulls. The elevated WBC, neutrophil, and eosinophil count in West Coast bulls may be linked to Theileriosis, a disease that induces systemic inflammation and immune activation (Rakwong *et al*., 2022). Furthermore, the high prevalence of strongyle and *Buxtonella sulcata* infestations in the region can lead to intestinal damage and secondary bacterial infections, further contributing to increased neutrophil and eosinophil levels (Chantip *et al*., 2024). The observed hematological variations between the west and east coasts of southern Thailand can be attributed to distinct environmental and ecological factors, including differences in monsoon patterns, humidity, and temperature, which influence physiology and immune responses. Regional variations in the prevalence of blood-borne parasites, such as *Babesia* spp. (Bohman *et al*., 2024), and seasonal weather patterns, including rainfall and vector activity, further contribute to these differences. These factors highlight the complex interplay between environmental conditions and the hematological profiles of fighting bulls.

Seasonal differences were also apparent, with bulls sampled during the rainy season showing elevated MCHC, PDW, monocyte count, BUN, CREA, and TP levels compared to those sampled during the summer. These findings are consistent with earlier studies that emphasized the influence of environmental factors on hematological and biochemical parameters in cattle (Hristev and Ivanova, 2022; Dar *et al*., 2019). However, our results contrast with the findings of Aengwanich *et al*. (2009), who reported no significant seasonal variations in hematological values in cattle from northeastern Thailand. Changes in dietary composition and availability during this period might influence protein metabolism and BUN levels (Mirzadeh *et al*., 2010a). Increased water intake due to the rainy season could lead to hemodilution, reflected in a wider PDW (Meeusen *et al*., 2000). Additionally, the rainy season might be associated with higher exposure to ectoparasites or pathogens, potentially elevating CREA and TP levels associated with inflammatory responses (Mirzadeh *et al*., 2010a). CREA is associated with muscle mass (Megahed *et al*., 2019), while albumin and globulin serve as markers of inflammation (Yurtseven *et al*., 2009). However, as these parameters were not assessed in this study, future research should incorporate body weight, body condition score, and muscle condition score alongside creatinine to better elucidate its relationship with muscle mass. Furthermore, integrating albumin and globulin measurements would provide a more comprehensive evaluation of inflammatory status and overall physiological health. Regional and seasonal variations necessitate the establishment of region-specific reference intervals for accurate health assessments. Further research is warranted to explore the specific mechanisms underlying these variations, including detailed dietary analysis, ectoparasite burden evaluation, and a deeper understanding of management practices across different regions and seasons.

This study created the first comprehensive reference ranges for hematology and biochemistry in over 1,400 healthy Southern Thai fighting bulls. While some values differed slightly from existing cattle references, age, location, and season also influenced results. Older bulls had lower RBC and WBC indices. Bulls from the West Coast had higher PLT, specific WBC types, and TP compared to the East Coast. Additionally, rainy-season bulls showed elevated values in certain tests. These findings emphasize the need to consider these factors when interpreting blood tests in fighting bulls. The established region-specific reference intervals can be a valuable tool for veterinarians to improve health assessments and management strategies for these animals.

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